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Tesis para la obtención del Título de Doctora en Biotecnología

CARACTERIZACIÓN Y MEJORA GENÉTICA DE LA BERENJENA (*S. melongena* L.) PARA COMPUESTOS BIOACTIVOS

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" Lo que no se empieza,
nunca tendrá un final"

Johan Wolfgang von Goethe

A Mario y a Vega
A mis padres
A Jorge

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SUMMARY

Fruits and vegetables contain bioactive compounds beneficial for human health. The development of varieties with a higher content in these compounds is of interest, as it contributes to satisfying an increasing demand by consumers of products with functional activity. Among other vegetables, eggplant (*Solanum melongena*) has a high antioxidant activity, mostly derived from its high content in polyphenols, and it has been demonstrated to have beneficial effects for human health. Amongst the phenolic compounds of eggplant, chlorogenic acid outstands, as it is the most abundant phenolic compound in this crop and presents multiple beneficial properties for health.

This Doctoral Thesis deals with the characterization and breeding of eggplant in order to obtain relevant information and plant material for the development of eggplant varieties with a higher content in bioactive compounds, in particular, polyphenols, making use of the intraspecific and interspecific variation. On the other hand, an integral breeding approach must take into account not only the trait to be improved, but also those traits of interest for the success of a variety and, in consequence, we have studied other traits related to the increase in the phenolic content, like fruit browning, and also other traits of general interest for breeding.

In the first part of this Doctoral Thesis we focus on the study of the diversity of common eggplant and related species in traits of agronomic interest. The objective is to evaluate the diversity, identify sources of variation and to study relationships among traits. In a first study, we evaluate a collection of traditional eggplant varieties, in which we have found a high diversity for functional quality traits and browning. In this study we found that the content in chlorogenic acid is positively correlated with the antioxidant activity and the correlation with browning is low, demonstrating that it is feasible to select eggplant varieties with high content in chlorogenic acid and moderate browning. We also found that, even with a low polyphenol oxidase activity, there may be a significant browning, suggesting that polyphenol oxidase activity (PPO) is not the limiting factor for browning in the studied collection.

With the aim of increasing the genetic diversity for breeding eggplant for bioactive compounds and other traits of importance, we have studied the

diversity in a collection of scarlet (*S. aethiopicum*) and gboma (*S. macrocarpon*) eggplants. The morphological characterization with conventional descriptors and phenomic tools (Tomato Analyzer) has allowed us to study the relationships among the different cultivar groups and wild relatives and to determine that scarlet and gboma eggplant complexes are hypervariable. In this collection we have also studied the reducing capacity and the content in chlorogenic acid, and we have found a huge variability. In general, scarlet eggplant presents relatively low contents, while gboma eggplant, in particular its wild ancestor *S. dasyphyllum*, presents very high values. In macrophage cell cultures we have also found that the varieties with higher content in chlorogenic acid also display a greater inhibition of nitric oxide (NO) production indicating beneficial properties for health.

In the second part of this Doctoral Thesis we have evaluated the interest of interspecific hybridization for eggplant breeding, in particular for the content in bioactive compounds. We have obtained two families, including backcrosses, between common eggplant (*S. melongena*) on one side and cultivated scarlet eggplant (*S. aethiopicum*) and the wild relative *S. incanum* on the other. The results show that fertility of materials derived from the hybridization between *S. melongena* and *S. aethiopicum* is low and that there is a low efficiency in the backcrosses to *S. melongena*. In addition, the low content in polyphenols of *S. aethiopicum* is dominant. On the contrary, the backcross to *S. aethiopicum* results in many plants with higher levels of fertility. Therefore, we suggest that *S. melongena* may be a source of variation for the improvement of the content in polyphenols of scarlet eggplant.

The family obtained by interspecific hybridization between *S. melongena* and *S. incanum* displayed high levels of fertility and, in the first backcross to *S. melongena,* we found individuals morphologically similar to cultivated eggplant. The study of phenolic compounds revealed that *S. incanum* is a good source of variation for the improvement of common eggplant, with values much higher than those of the cultivated species. In the first backcross we already found individuals with high chlorogenic acid content and moderate browning, suggesting that it is possible to successfully introgress the high content in chlorogenic acid of *S. incanum* in the genetic background of cultivated eggplant.

In summary, the works performed in this Doctoral Thesis contribute to new knowledge on the diversity and relationship among traits involved in functional quality of eggplant and other traits of interest for the genetic improvement of this crop. The materials selected and obtained are of great interest for the development of commercial varieties of eggplant with improved bioactive properties.

RESUMEN

Las frutas y hortalizas presentan compuestos bioactivos beneficiosos para la salud humana. El desarrollo de variedades con un mayor contenido en este tipo de compuestos es de interés, ya que contribuye a satisfacer una demanda creciente por parte de los consumidores por productos con propiedades funcionales. Dentro de las hortalizas, la berenjena (*Solanum melongena*) presenta una alta actividad antioxidante, fundamentalmente derivada de su alto contenido en polifenoles, y se ha demostrado que presenta efectos beneficiosos para la salud humana. Entre los compuestos fenólicos de la berenjena destaca el ácido clorogénico, ya que se trata del más abundante en este cultivo y presenta múltiples propiedades beneficiosas para la salud.

Esta Tesis Doctoral trata de la caracterización y mejora de la berenjena para obtener información relevante y material vegetal para el desarrollo de variedades de berenjena con un mayor contenido en compuestos bioactivos, en particular polifenoles. Para ello utilizamos la variación intraespecífica e interespecífica. Por otra parte, en una mejora integral se debe tener en cuenta no solo el carácter a mejorar, sino también aquellos caracteres de interés para el éxito de una variedad, por lo que también hemos estudiado otros caracteres relacionados con el incremento del contenido en polifenoles, como puede ser el pardeamiento del fruto, y así como otros caracteres de interés general en mejora.

En una primera parte de esta Tesis Doctoral nos centramos en el estudio de la diversidad en berenjena común y especies relacionadas para los caracteres objeto de esta tesis y también para caracteres de interés agronómico. El objetivo es evaluar la diversidad, identificar fuentes de variación y estudiar las relaciones entre caracteres. En un primer estudio, evaluamos una colección de variedades tradicionales de berenjena, en la cual hemos encontrado una alta diversidad para caracteres de calidad funcional y pardeamiento. En este estudio encontramos que el contenido en ácido clorogénico está correlacionado positivamente con la actividad antioxidante y que la correlación con el pardeamiento es baja, demostrando que es posible seleccionar variedades de berenjena con alto contenido en ácido clorogénico y pardeamiento moderado. También comprobamos que incluso con baja actividad polifenol oxidasa (PPO) se puede producir pardeamiento significativo,

sugiriendo que la actividad PPO no es el factor limitante para el pardeamiento en la colección estudiada.

Con objeto de ampliar la diversidad genética de la berenjena para la mejora de compuestos bioactivos y otros caracteres de importancia hemos estudiado la diversidad en una colección de berenjenas escarlata (*S. aethiopicum*) y gboma (*S. macrocarpon*). La caracterización morfológica mediante descriptores convencionales y herramientas fenómicas (Tomato Analyzer) nos ha permitido estudiar las relaciones entre los distintos grupos de cultivares y especies silvestres relacionadas y determinar que los complejos berenjena escarlata y gboma son hipervariables. En esta colección hemos estudiado también la actividad reductora y el contenido en ácido clorogénico, encontrado una enorme variabilidad. En general, la berenjena escarlata presenta contenidos relativamente bajos, mientras que la berenjena gboma, en particular su ancestro silvestre *S. dasyphyllum*, presentan valores muy elevados. También hemos comprobado en cultivos celulares de macrófagos que las variedades con mayor contenido en ácido clorogénico presentan una mayor inhibición de la producción de óxido nítrico (NO) indicando propiedades beneficiosas para la salud.

En la segunda parte de la Tesis Doctoral hemos evaluado el interés de la hibridación interespecífica para la mejora de la berenjena, en particular para el contenido en compuestos bioactivos. Hemos obtenido dos familias, incluyendo retrocruzamientos, entre la berenjena común (*S. melongena*) por una parte y la berenjena escarlata cultivada (*S. aethiopicum*) y la especie silvestre *S. incanum* por otra. Los resultados muestran que la fertilidad de los materiales derivados de la hibridación entre *S. melongena* y *S. aethiopicum* es baja y que se obtiene una baja eficiencia en los retrocruzamientos hacia *S. melongena*. Además, el bajo contenido en polifenoles de *S. aethiopicum* se comporta como dominante. En cambio el retrocruzamiento hacia *S. aethiopicum* proporciona muchas plantas con mayores niveles de fertilidad. Sugerimos, por tanto, que S. melongena puede ser una fuente de variación para la mejora en contenido en polifenoles de la berenjena escarlata.

La familia obtenida por hibridación interespecífica entre *S. melongena* y *S. incanum* mostró altos niveles de fertilidad y en el primer retrocruce hacia *S. melongena* se encuentran individuos morfológicamente similares a la

berenjena cultivada. El estudio de los compuestos fenólicos mostró que *S. incanum* es una buena fuente de variación para la mejora de la berenjena común, con valores muy superiores a los de la especie cultivada. En el primer retrocruce se encuentran ya individuos con alto contenido en ácido clorogénico y pardeamiento moderado, lo cual sugiere que es posible introgresar exitosamente el alto contenido en ácido clorogénico de *S. incanum* en el fondo genético de la berenjena cultivada.

En definitiva, los trabajos realizados en esta Tesis Doctoral aportan nuevo conocimiento sobre la diversidad y relaciones entre caracteres implicados en la calidad funcional de la berenjena y otros caracteres de interés en la mejora genética de este cultivo. Los materiales seleccionados y obtenidos son de gran interés para el desarrollo de variedades comerciales de berenjena con propiedades bioactivas mejoradas.

RESUM

Les fruites i hortalisses presenten compostos bioactius beneficiosos per a la salut humana. El desenvolupament de varietats amb un major contingut en aquest tipus de compostos és d'interés, ja que contribueix a satisfer una demanda creixent per part dels consumidors per productes amb propietats funcionals. Dins de les hortalisses, l'albergina (*Solanum melongena*) presenta una alta activitat antioxidant, derivada fonamentalment del seu alt contingut en polifenols, i s'ha demostrat que té efectes beneficiosos per a la salut humana. Entre els compostos fenòlics de l'albergina destaca l'àcid clorogènic, el més abundant en aquest cultiu i amb múltiples propietats beneficioses per a la salut.

Aquesta Tesi Doctoral tracta de la caracterització i millora de l'albergina per a obtenir informació rellevant i material vegetal per al desenvolupament de varietats d'albergina amb un major contingut en compostos bioactius, en particular polifenols. Per a això utilitzem la variació intraespecífica i interespecífica. D'altra banda, en una millora integral s'ha de tindre en compte no sols el caràcter que cal millorar, sinó també aquells caràcters d'interés per a l'èxit d'una varietat, per la qual cosa també hem estudiat altres caràcters relacionats amb l'increment del contingut en polifenols, com pot ser l'enfosquiment del fruit, a més d'altres caràcters d'interés general en millora.

En la primera part d'aquesta Tesi Doctoral ens centrem en l'estudi de la diversitat en l'albergina comuna i espècies relacionades per als caràcters objecte d'aquesta tesi, i també per a caràcters d'interés agronòmic. L'objectiu és avaluar la diversitat, identificar fonts de variació i estudiar les relacions entre caràcters. En un primer estudi, avaluem una col•lecció de varietats tradicionals d'albergina, en la qual hem trobat una alta diversitat per a caràcters de qualitat funcional i enfosquiment. En aquest estudi trobem que el contingut en àcid clorogènic està correlacionat positivament amb l'activitat antioxidant i que la correlació amb l'enfosquiment és baixa, la qual cosa demostra que és possible seleccionar varietats d'albergina amb alt contingut en àcid clorogènic i enfosquiment moderat. També comprovem que en una de baixa activitat polifenol oxidasa (PPO) es pot produir un enfosquiment

significatiu, suggerint que l'activitat PPO no és el factor limitant per a l'enfosquiment en la col•lecció estudiada.

A fi d'ampliar la diversitat genètica de l'albergina per a la millora de compostos bioactius i altres caràcters d'importància, hem estudiat la diversitat en una col•lecció d'albergines escarlata (*S. aethiopicum*) i gboma (*S. macrocarpon*). La caracterització morfològica mitjançant descriptors convencionals i eines fenòmiques (Tomato Analyzer) ens ha permés estudiar les relacions entre els distints grups de cultivars i espècies silvestres relacionades i determinar que els complexos albergina escarlata i gboma són hipervariables. En aquesta col•lecció hem estudiat també l'activitat reductora i el contingut en àcid clorogènic, amb una enorme variabilitat. En general, l'albergina escarlata presenta continguts relativament baixos, mentre que l'albergina gboma, en particular l'avantpassat silvestre *S. dasyphyllum*, presenta valors molt elevats. També hem comprovat en cultius de cèl•lules de macròfags que les varietats amb un major contingut en àcid clorogènic mostra una major inhibició de la producció d'òxid nítric (NO), la qual cosa indica propietats beneficioses per a la salut.

En la segona part de la Tesi Doctoral hem avaluat l'interés de la hibridació interespecífica per a la millora de l'albergina, en particular per al contingut en compostos bioactius. N'hem obtingut dues famílies, incloent-hi retrocreuaments, entre l'albergina comuna (*S. melongena*) d'una banda i l'albergina escarlata cultivada (*S. aethiopicum*) i l'espècie silvestre *S. incanum* per una altra. Els resultats mostren que la fertilitat dels materials derivats de la hibridació entre *S. melongena* i *S. aethiopicum* és baixa, i que s'obté una baixa eficiència en els retrocreuaments cap a *S. melongena*. A més, el baix contingut en polifenols de S. aethiopicum es comporta com a dominant. En canvi, el retrocreuament cap a *S. aethiopicum* proporciona moltes plantes amb majors nivells de fertilitat. Suggerim, per tant, que *S. melongena* pot ser una font de variació per a la millora en contingut en polifenols de l'albergina escarlata.

La família obtinguda per hibridació interespecífica entre *S. melongena* i *S. incanum* va mostrar uns alts nivells de fertilitat, i en el primer retrocreuament cap a S. melongena es troben individus morfològicament semblants a l'albergina cultivada. L'estudi dels compostos fenòlics mostrà que S. incanum és una bona font de variació per a la millora de l'albergina comuna,

amb valors molt superiors als de l'espècie cultivada. En el primer retrocreuament es troben ja individus amb un alt contingut en àcid clorogènic i enfosquiment moderat, la qual cosa suggereix que és possible introgressar reeixidament l'alt contingut en àcid clorogènic de *S. incanum* en el fons genètic de l'albergina cultivada.

En definitiva, els treballs realitzats en aquesta Tesi Doctoral aporten nous coneixements sobre la diversitat i les relacions entre caràcters implicats en la qualitat funcional de l'albergina i altres caràcters d'interés en la millora genètica d'aquest cultiu. Els materials seleccionats i obtinguts són de gran interés per al desenvolupament de varietats comercials d'albergina amb propietats bioactives millorades.

Introducción

1.1 Breeding Vegetables with Improved Bioactive Properties

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Abstract

Vegetable crops contain significant amounts of many bioactive compounds which prevent and/or protect against chronic diseases. Consumers increasingly demand vegetables with improved bioactive properties and this is stimulating the development of new cultivars with enhanced content in bioactive compounds. Generally, breeding programmes of specific crops are aimed at increasing the most relevant bioactive compounds of each crop. The success of these breeding programmes depends on the availability of sources of variation for bioactive compounds. Traditional varieties and wild relatives' collections are generally very variable for these compounds and in many cases it is possible to identify sources of variation of great interest among these materials. There are several breeding strategies for improving the content in bioactive compounds, including conventional strategies based on phenotyping, as well as modern strategies that rely on marker assisted selection or genetic transformation. Breeding for the enhancement of bioactive compounds may affect vegetables in a positive (e.g., extended shelf-life) or negative (e.g., browning, bitterness) way other relevant traits for the success of a cultivar. The negative side effects may be circumvented by using complementary breeding strategies aimed at reducing or removing the negative impact on the characteristics and performance of a new cultivar. In summary, breeding can contribute to the development of a new generation of vegetable crops with enhanced bioactive properties and therefore to the development of the horticultural sector.

Introduction

Many epidemiological studies reveal that people having a high level of consumption of vegetables presents a better health and lower risk of chronic diseases, including cardiovascular diseases and different types of cancer (Hung *et al*., 2004; Boeing *et al*., 2012). Vegetables contain many bioactive compounds and represent a major source of antioxidants and other compounds that are beneficial to human health (Terry, 2011; Rajarathnam *et al*., 2014). Consumers are increasingly demanding vegetables with bioactive properties that contribute to maintaining a good health and preventing diseases (Weatherspoon *et al*., 2014). In consequence, breeding programmes in vegetables are increasingly considering the content in bioactive compounds as a major breeding objective (Diamanti *et al*., 2011).

In many vegetable crops, breeding programmes have been devoted to improving yield, resistance to diseases, produce uniformity or apparent quality (Prohens and Nuez, 2008a, 2008b). Other important traits, like those related to organoleptic quality have generally been considered of second rank compared to breeding for yield, although in some crops breeding for organoleptic quality has also been considered an important trait in breeding programmes (Casañas and Costell, 2006). The content in bioactive compounds has been usually considered of low priority in breeding programmes, and few cultivars have been developed having dramatically improved contents in bioactive compounds. Among them, some new varieties have been released that are characterized (and are advertised as such) with a higher content in bioactive compounds. Among them there are some prominent examples, like the 'Fashion' watermelon, which has a high content in lycopene and citrulline, the 'Lycomate' and 'Doublerich' tomatoes, which have, respectively, a high content in lycopene and vitamin C, the Almagro eggplant, with high contents in chlorogenic acid (Watada *et al*., 1986; Tarazona-Díaz *et al*., 2011; Hurtado *et al*., 2014). As a consequence of this increased content in bioactive constituents these vegetable varieties have a high added value and reach a higher price in the market.

Given the increased demanding by consumers for vegetables with increased content in bioactive compounds, researchers and breeders are developing new knowledge and tools for an efficient breeding of the content in bioactive compounds in vegetables (Cámara, 2006; Diamanti *et al*., 2011). In this way, there is an increasing number of breeding programmes and scientific studies aimed at improving the content in bioactive compounds of vegetables, and the trend seems that will continuing in the coming years. In this respect, the development of genomics is greatly contributing to improve marker assisted selection as well as to develop tools for an efficient breeding (Pérez-de-Castro *et al*., 2012).

In this paper we deal with some relevant issues related to breeding for the content in bioactive compounds in vegetables, including breeding objectives, diversity and sources of variation, breeding strategies, and collateral effects on other traits of interest for the success of a cultivar. The

objective is to provide general and comprehensive information for the development of vegetables with improved bioactive properties.

Breeding objectives for improving bioactive properties

Plant breeding is aimed at exploiting the genetic potential of plants for benefit of humans (Rodríguez-Burruezo *et al*., 2009; Acquaah, 2012). Therefore, breeding programmes aimed at improving the bioactive properties of vegetables will be devoted to developing new varieties with contents of bioactive compounds higher than those of the predominant varieties (Cámara, 2006; Diamanti *et al*., 2011). In this respect, breeding efforts can be devoted to improve a specific compound (e.g., chlorogenic acid, β-carotene, glucoraphanin, etc.), a group of compounds (e.g., total phenolics, total carotenoids, total glucosinolates, etc.), or an aggregate property (e.g., antixodant activity, anticarcinogenic activity, etc.). Each of these levels has different levels of complexity from the point of view of breeding. Breeding for specific compounds generally will be less complex from the genetic point of view than breeding for groups of compounds or aggregate properties, in which the genetic control is usually more complex (Rodríguez-Burruezo *et al*., 2009; Acquaah, 2012).

Within vegetable crops there are many compounds with bioactive properties, like phenolics, carotenoids, glucosinolates, vitamins, folates, phytosterols, etc. (Cámara, 2006; Rajaranthnam *et al*., 2014). However, each of these groups contains many compounds, and there are important differences in the activity of individual compounds within each group (Ignat *et al*., 2011; Fernández-García *et al*., 2012). Also, given that there are important differences among vegetables in the compounds responsible for the bioactive properties (Cámara, 2006; Tsao *et al*., 2006; Prohens and Nuez 2008a, 2008b), breeding programmes are usually directed to increase the levels of those compounds or groups of compounds that are responsible of the most relevant properties for each vegetable crop (Table 1).

Table 1. Some important vegetable crops and major bioactive groups of compounds and specific compounds for which breeding programmes are being performed.

Vegetable crop	Compounds with bioactive properties	References
Artichoke (*Cynara cardunculus* var. *scolymus* L.)	Phenolics, in particular chlorogenic acid	Pandino *et al*. (2012)
Asparagus (*Asparagus officinalis* L.)	Phenolics, in particular phenolic acids, flavonoids, flavanols and ascorbic acid	Lee *et al*. (2014)
Cabbage and cauliflower (*Brassica oleracea* L.)	Glucosinolates, carotenoids and anthocyanins	Padilla *et al*. (2007)
Carrot (*Daucus carota* L.)	Carotenoids and phenolics, in particular cholorogenic acid and anthocyanins	Baranski *et al*. (2012)
Celery (*Apium graveolens* L.)	Phenolics	Yao *et al*. (2010)
Cucumber (*Cucumis sativus* L.)	Carotenoids, in particular β-carotene	Navazio and Simon (2001)
Eggplant (*Solanum melongena* L.)	Phenolics, in particular chlorogenic acid and anthocyanins	Prohens *et al*. (2007)
Leek (*Allium porrum* L.)	Phenolics, lutein, β-carotene, ascorbic acid and vitamin E	Bernaert *et al*. (2012)
Lettuce (*Lactuca sativa* L.)	Carotenoids, in particular β-carotene and lutein, and anthocyanins	Mou (2005)
Melon (*Cucumis melo* L.)	Carotenoids	Harel-Beja *et al*. (2010)
Onion (*Allium cepa* L.)	Phenolics, in particular flavonoids, flavonols and anthocyanins, and ascorbic acid	Yang *et al*. (2004)
Pepper (*Capsicum annuum* L.)	Carotenoids, phenolics, and ascorbic acid	Rodríguez-Burruezo *et al*. (2011)
Pumpkin, squash and zucchini (*Cucurbita* spp.)	Carotenoids, tocopherol, ascorbic acid	de Carvalho *et al*. (2012)
Spinach (*Spinacia oleracea* L.)	Lutein and phenolics	Pandjaitan *et al*. (2005)
Table beet (*Beta vulgaris* subsp. *vulgaris* L.)	Betalains	Gaertner *et al*. (2005)
Tomato (*Solanum lycopersicum* L.)	Carotenoids, in particular lycopene, phenolics, and ascorbic acid	Adalid *et al*. (2010)
Watermelon (*Citrullus lanatus* (Thunb. Matsum. & Nakai)	Carotenoids, in particular lycopene, and ascorbic acid	Yoo *et al*. (2012)

Diversity and sources of variation

As occurs with any breeding programme, the success of a breeding programme for improving the bioactive properties of a vegetable crop requires having genetic diversity available for the target trait/s (Rodríguez-Burruezo *et*

al., 2009; Diamanti *et al*., 2011; Acquaah, 2012). Identification of genetic diversity in collections of germplasm or populations for bioactive compounds can be done using conventional methods based on classical genetics and quantitative genetics methods or with modern biotechnologies (Rodríguez-Burruezo *et al*., 2012; Acquaah, 2012; Pérez-de-Castro *et al*., 2012).

As occurs with nutrients (e.g., carbohydrates, proteins, minerals, etc.) in which modern breeding has led to the undesirable effect of "dilution of nutrients" (Davis, 2009), for bioactive compounds there has also been a reduction in the levels in modern varieties when compared with traditional varieties. In this way, increases in yield have frequently been associated to a reduction in the content of compounds with bioactive properties. Similarly, the introduction of long shelf-life genes, which alter ripening, may produce a reduction in the content of bioactive compounds. In this respect, in the case of tomato, gene *rin*, which is present in many long shelf-life varieties of tomato (Marín, 2013), causes a reduction in the content in lycopene in the fruit (Vrebalov *et al*., 2002). This indicates that very often breeding programmes aimed at improving the bioactive properties of vegetables will need to identify sources of variation in materials other than élite modern varieties. Furthermore, modern varieties usually have a narrow genetic base (Simmonds, 1997; Rodríguez-Burruezo *et al*., 2009; Acquaah, 2012) and in order to improve the bioactive properties breeders very frequently will have to turn to materials like traditional varieties and wild relatives.

Traditional varieties usually present a high variation for the content in bioactive compounds, with values much higher than those of modern commercial varieties (Koch and Goldman, 2005; Mou, 2005; Rodríguez-Burruezo *et al*., 2005; Burger *et al*., 2006; Prohens *et al*., 2007; Perkins-Veazie *et al*., 2010). Traditional varieties have the advantage that hybridizations with modern élite materials are fertile and hybrids and subsequent generations present the typical characteristics of the domesticated species (Rodríguez-Burruezo *et al*., 2009). On occasion, related wild species represent an additional source of variation of great interest as they present values much higher (frequently several times higher) than those present in the cultivated species (Willits *et al*., 2005; Prohens *et al*., 2013). However, in these cases, breeding programmes can encounter some difficulties in hybridization, hybrid

sterily or reduced fertility, and the need of high number of backcross generations to remove the undesirable part of the genetic background of the donor wild relative (Kalloo and Chowdhury, 1992; Rodríguez-Burruezo *et al*., 2009). In any case, the availability of adequate sources of variation usually requires the screening of large germplasm collections in order to identify materials of interest (Rodríguez-Burruezo *et al*., 2005; Prohens *et al*., 2007). Once these sources of variation have been identified, an adequate and efficient breeding strategy has to be applied in order to introgress it into an appropriate genetic background in order to obtain a commercially valuable cultivar (Simmonds, 1997; Rodríguez-Burruezo *et al*., 2009; Acquaah, 2009).

Breeding strategies

Although some bioactive properties of specific vegetable crops may be qualitative (i.e., presence/absence), in most cases the traits responsable of the bioactive properties are quantitative. Also, apart from genetic differences, the high environmental influence in the expression of this type of traits favours the existence of continuous variation, even when the trait has an oligogenic control (Tsao *et al*., 2005). This implies that usually strategies for breeding for bioactive properties are those used for quantitative traits. Depending on the type of strategy to be used we can distinguish between conventional strategies based on phenotyping, marker assisted selection, and strategies derived from genetic transformation (Gepts, 2002; Collard and Mackill, 2008).

Conventional strategies are based on selection in genetically variable populations for the trait of interest and on hybridization and selection in segregating generations (Rodríguez-Burruezo *et al*., 2009; Acquaah, 2012). The success of plant breeding in the XXth century has mostly relied on these conventional strategies, which have proved highly successful and efficient for yield traits (Pérez-de-Castro *et al*., 2012). Application of these breeding methods to traits related to bioactive properties shows that for these traits it is possible to achieve important genetic advances. For example, we have found that in eggplant the narrow-sense heritability for total phenolics was of 0.5 (Prohens *et al*., 2007), which together with the wide diversity for this trait in the germplasm collections indicates that it is possible to achieve considerable genetic advances for this trait (Plazas *et al*., 2013).

The increasing availability of molecular and genomic tools is fostering, as occurs with other traits, a revolution in breeding for bioactive properties (Pérez-de-Castro *et al*., 2012). In this way, thanks to the new developments it has been possible to identify quantitative trait loci (QTL) as well as genes and allelic variants of these genes involved in the synthesis of compounds responsible for bioactive properties as well as molecular markers linked to them (Just *et al*., 2009; Kinkade and Foolad, 2013a; Sotelo *et al*., 2014). This makes feasible in vegetable crops the marker assisted selection for traits related to bioactive properties (Kinkade and Foolad, 2013b; Plazas *et al*., 2013). Therefore, once the genes or QTLs involved in the target bioactive compound/s are identified selection can be done of the individuals of interest without the need of phenotyping (Collard and Mackill, 2008). This strategy can also be very useful for gene pyramiding for different favourable alleles involved in the biosynthetic pathways of the target compounds (Ishii and Yonezawa 2007a, 2007 b; Plazas *et al*., 2013).

The improvement in the content of bioactive compounds can also be achieved by means of genetic transformation, which allows important increases in a short period of time (Díaz de la Garza *et al*., 2007; Guo *et al*., 2012). Genetic transformation requires the introduction using different transformation techniques of one or several genes from different organisms in the genome of the target species in order to achieve transgenesis (Kole *et al*., 2010). However, transgenic varieties are suffering from an important rejection at the social level and it seems difficult that they represent at a short-medium term a realistic alternative for the development of commercially accepted variaties, at least in Europe (nicolia *et al*., 2014). Cisgenesis, which consists in the genetic transformation resulting only in the introduction of genes obtained from materials sexually compatible with the donor variety (Jacobsen and Schouten, 2007), is an alternative that is free from most of the critics of transgenesis. However, given that cisgenesis uses genetic transformation techniques its utilization is not free of criticism and it is unlikely that it becomes approved soon in Europe.

Effects of breeding for bioactive properties on other traits

The success of a new cultivar requires that all the actors involved in the chain that goes from the production to the consumer become satisfied with

the performance of the new variety (Rodríguez-Burruezo *et al*., 2009; Acquaah, 2012). In this respect, the improvement in the content of bioactive compounds, apart from an increase in the compound/s of interest may have other collateral effects, which can be positive or negative, on other agronomic or quality traits that may affect the success of the new cultivar.

An example of a positive effect is the increase in the shelf-life of tomato fruits with high levels of anthocyanins in the fruit (Zhang *et al*., 2013). In this respect, the antioxidant properties of many bioactive compounds may have a role in extending shelf-life, as they are able to neutralize the free radicals that are generated during the periods of senescence or as a consequence of infection (Davey and Keulemans, 2004; Singh *et al*., 2010; Zhang *et al*., 2013). Regarding negative effects, the increase in phenolics content can result in an increase of browning in vegetables like artichoke or eggplant (Prohens *et al*., 2007; Cefola *et al*., 2012). However, selection of allelic variants of polyphenol oxidases (necessary for the development of enzymatic browning) with reduced activity makes possible the selection of varieties with high content in phenolics and low browning (Plazas *et al*., 2013; Chi *et al*., 2014). Another example of a negative effect associated to the increase in bioactive compounds corresponds to glucosinolates, which have a bitter flavour, in brassicas (Drewnowski and Gomez-Carneros, 2000). In this case, the perception of the bitter flavour for different glucosinolates is different (Williams and Pun, 2010), and with positive selection for glucosinolates with low bitterness and negative selection for glucosinolates with high bitterness it might be possible to improve the content in glucosinolates without increasing bitterness (Wricke and Weber, 1986). These two examples show that there are strategies that allow combining an increase in the content in compounds with bioactive properties and reduce the undesirable effects on other traits important for the success of a cultivar.

Conclusions

Breeding for bioactive properties in vegetables is increasingly becoming important in breeding programmes in vegetable crops. There are many bioactive compounds in vegetables and, therefore, there are many possibilities for the development of new cultivars with improved bioactive properties. The utilization of a wide diversity in breeding programmes, in

particular from traditional varieties and wild relatives, on which applying adequate strategies for increasing the content of bioactive compounds will lead to the development of new vegetable crops cultivars with improved bioactive properties compared to present cultivars. At the same time, these strategies will strengthen the positive effects of the increase in these bioactive compounds on other traits of agronomic or commercial interest and to reduce the negative effects that may have on other characteristics. In summary, breeding for bioactive properties will allow the development of a new generation of cultivars with improved bioactive properties.

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1.2 La berenjena como cultivo objetivo para la mejora de compuestos bioactivos

1.2.1. Las berenjenas cultivadas y su importancia

La berenjena (*Solanum melongena* L.) es, después del tomate, la segunda solanácea más importante cultivada por su fruto. Su origen ha sido muy discutido debido a que el género *Solanum* es bastante extenso (Figura 1) y muchas especies no se encuentran en bancos de germoplasma o no todas han sido correctamente clasificadas. Las teorías más extendidas acerca del origen de la berenjena la sitúan en la zona Indo birmana, desde donde se cree que se fue distribuyendo, siendo China y la zona mediterránea centros secundarios de diversidad (Lester and Hasan, 1991; Furini y Wunder, 2004; Hurtado *et al.*, 2012; Cericola *et al.*, 2013). Sin embargo, recientemente se han obtenido evidencias de que la berenjena surgió en África y se dispersó hacía del Medio Oriente hasta la India (Weese y Bohs, 2010).

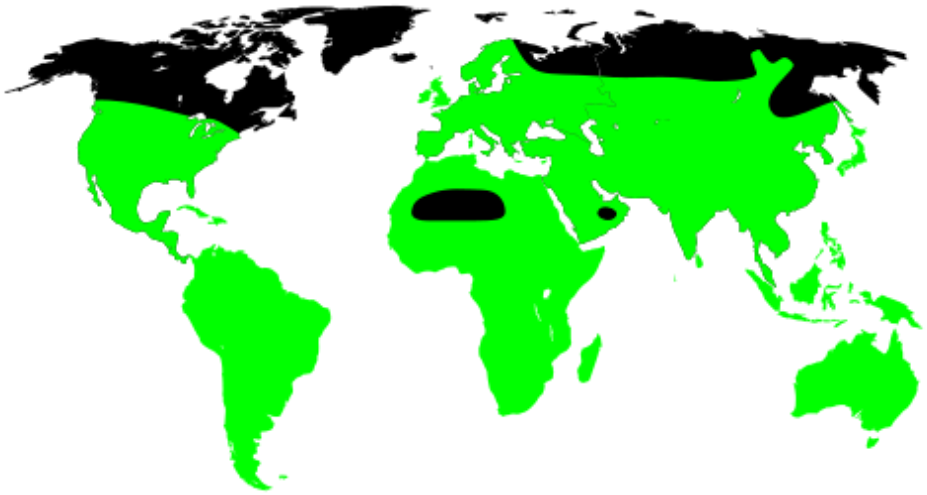

Figura 1. Distribución de miembros de la familia de las *Solanaceae* en el mundo (zona verde).

La relación entre las especies silvestres, formas adventicias y la cultivada de *S. melongena* ha sido siempre motivo de controversia. Hay varias teorías sobre cuál fue su origen: algunos autores afirman que la berenjena actual es una especie que procede de la domesticación de la especie silvestre

S. incanum; otras afirman que probablemente la berenjena viene de la domesticación de la especie silvestre *S. insanum* (hay quien clasifica *S. insanum* como *S. melongena* var. insanum) (Prohens *et al.,* 2013; Knapp *et al.*, 2013). Sin embargo, es muy probable que la berenjena que se consume en la actualidad sea consecuencia de la domesticación de una de estas dos especies silvestres, ya que las tres especies tienen muchas similitudes morfológicas y, además, entre ellas se obtienen híbridos completamente fértiles y con meiosis regular (Anis *et al.*, 1994; Knapp *et al.*, 2013).

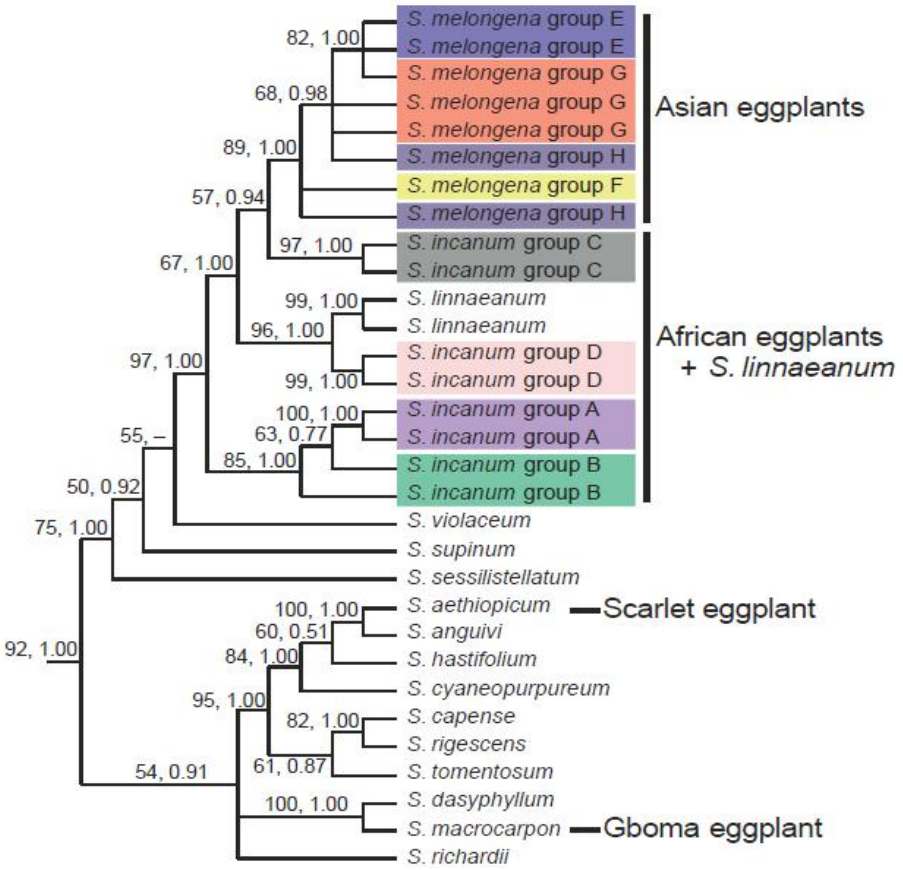

Figura 2. Árbol consenso basado en ITS, waxy, y los cloroplastostrnT-L y trnL-F (figura modificada de Weese y Bohs, 2010). Los números en las ramas indican los valores de bootstrap y las probabilidades posteriores Bayesianas por encima del 50%.

Según un árbol de consenso construido por Weese y Bohs (2010) la especie más estrechamente emparentada con *S. melongena* es *S. incanum* (grupo c) (Figura 2).

La berenjena común, S. melongena

La berenjena común se cultiva por sus frutos, los cuales presentan una gran diversidad en cuanto a formas y colores (Figura 3). Sus flores son autógamas, pentámeras, de color blanco a morado, en ramillete con una flor principal que actúa normalmente como flor hermafrodita y otras que normalmente tienen el estigma inserto y actúan principalmente como flores masculinas. Es un cultivo de porte erecto, perenne y que puede incluso producir varios años seguidos.

Figura 3. Diversidad del fruto en la berenjena común (*S. melongena*).

La berenjena escarlata (S. aethiopicum) y la berenjena gboma (S. macrocarpon)

Además de la berenjena común, existen otros dos tipos de berenjenas cultivadas, la berenjena escalata (*S. aethiopicum* L.) y la berenjena gboma (*S. macrocarpon* L.) originarias de África y con gran importancia en cuanto a su consumo en la zona subsahariana de África. Ambos cultivos son hipervariables en cuanto a forma, en particular la berenjena escarlata, donde podemos reconocer cuatro grupos: Aculeatum (usada como ornamental), Gilo (usada por sus frutos), Kumba (usada tanto por sus frutos como por sus hojas), y Shum (usada por sus hojas) (Lester, 1986; Lester *et al.*, 1986; Plazas *et al.*, 2014). Una clave de clasificación muy útil que podemos utilizar para

distinguirlas es la elaborada por Lester y Niakan (1986) con la que las accesiones individuales se pueden clasificar correctamente en cada uno de los grupos.

La **berenjena escarlata** (Figura 4) procede de la domesticación de la especie silvestre *S. anguivi* Lam., planta herbácea de gran tamaño que se caracteriza por tener unos frutos verdes muy pequeños en estado inmaduro y rojos en su madurez fisiológica. Debido a que estas plantas todavía se pueden encontrar en estado silvestre o como malas hierbas en zonas no modificadas por los humanos, se han descrito plantas con unas características intermedias entre las dos especies y que son difíciles de clasificar en los grupos descritos, por lo que hemos decidido tratarlas como "intermedias". Son, por tanto, plantas con características intermedias entre *S. anguivi* y *S. aethiopicum*, debidas probablemente a cruces espontáneos obtenidos de forma natural. Además de África, esta especie se cultiva ocasionalmente en el Caribe y en Brasil (Schippers, 2000) probablemente llevada allí por esclavos, además de en algunas zonas del sur de Italia (Sunseri *et al.*, 2010).

Figura 4. Diversidad en la berenjena escarlata (*S. aethiopicum*) y grupos que componen la especie *S. aethiopicum* (a. Shum, b. Aculeatum, c. Kumba, d. Gilo).

La **berenjena gboma** (Figura 5) procede de la domesticación de la especie silvestre *S. dasyphyllum* Schum. y Thonn., especie herbácea de abundantes frutos redondos de tamaño medio que se caracteriza por tener un cáliz envolvente del fruto redondo y muchas espinas en planta, hojas y cáliz.

Figura 5. Diversidad en el fruto de la berenjena Gboma (*S. macrocarpon*).

La polinización de *S. macrocarpon* y *S. dasyphyllum* es generalmente autógama, por lo que suele llevarse a cabo con el polen de la propia flor o de la misma planta, aunque no debe descartarse la polinización cruzada a través de insectos, pudiendo alcanzar valores de alogamia elevados (Bukenya y Carasco, 1994).

Importancia económica

La berenjena común (*S. melongena*) es un cultivo ampliamente distribuido por todo el mundo y es la séptima hortaliza a nivel mundial en producción. China encabeza la producción mundial, siendo España la séptima productora mundial y la primera en Europa, superando a Italia tras más de una década a su estela (Figura 6). No se han encontrado datos de producción para la berenjena escarlata ni para la gboma, aunque se considera que la berenjena escarlata es la quinta hortaliza en nivel de consumo en el centro y este de África, por detrás del pimiento, patata, cebolla y la okra (Schippers, 2000; Maundu *et al.*, 2009).

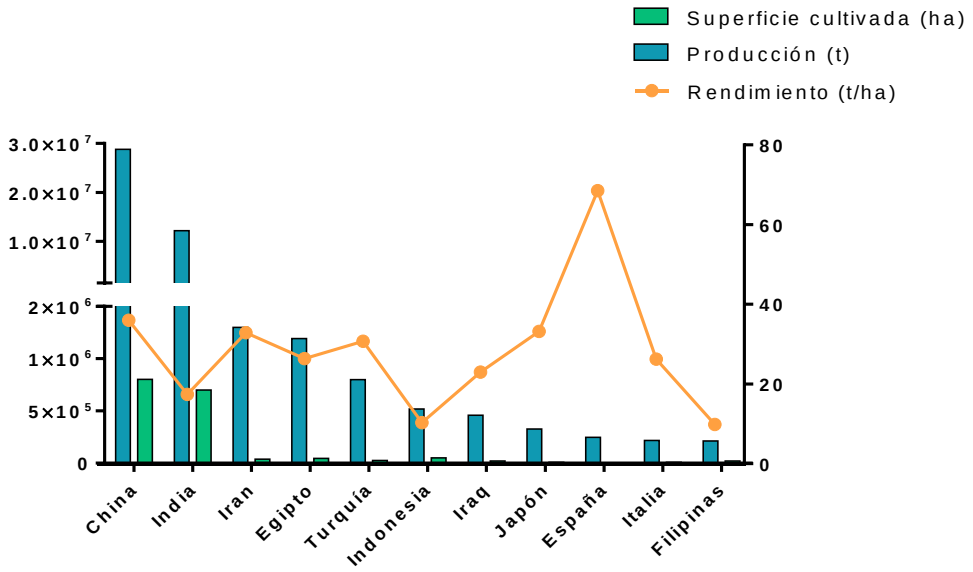

Figura 6. Producción, área cultivada y rendimiento del cultivo de la berenjena en los principales países productores en el año 2012 (datos FAO, 2014).

1.2.2. Composición y usos de las berenjenas

La berenjena es un cultivo bajo en calorías debido a que está compuesto principalmente por agua, sales minerales y vitaminas (vitamina A, ácido ascórbico y vitamina B) (Tabla 1). Esta elevada cantidad de agua es lo que proporciona a las berenjenas las propiedades adelgazante y diurética. Además es una buena fuente de calcio, fósforo y hierro, por lo que se ha recomendado el uso de esta hortaliza para combatir la anemia, ya que su consumo fortalece las defensas además de mejorar el funcionamiento de músculos y corazón. También se recomienda a personas con problemas reumáticos y a pacientes hipercolesterolémicos ya que tiene altas concentraciones de ácidos poliinsaturados como el linoleico y el linolénico, que tienen una acción hipolipemiante (Cho *et al.*, 2010). El potasio le confiere propiedades desintoxicantes ya que ayuda a la eliminación de toxinas, la fibra le aporta un efecto laxante natural y la vitamina A protege la piel y retrasa los signos de envejecimiento. La berenjena también tiene propiedades hipoglucemiantes, reduciendo los niveles de glucosa en sangre, por lo que se recomienda su

consumo en personas con diabetes Tipo II (Coman *et al.*, 2012). De hecho, en la India se consumen tradicionalmente las variedades de frutos blancos porque existe la creencia de que previenen esta enfermedad (Choudhury, 1976). También en la India, además de en Nigeria o Guinea, se utilizan las raíces por sus propiedades antiasmáticas y como analgésico (Chadha, 1993; Choudhury, 1995).

Una de las características que confieren el mayor atractivo a la berenjena es el alto contenido en polifenoles, principalmente en ácido clorogénico, que le proporciona un alto poder antioxidante (Cao *et al*., 1996; Stommel y Whitaker, 2003; Prohens *et al*., 2007). El ácido clorogénico está presente en la carne del fruto y no se degrada con el cocinado (Lo Scalzo *et al.*, 2010). Estos ácidos fenólicos le confieren a la berenjena un valor añadido muy importante, ya que se piensa que estos compuestos bioactivos ayudan a prevenir el desarrollo de enfermedades cardiovasculares, degenerativas y de ciertos tipos de cáncer (Surh, 2003; Rice-Evans *et al.*, 1996; Suzuki *et al.*, 2006; Ahn *et al.*, 2011; Zhao *et al.*, 2012). Esto hace que los programas modernos de mejora de este cultivo tengan como uno de sus objetivos la obtención de híbridos con alto contenido en polifenoles.

Dentro de la berenjena escarlata, el grupo que tiene un mayor número de polifenoles es el grupo Kumba. En la berenjena gboma la especie que tiene una mayor cantidad es *S. dasyphyllum* (Figura 7), que tiene más del doble que el resto de las especies anteriormente mencionadas.

El ancestro de la berenjena que más cantidad de ácido clorogénico posee es *S. incanum*, ya que probablemente éste haya sufrido una menor presión de selección. Las variedades cultivadas tienden a tener una menor cantidad debido a que los polifenoles provocan, por acción de las polifenoloxidasas (PPO), el pardeamiento del fruto nada más cortarlo, lo cual es una característica no deseable comercialmente (Prohens *et al.*, 2007). Por ello, se han seleccionado a lo largo del tiempo frutos con bajo pardeamiento, con lo cual, indirectamente, se han seleccionado frutos con un nivel inferior de polifenoles.

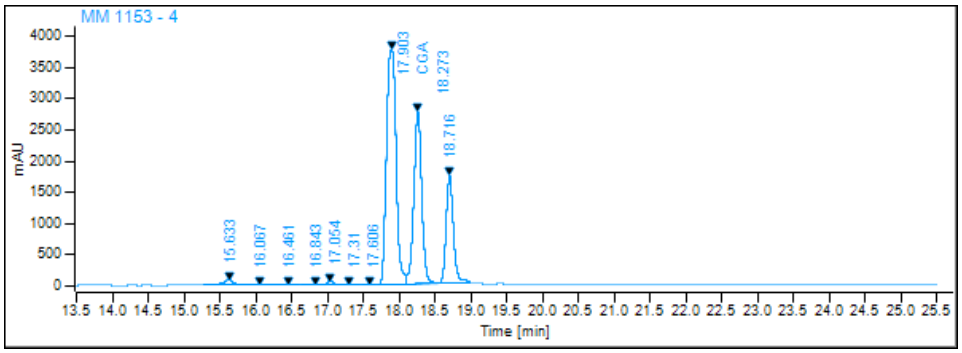

Figura 7. Ejemplo de un cromatograma de *S. dasyphyllum* donde aparece el pico correspondiente al ácido clorogénico y otros dos polifenoles.

Los tres tipos de berenjenas cultivadas se recolectan en estado de madurez comercial, que técnicamente es cuando el fruto todavía está inmaduro, ya que este tipo de hortalizas son depreciadas si tienen la semilla totalmente formada, cosa que provoca que el fruto sea prácticamente incomestible. Se consumen habitualmente cocinadas o encurtidas (como es el caso de la berenjena de Almagro). Además de utilizarse en la industria conservera, se pueden encontrar en el mercado en preparados precocinados, enlatados, congelados, etc. Como excepción a esto, dentro de la berenjena escarlata destacan los grupos Kumba y Shum que, además de por los frutos se cultivan para consumir sus hojas (Schippers, 2000).

Tabla 1. Composición nutricional de la berenjena correspondiente a frutos de *S. melongena, S. aethiopicum y S. macrocarpon* (Flick *et al*., 1978; Sánchez-Mata *et al.*, 2010; San José, 2010).

	S. melongena	*S. aethiopicum*	*S. macrocarpon*
Composición (por cada 100 g)			
Humedad (g)	92.2 – 94.2	85,80 – 87,55	85,99 – 88,26
Energía (kcal)	26	32	40
Proteína (g)	1,1	1,35 – 1,67	0,86 – 1,59
Grasas (g)	0,18 – 0,2	0,1	1
Carbohidratos (g)	6,3	2,89 - 4,64	4,94 – 8,04
Azucares solubles (g)	3,4	0,28 – 0,55	0,21 – 0,36
Fibra (g)	1,0	3,35 – 5,58	2,39 – 3,70
Ácido oxálico (mg)	18	-	-
Vitaminas (por cada 100 g)			
Vitamina A (IU)	70	35	-
Tiamina (mg)	0,09	-	-
Riboflavina (mg)	0,02	-	-
Niacina (mg)	0,60	-	-
Ácido ascórbico (mg)	1,60	9,24 – 14,14	15,02 – 22,03
Vitamina B_1 (mg)	0,04	0,07	-
Vitamina B_2 (mg)	0,05	0,06	-
Vitamina B_6 (mg)	0,09	0,8	-
Ácido nicotínico (mg)	0,09	-	-
Glicoalcaloides (por cada 100 g)			
α- Solasonina (mg)	0,17 – 0,40	0,41 – 1,02	16 – 23,5
α- Solamargina (mg)	0,85 – 1,61	0,58 – 4,86	124 - 197
Minerales (ppm)			
Aluminio	76,9 – 132,5	-	-
Calcio	1068 - 1450,1	2800	1300
Cloro	2060 - 3590	-	-
Cobre	13,2 - 21,8	-	-
Hierro	157 - 180	150	-
Potasio	17390 - 28220	-	-
Magnesio	1245 - 1690	-	-
Manganeso	10 – 14,8	-	-
Sodio	211 - 306	-	-

Azufre	3800 - 9950	-	-
Selenio	1,1 - 2,0	-	-
Vanadio	1,0	-	-
Zinc	5,8 - 8	-	-
Aminoácidos (mg/100 g)			
Lisina	0,541 - 0,769	-	-
Histidina	0,332 - 0,475	-	-
Arginina	0,724 - 1,206	-	-
Ácido aspártico	1,969 - 3,274	-	-
Treonina	0,493 - 0,776	-	-
Serina	0,562 - 0,815	-	-
Ácido glutámico	2,405 - 3,582	-	-
Prolina	0,534 - 0,784	-	-
Glicina	0,542 - 0,776	-	-
Alanina	0,658 - 0,995	-	-
Valina	0,795 - 1,212	-	-
Isoleucina	0,638 - 0,722	-	-
Leucina	0,944 - 1,266	-	-
Tirosina	0,287 - 0,419	-	-

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1.3 Diversidad en berenjena como materia prima para la mejora

1.3.1. El complejo berenjena común

La berenjena cultivada (*S. melongena*) junto con formas adventicias adaptadas del sudeste asiático (*S. insanum* y la especie silvestre *S. incanum*) forman el denominado "complejo berenjena" (Pearce y Lester, 1979; Lester y Hasan, 1990, 1991; Daunay *et al*., 1997; Knapp *et al*., 2013). Estos materiales incluidos en el "complejo berenjena" cruzan y dan híbridos fértiles con la berenjena cultivada sin dificultad, constituyendo el **germoplasma primario** de berenjena (Figura 1).

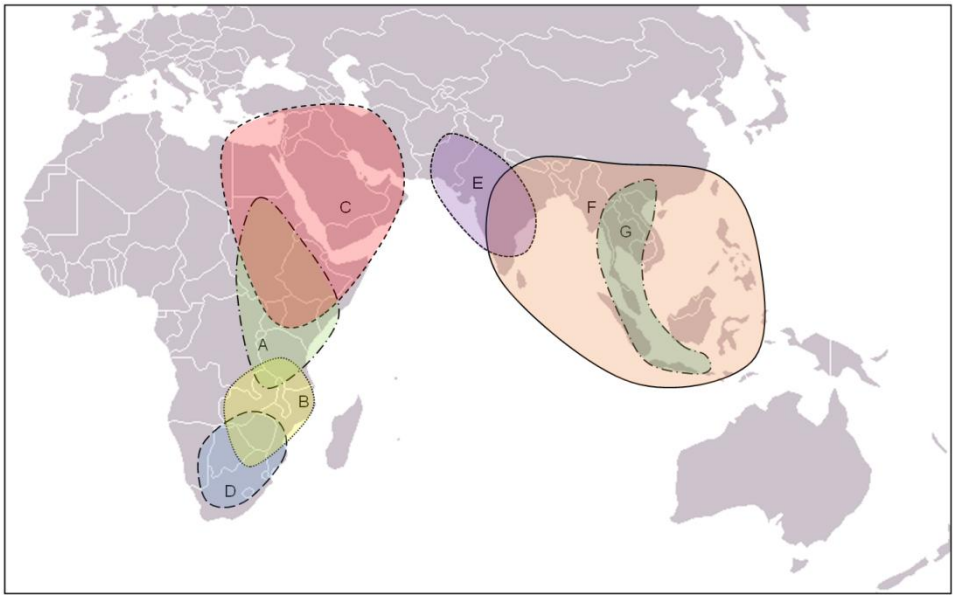

Figura 1. Distribución de las formas del "complejo berenjena" (basada en Daunay *et al*., 1997). El grupo H no se incluye ya que corresponde a las variedades modernas de berenjena con distribución mundial.

Basándonos en el sistema de Lester, éste reconoce dos especies botánicas, *S. incanum* y *S. melongena*, cada una con cuatro grupos, identificados por una letra mayúscula (Lester y Hasan, 1991; Daunay *et al*., 2001). La especie *S. incanum* incluye, en sentido amplio, a los grupos A, B, C y D, mientras que todas las formas de *S. melongena* se encuentran recogidas dentro de los grupos E, F, G y H.

- Los grupos A y B están integrados por formas de *S. incanum* del este y sur de África. *S. incanum* en sentido estricto constituye el grupo C, que se encuentra en hábitats no modificados por los humanos, como la sabana, y en cauces de escorrentía de zonas desérticas del noreste de África y Oriente Medio. En el grupo D se incluyen las formas que crecen en ambientes más xerofíticos del sudeste africano.

- *Solanum melongena* se compone de 4 grupos etiquetados con las letras de la E a la H (Lester y Hasan, 1991), generalmente fértiles entre sí. Los grupos E y F corresponden a las formas silvestres y adventicias de berenjena procedentes de India y de la parte central de Asia (E) y del sudeste de Asia (F). Al grupo E pertenece *S. insanum,* muy espinosa y de poca altura, que crece de forma adventicia en zonas abiertas de campos de cultivo. El grupo F contiene formas moderadamente espinosas, que crecen como adventicias en huertos, zonas de vegetación modificada y bordes de caminos. El grupo G engloba los cultivares primitivos, de frutos pequeños procedentes también del sudeste asiático. La inmensa mayoría de frutos grandes (normalmente entre 10-20 cm de largo y entre 7-12 cm de diámetro) cultivados en la actualidad y repartidos por todo el mundo pertenecen al grupo H (Lester y Hasan, 1990, 1991; Daunay, 2008; Weese y Bohs, 2010). El grupo H es el grupo de berenjenas económicamente relevante por lo que en la literatura científica cuando se nombra la berenjena normalmente se refiere al grupo H, a no ser que se especifique lo contrario.

Aunque en Europa, América y Oriente Medio la mayoría de accesiones de berenjena que se cultivan pertenecen al grupo H, en Asia, además de éstas también se encuentran cultivares más primitivos del grupo G, con frutos más pequeños y espinosos que se utilizan para el consumo (Hennart, 1996; Muñoz-Falcón *et al*., 2005; Hurtado *et al.*, 2012).

Centrándonos en los tipos de berenjena cultivada (grupo H), la clasificación más utilizada es la proporcionada por Bailey en 1947, donde se diferencian tres tipos de variedades botánicas (Figura 2): *esculentum*, la más común, variedades de frutos grandes y ovalados; *depressum*, contiene los

grupos de frutos más pequeños y precoces, que normalmente pueden tener un cáliz envolvente; y *serpentinum*, engloba las variedades de frutos largos.

Figura 2. Diversidad en la morfología del fruto de berenjena común: *esculentum* (a), *depressum* (b) y *serpentinum* (c).

Además de esta clasificación hay otras, como la que describieron Martin y Rhodes en 1979, de 11 tipos de cultivares basados en 18 caracteres morfológicos y agronómicos. A su vez, en 2005, Prohens y colaboradores clasificaron las variedades en cuatro grupos de cultivares: redondas, semilargas, largas y Listadas de Gandía. Por otra parte, también podemos clasificar las variedades teniendo en cuenta dónde se cultivan, de ahí que se pueda agrupar las variedades en grupos occidentales y orientales.

Los tipos varietales Occidentales suelen destinarse a Europa, Norteamérica y Oriente Medio y suelen corresponder a plantas vigorosas de frutos grandes, pertenecientes al grupo H, (Prohens *et al*., 2005b) (Figura 3), teniendo preferencia por frutos de color negro brillante y de forma semi-ovalada. Alguna de las variedades más conocidas son: Black Beauty o Bellezza Nera, Long Purple, Dourga, Florida Market, Redonda Violeta, De Barbentane, Rosa Bianca y la Turkish Orange.

Por otra parte, los tipos Orientales se destinan a variedades consumidas en el este y sudeste Asiático, donde se aprecia mucho más la diversidad en colores, tamaños y formas, utilizándose variedades negras, violetas, verdes, blancas o estriadas. Suelen ser plantas menos vigorosas, más espinosas y de color verde con estrías o vetas oscuras (Costa, 1978; Chadha, 1993). Tradicionalmente, suelen ser variedades de polinización abierta (Chadha, 1993) aunque últimamente se están empezando a introducir variedades híbridas. Las principales variedades orientales son: Long White, Thai Long Green, Ping Tung Long, Purple Ball, Kermit y la Pursa Purple Long.

Figura 3. Frutos de distintos tipos de variedades comerciales con distintas formas, colores y tamaños.

1.3.2. El complejo berenjena escarlata

El complejo berenjena escarlata está compuesto por la especie cultivada *S. aethiopicum*, la cual está dividida en cuatro grupos. Esta especie es el resultado de la domesticación de la especie silvestre *S. anguivi*. Este complejo también incluye un grupo de accesiones asilvestradas que tienen características intermedias entre las dos especies, que durante este documento llamaremos “grupo Intermedio”, que probablemente se generaron a partir de cruces interespecíficos entre ellas.

S. aethiopicum pertenece a la sección Oliganthes (Lester, 1986; Lester y Niakan, 1986). Es una especie hipervariable que se caracteriza por tener muchos tipos y formas morfológicamente diferentes, además de cientos de variedades locales (Lester *et al.*, 1986) que a menudo dificultan la correcta clasificación de la especie. Podemos encontrarla nombrada de más de 20 maneras distintas a lo largo del tiempo (Lester, 1986). Dentro de *S. aethiopicum* se distinguen cuatro grupos de cultivares completamente interfértiles entre ellos (Lester y Niakan, 1986): Gilo, Kumba, Shum y Aculeatum (Polignano *et al.*, 2010; Adeniji *et al*., 2013), que aunque muchos autores los han tratado como especies distintas, se han aceptado como una única especie (Lester *et al.*, 1986, 2011; Edmonds, 2012).

Además de ser grupos distintos en apariencia, los usos de los distintos cultivares también lo son. El grupo Gilo se utiliza mayoritariamente por sus frutos, el Kumba tanto por sus frutos como por sus hojas, el grupo Shum principalmente por sus hojas y Aculeatum suele emplearse como ornamental (Lester, 1986; Schippers, 2000; Lester y Daunay, 2003). Estas accesiones se han ido adaptando a las diferentes climatologías de las distintas zonas, distribuyéndose en las áreas según requerimientos. El grupo Shum se adoptó mejor a las zonas más húmedas de África, el grupo Kumba dio mejores resultados en las zonas semiáridas del occidente del Sahel llegando incluso al norte de Nigeria y en las zonas más lluviosas es donde se localiza más frecuentemente el grupo Gilo. Además de estos usos específicos, *S. aethiopicum* y los cruces interespecíficos que se obtienen al cruzar con la berenjena común (*S. melongena* L.) (Daunay *et al.*, 1991; Oyelana y Ugborogho, 2008; Prohens *et al.*, 2012) pueden utilizarse como portainjertos (Gisbert *et al*., 2011).

El **grupo Gilo** también conocido como “garden eggs”, es el más ampliamente utilizado y cultivado. Su amplia gama en cuanto a formas de fruto puede ser encontrada dispersa dependiendo del criterio local de selección.

Suelen ser plantas de tipo arbustivo de hasta 2 metros de altura, aunque las variedades comerciales rondan de 65 a 110 cm. Las variedades comerciales suelen tenerse en campo aproximadamente 6 meses sacándole el máximo rendimiento a la planta, aunque en determinadas zonas puede

tenerse hasta 3 años disminuyendo la calidad y la cantidad de frutos obtenidos en estas plantas más viejas en comparación con las jóvenes (Schippers, 2000).

Las hojas son largas, con pelos foliares estrellados especialmente en el envés y pueden tener espinas, aunque la mayoría de variedades del grupo Gilo que se emplean para el consumo no suelen presentarlas.

Las flores son normalmente pentámeras, blancas y pequeñas de menos de 25 mm. Podemos encontrar de 1 a 3 frutos por nudo con una medida que puede variar de 2 a 10 cm de diámetro unidos a la planta firmemente y con el pedicelo curvado hacia abajo. El color del fruto puede virar de blanco a verde, o incluso morado cuando están inmaduros, y entre naranja, rojo oscuro o marrón brillante cuando el fruto ha alcanzado la madurez fisiológica. Muchos de ellos tienen 2 o 3 lóculos, pero algunas variedades pueden tener hasta 6.

Los frutos de este grupo van de ovalados a esféricos aunque podemos encontrar algunas accesiones locales de frutos alargados y de cilíndricos a aplanados. La superficie de la piel puede ser estriada o lisa, dependiendo de las variedades (Figura 4). Kouassi *et al.* (2014) han dividido en tres subgrupos el grupo Gilo, utilizando caracteres morfológicos y agronómicos, confirmando lo que los agricultores en Costa de Marfil vienen distinguiendo en sus campos de cultivo.

Figura 4. *S. aethiopicum* grupo Gilo.

El **grupo Kumba (o jakatu)** se encuentra localizado en las zonas semiáridas del occidente de Sahel hasta el norte de Nigeria. Aunque en Europa es un cultivo apenas conocido, en Senegal su producción alcanza los niveles del tomate y la cebolla, produciéndose todo el año en condiciones de regadío, consumiéndose tanto sus frutos como sus hojas.

Estas plantas pueden alcanzar el metro de altura, aunque normalmente suelen medir en torno a los 50 cm (Plazas *et al.*, 2014), anuales y ocasionalmente perennes. La inflorescencia es similar al grupo Gilo aunque tiene el ovario más engrosado. Las hojas son glabras sin espinas, aunque pueden tener pequeños pelos glandulares, son grandes, y su tamaño va desde 15 a 30 cm de largo. Algunas de las variedades de este grupo se cultivan por sus hojas, que se consumen en hervidos, pero solo durante las primeras etapas de desarrollo, ya que después maduran y se endurecen. Los frutos son grandes, de verdes a blancos en su madurez comercial y muy acostillados (normalmente más de 5 surcos y entre 10 y 15 lóculos), achatados de aproximadamente 10 cm de diámetro (Figura 5). Su sabor normalmente es dulce.

Figura 5. *S. aethiopicum* grupo Kumba.

El **grupo Shum (o nakati)** se localiza en las zonas más altas y lluviosas del oeste y centro de África. Podemos encontrar este cultivo en prácticamente todos los mercados de Uganda y en la mayoría de los del sureste de Nigeria.
En apariencia son plantas muy parecidas a las del grupo Kumba, pero con hojas y frutos más pequeños que pueden llegar a medir un máximo de 80 cm.

Las hojas son glabras y hay variedades con muy pocos pelos foliares que se comen.

Las flores de esta variedad son las típicas de *S. aethiopicum*. Los frutos miden aproximadamente 2 cm de diámetro, ligeramente más anchos que largos y verdes en la madurez comercial, con un sabor normalmente amargo, por lo que no suelen utilizarse para alimentación. Pueden encontrarse solitarios o en grupos de hasta 8 frutos, con 2 o 4 lóculos (Figura 6).

Figura 6. *S. aethiopicum* grupo Shum.

El **grupo Aculeatum** es el de más reciente clasificación. No suele encontrarse en África de forma espontánea. Se cree que se desarrolló en Europa con fines ornamentales a partir del cruce interespecífico entre *S. anguivi* y *S. aethiopicum* grupo Kumba (Lester *et al*., 1986; Schippers, 2000).

Las plantas que pertenecen a este grupo en apariencia son similares a las del grupo Gilo, plantas que pueden alcanzar hasta 150 cm de altura, más espinosas en tallo y hojas. Las hojas son pubescentes, con pelos estrellados y flores blancas y pequeñas, típicas de la especie *S. aethiopicum.*

Las inflorescencias pueden tener de 5 a 10 flores por raquis, no superior a 2 cm de largo. Los frutos de este grupo suelen estar entre los 3 a 8 cm de diámetro, esféricos o subesféricos, con 4 o más costillas y de 4 a 10 lóculos como puede observarse en la Figura 7.

Figura 7. *S. aethiopicum* grupo Aculeatum.

S. anguivi Lam. se considera una hierba medicinal rara que pertenece a la familia Solanaceae. Podemos encontrarla en muchos lugares en las zonas no

áridas de África. Es una especie altamente polimórfica y variable tanto para estructura de planta, frutos y caracteres de hoja. No siempre es fácil distinguirla de algunos de sus híbridos. Se considera el ancestro silvestre de la especie cultivada *S. aethiopicum* (Sunseri *et al.*, 2010). Los híbridos entre estas dos especies se obtienen fácilmente y son fértiles (Lester y Niakan, 1986; Lester y Thiati, 1989), aunque la mortalidad de las plantas resultantes es muy alta. En la literatura también aparece nombrada como *S. indicum* o *S. anomalum.*

S. anguivi se caracteriza por sus hojas pubescentes con pelos estrellados, al igual que Aculeatum y Gilo, de apariencia leñosa y de tipo arbustivo, pueden ser plantas espinosas, sobre todo cuanto más silvestres se encuentren. Su planta llega a medir hasta 3 metros de altura. En un mismo raquis de aproximadamente 2 cm podemos encontrar hasta 10 flores por inflorescencia. Estas plantas dan abundante número de frutos pequeños de 1 a 2 cm (Figura 8), esféricos de color verde cuando están inmaduros y anaranjados o rojos cuando alcanzan la madurez fisiológica. Los frutos son jugosos aunque amargos y se separan fácilmente del pedícelo, que es fino y erecto o deflexo.

Figura 8. foto detalle de *S. Anguivi.*

El interés de esta especie radica sobretodo en su uso medicinal, utilizado como antianémico, expectorante, antiasmático, etc. (Elekofehinti *et al*., 2012; Johnson *et al*., 2010), y su uso como portainjerto, ya que esta especie confiere a la berenjena cultivada resistencia a *Ralstonia solanacearum* y a otras enfermedades (Collonnier *et al*., 2001). Además de todo esto, *S. anguivi* se utiliza para preparar sopas y, como es una especie muy rica en antioxidantes, puede ser usado como aditivo alimentario para prevenir enfermedades asociadas al estrés oxidativo (Elekofehinti *et al.*, 2012 y 2013)

Las accesiones que hemos denominado como **grupo intermedio** presentan características morfológicas intermedias entre *S. anguivi* y *S. aethiopicum* grupo Gilo, siendo muy complicada la clasificación de las mismas en un grupo o en otro. En la Figura 9 puede observarse que los frutos del grupo intermedio se parecen mucho a los frutos de *S. anguivi,* mientras que el aspecto y tamaño de la planta es muy similar a *S. aethiopicum*. Esta accesión guarda mucha similitud con el tercer subgrupo del grupo Gilo que se describe en el trabajo de Kouassi *et al.* (2014) y que denominan *Gnangnan.*

Figura 9. Grupo intermedio entre *S. aethiopicum* y *S. anguivi.*

1.3.3. El complejo berenjena gboma

El complejo berenjena gboma está compuesto por la especie cultivada *S. macrocarpon* y por su ancestro silvestre *S. dasyphyllum*. Entre ellas cruzan fácilmente, obteniéndose híbridos fértiles (Schippers, 2000). A su vez, son especies emparentadas con la berenjena común (*S. melongena*) con la que se han podido obtener híbridos interespecíficos (Daunay *et al*., 1991; Khan *et al*., 2013). Se pueden encontrar accesiones con características intermedias normalmente en zonas donde ambos cultivares están presentes de manera conjunta.

Solanum macrocarpon pertenece a la sección Melongena (Lester *et al.*, 1990; Lester y Daunay, 2003; Lester *et al.*, 2011). Es una especie que crece en las zonas cálidas y no áridas de África, aunque también puede encontrarse en Sudamérica, El Caribe y el sudeste de Asia. Es un cultivo muy importante en Benin y en las regiones de la selva tropical del África costera y el río Congo (Lester *et al*., 1990; Dansi *et al*., 2008).

Las flores de esta especie se distinguen por tener los pétalos soldados, generalmente de color violeta y de tamaño bastante más grandes que las que presenta *S. aethiopicum* entre 25 y 45 mm de diámetro. Se pueden encontrar hasta 10 flores por inflorescencia aunque lo normal es que tengan entre 2 y 6 flores. Como ocurre en muchas solanáceas las primeras flores son hermafroditas y el resto suelen actuar de flor masculina.

Los frutos son muy característicos en aspecto, achatados y cubiertos en gran parte por el cáliz (Daunay *et al.*, 1997) de colores que van desde el blanco al verde, volviéndose marrones con grietas cuando han alcanzado la madurez fisiológica (Figura 10). Las hojas son brillantes y sin pelos foliares de distintas formas y tamaños.

De esta especie se consumen tanto sus frutos como sus hojas y se han descrito seis variedades, los cultivares Gboma, Mankessim, Akwaseho, Kade, Sarpeiman y Bui. Tienen distintas procedencias y se pueden distinguir fácilmente por su morfología (Bukenya y Carasco, 1994).

Figura 10. Distintas morfologías dentro de *S. macrocarpon*

S. dasyphyllum o también *S. macrocarpon* subp. *dasyphyllum* se encuentra localizada en el este de África. Es una especie adventicia que se encuentra normalmente como mala hierba en zonas poco transformadas por el ser humano. Se cree que *S. sessilistellatum*, parte de la vegetación primaria en Kenia, es su ancestro silvestre. Normalmente tiene pelos y espinas en tallos, hojas y cáliz, lo que la hace una especie poco atractiva tanto para animales como para humanos.

Son plantas que no suelen medir más de 1 m de altura, muy espinosas y pilosas (Lester *et al*., 1990). Aunque en las zonas donde todavía se cultiva la finalidad es conseguir frutos y hojas comestibles, se sigue manteniendo por los muchos usos medicinales que se le da a esta especie. (Bukenya y Carasco, 1994).

Las flores son muy parecidas a la de *S. macrocarpon* pero más blancas, aunque con abundantes espinas en los sépalos. El fruto es redondeado y verde en la madurez fisiológica, rodeado en gran parte por el cáliz lleno de espinas. Son ligeramente más pequeños que los frutos de *S. macrocarpon,* de aproximadamente 5 cm de diámetro y también se tornan marrones con grietas cuando están totalmente maduros (Figura 11).

Figura 11. Detalle del fruto y la flor de *S. dasyphyllum*.

1.3.4. Hibridación interespecífica entre berenjenas

La controvertida identificación de los ancestros de berenjena y su centro de origen y domesticación (Weese y Bohs, 2010; Meyer *et al.*, 2012) ha sido un obstáculo a la hora de buscar variabilidad genética útil en las colecciones de germoplasma. De hecho, teniendo en cuenta la enorme contribución del uso de especies silvestres y especies relacionadas en la mejora genética de solanáceas (como en el tomate y en el pimiento), queda patente que, en el caso de la berenjena, el potencial que presentan sus especies relacionadas no ha sido explotado todavía (Daunay y Hazra, 2012). Por ello, es muy importante el uso de híbridos interespecíficos, ya que dentro de las otras dos especies cultivadas relacionadas con la berenjena (*S. aethiopicum* y *S. macrocarpon*) podemos encontrar caracteres de interés, como la tolerancia a distintos hongos, nematodos, etc. y resistencias a enfermedades (ejemplo, *Ralstonia solanaceae, verticillium*) que nos serían fácilmente introgresables en la berenjena común mediante cruces entre estas

especies y *S. melongena* (Daunay *et al.*, 1991; Daunay y Hazra, 2012; Rotino *et al.*, 2014).

Por regla general, las flores en berenjena están distribuidas en ramilletes con una flor principal y varias secundarias, o solamente una flor principal. La flor principal suele ser hermafrodita, su tamaño es superior al resto y tiene el estigma exerto. En las condiciones de cultivo habituales, la berenjena es una planta autógama aunque su alogamia puede llegar al 20 %. La berenjena mejora su cuajado cuando la polinización de realiza de forma manual, aumentando de un 67% a un 85% de éxito (Rao, 1980).

Los cruces en berenjena se hacen tal como se muestra en la Figura 12. Es importante elegir bien la hora y temperatura que habrá en el lugar donde se van a realizar los cruces. Es mejor emplear las primeras horas de la mañana, normalmente antes de las 11:00 a.m., no superar los 27ºC y que la humedad relativa no sea superior a 55 %, ya que si esto ocurre habrá problemas en el desprendimiento de polen de las anteras (Daunay y Hazra, 2012).

Figura 12. Pasos en la hibridación en berenjena: 1. material utilizado, 2. extracción de polen sobre placa Petri, 3. castrado de la flor antes de su apertura, 4. colación del polen en el estigma floral, 5. etiquetado y estado de la flor después de la emasculación.

Lo más importante a la hora de realizar los cruces interespecíficos, es decidir con qué especie vamos a cruzar nuestras plantas y cuál es el carácter de interés a introgresar. Por otra parte, la utilización de la berenjena cultivada como parental femenino, permite recuperar el genoma de los orgánulos citoplasmáticos en una sola generación, lo cual evita el problema de esterilidad en subsiguientes generaciones de retrocruzamientos (Yoshimi *et al.,* 2013). En la Figura 13 se muestran algunos ejemplos de especies que cruzan con la berenjena y que tipo de híbridos se obtienen.

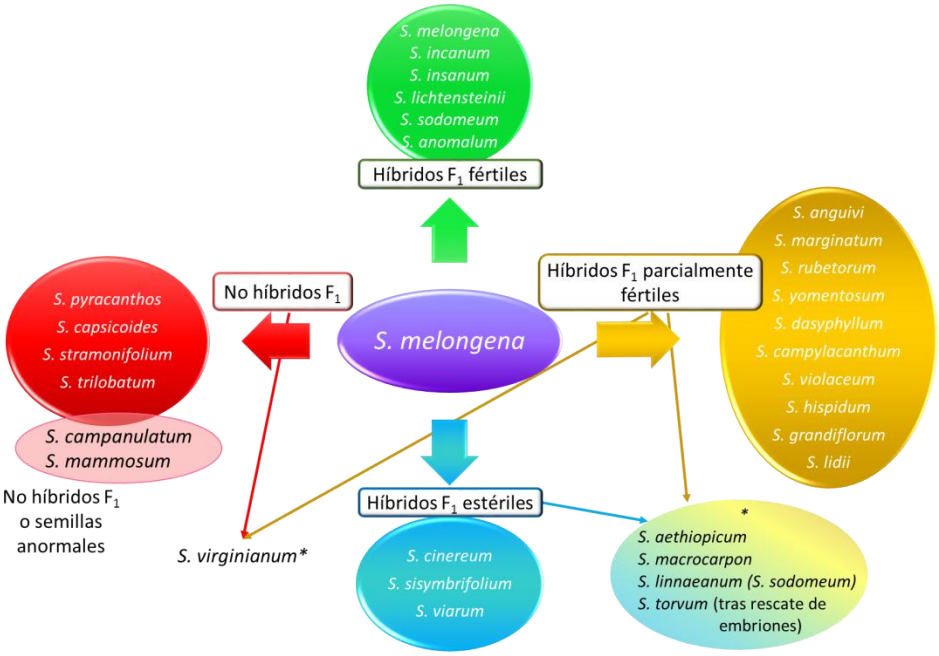

Figura 13. Esquema de cruces interespecíficos entre la berenjena cultivada (*S. melongena* L.) y otras especies de *Solanum*. Basada en Daunay y Harza, 2012 y Rotino *et al*., 2014. * En función de la fuente consultada, los datos de cruzamiento son diferentes.

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1.4 Breeding for chlorogenic acid content in eggplant: interest and prospects

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Abstract

Chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA) is an ester of caffeic acid and (-)-quinic acid. with many beneficial properties for human health, such as anti-oxidant, anti-inflammatory, cardioprotective, anti-carcinogenic, anti-obesity, and anti-diabetic properties. This has raised an interest for the development of new crop cultivars with increased CGA content. One of the crops with higher CGA content is eggplant (*Solanum melongena*). There is a wide diversity for CGA content in cultivated eggplant germplasm, which is influenced by the fruit developmental stage, storage conditions, and environmental factors. Therefore, appropriate experimental designs are required for an efficient breeding. Several strategies are proposed for breeding for high CGA content such as intraspecific variation, selection among accessions, development of hybrids and lines with good agronomic and commercial characteristics, or introgression of the high CGA trait in élite lines. Some wild relatives, like *S. incanum*, present higher CGA contents than those of eggplant. Interspecific hybridization can be used to introgress favorable alleles from the wild species into the genetic background of cultivated eggplant. Fruit flesh browning, as a result of CGA oxidation by polyphenol oxidases, could be a side effect of increasing the CGA content in eggplant. However, experimental results indicate that the relationship between CGA content and fruit flesh browning is low or moderate. Furthermore, selection for low polyphenol oxidase activity might result in reduced fruit flesh browning. Overall, the available data suggest that the development of eggplant cultivars with improved functional quality resulting from a higher CGA content is feasible.

What is chlorogenic acid?

Chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA) is a phenolic compound resulting from the esterification of caffeic acid and the aliphatic alcohol (–) quinic acid (1L-1(OH)-3,4/5-tetrahydroxycyclo-hexane carboxylic acid) (Figure 1). CGA is present in many plants where it plays a role in plant defense, as well as an antioxidant (Korkina, 2007; Leiss *et al.*, 2009; Ngadze *et al.*, 2012). The broader term “chlorogenic acids” has also been used to refer to a family of esters formed between certain *trans*-cinnamic acids (caffeic, ferulic and *p*-coumaric acids) and quinic acid (Clifford, 2000). The main subgroups of chlorogenic acids include: mono-esters of caffeic acid (caffeoylquinic acids, *p*-

coumaroylquinic acids and feruloylquinic acids), di-esters, tri-esters, a single tetra-ester of caffeic acid, and mixed di-esters of caffeic and ferulic acid (caffeoylferuloylquinic acids) or caffeic and sinapic acid (caffeoylsinapoylquinic acids). Mixed esters involving various permutations of between one and three residues of caffeic acid with one or two residues of a dibasic aliphatic acid (such as glutaric, oxalic, succinic) have also been denominated chlorogenic acids (Clifford, 2000). However, for the purposes of the present paper we use the term chlorogenic acid (CGA) to refer specifically to 5-*O*-caffeoyl-quinic acid.

CGA is included in the broad category of polyphenols, which are typically classified into one of either two categories: flavonoids and phenolic acids (Macheix, 1990). Among the latter, hydroxycinnamic acids, of which CGA is a major representative, is considered the main class.

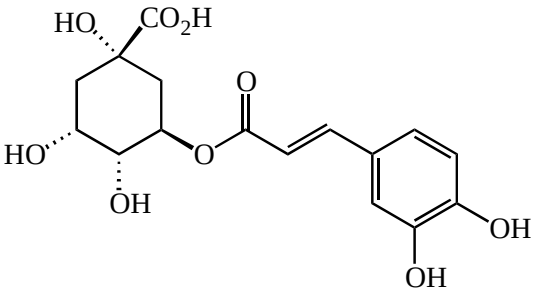

Figure 1. Chemical structure of chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA).

The interest for breeding for CGA content: bioactive properties.

Dietary polyphenols from numerous plant species have shown to be beneficial for human health due to its known biological activities, which include free-radical scavenging, regulation of enzymatic activity, and modulation of several cell signaling pathways (Sato *et al.*, 2011). In fact, many of them are being actively studied as potential treatments for various metabolic and cardiovascular diseases. For example, resveratrol from red wine, epigallocathechin-3-gallate from green tea, curcumin from turmeric, and quercetin from different sources have all been studied as potential therapeutic agents to induce weight loss, lower blood pressure, attenuate glucose levels

and insulin resistance, and improve hemoglobin A1c and lipid profile in humans (Andújar *et al.*, 2012).

CGA is found in many edible and medicinal plants, and is well known for having various biological properties of interest for human health. These include anti-oxidant, anti-inflammatory and analgesic properties demonstrated both *in vitro* and *in vivo* (dos Santos *et al.*, 2006; Jin *et al.*, 2006; Morishita and Ohnishi, 2001; Sato *et al.*, 2011; Sheu *et al.*, 2009), as well as strong anti-microbial activity (Almeida *et al.*, 2006). In relation to this anti-oxidant/anti-inflammatory activity, several studies also highlight CGA neuroprotective (Ahn *et al.*, 2011) and cardioprotective (Chen *et al.*, 2009; Zhao *et al.*, 2012) effects.

A number of animal studies have indicated that CGA is hypotensive (Suzuki *et al.*, 2002, 2006). This blood pressure-lowering activity also occurs in humans, as confirmed by clinical trials: the administration of 140 mg/day of CGA to mildly hypertensive subjects decreased both systolic and diastolic blood pressure significantly (Watanabe *et al.*, 2006).

CGA is also known to exert selective anti-carcinogenic effects via induction of apoptosis in many human cancer cells, such as leukemia cells (Yang *et al.*, 2012) and lung cancer cells (Burgos-Morón *et al.*, 2012). Other biological activities of CGA include its anti-obesity effect with improvement of lipid metabolism (Cho *et al.*, 2010), and a delay in intestinal glucose absorption and inhibition of gluconeogenesis (Ong *et al.*, 2012), which contributes to an anti-diabetic effect (Coman *et al.*, 2012).

CGA is one of the most abundant polyphenols in the human diet and is highly bioavailable in nature (dos Santos *et al.*, 2006). This fact, together with its numerous bioactive properties potentially beneficial for human health, encourages the use of breeding approaches in order to increase its level in food crops (Niggeweg *et al.*, 2004).

Eggplant as a source of CGA in the diet

The major dietary sources of CGA are vegetables, fruits, and beverages like coffee (Azuma *et al.*, 2000). It is estimated that humans consume up to 1 g of CGA per day (Chen *et al.*, 2009). Although coffee is considered a major source of CGA in the human diet, as regular coffee drinkers may consume up

to 0.5 – 1 g of CGA per day (Olthof *et al.*, 2001), fruits and vegetables also make a substantial contribution to CGA intake (Olthof *et al.*, 2001). In this respect, eggplant is one of the vegetables with a higher content in CGA (Table 1).

CGA is, by far, the major phenolic compound of the eggplant fruit, and typically makes between 80% and 95% of the total hydroxycinnamic acids present in the fruit flesh (Prohens *et al.*, 2013; Stommel and Whitaker, 2003; Whitaker and Stommel, 2003). Also, it has been found that concentrations of CGA in the eggplant fruit skin are similar to those present in the fruit flesh (Gajewski *et al.*, 2009). When compared with the estimation of the total phenolics content by means of the spectrophotometric method of Folin-Ciocalteu, CGA typically represents between 30% and 75% of the total phenolics of the fruit when harvested at the commercially mature stage (Luthria, 2012; Mennella *et al.*, 2012). CGA content in eggplant flesh is highly correlated with total phenolics and antioxidant activity, with r^2 values of 0.87 and >0.95, respectively (Luthria *et al.*, 2010, 2012). These results confirm that CGA is the most relevant phenolic compound in the eggplant fruit, and the major contributor to the high antioxidant capacity of eggplant. In fact, eggplant ranks among the vegetables with highest oxygen radical absorbance capacity due to its high content in phenolics (Cao *et al.*, 1996).

The multiple health benefits of eggplant, which include anti-oxidant, anti-diabetic, hypotensive, cardioprotective, and hepatoprotective effects (Akanitapichat *et al.*, 2010; Das *et al.*, 2011; Kwon *et al.*, 2008), are largely attributed to its phenolic content, in particular to CGA. In addition, the content of CGA in eggplant increases after the thermal treatments normally used for eggplant cooking (Lo Scalzo *et al.*, 2010). Also, it is worth mentioning that, although some phenolic compounds are bitter (Macheix, 1990), bitterness present in some cultivars of eggplant is caused by saponins and glycoalkaloids (Aubert *et al.*, 1989; Sánchez-Mata *et al.*, 2010) and not by CGA, which does not cause appreciable bitterness at the concentrations present in eggplant (Nagel *et al.*, 1987). Therefore, breeding new cultivars of eggplant with enhanced CGA content is of interest, as these new cultivars would have a high added value derived from its improved nutraceutical properties without affecting its organoleptic properties.

Table 1. Comparison of contents in chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA) in eggplant with other major vegetables, fruits, and plant products providing significant amounts of CGA to the diet.

Plant source	**CGA ($g \cdot kg^{-1}$ dw)**	**References**
Eggplant	4.9-21.6	Stommel and Whitaker (2003)
	4.2-9.5	Whitaker and Stommel (2003)
	1.5-2.2	Gajewski *et al*. (2009)
	5.0-8.1	Singh *et al*. (2009)
	2.6-6.7	Luthria *et al*. (2010)
	11.2-24.0	Mennella *et al*. (2010)
	1.4-8.4	Luthria (2012)
	14.1-28.0	Mennella *et al*. (2012)
Other vegetables		
Artichoke	1.1-1.8	Lutz *et al*. (2008)
Carrot	0.3-18.8	Sun *et al*. (2009)
Pepper	0.7-0.9	Hallmann and Rembialkowska (2013)
Tomato	0.2-0.4	Hallmann (2012)
Fruits		
Apple	0.4-1.2	van der Sluis *et al*. (2001)
Apricot	0.02-0.51	Madrau *et al*. (2009)
Cherry	0.02-0.09	Serra *et al*. (2011)
Peach	0.1-1.6	Andreotti *et al*. (2008)
Plum	0.4	Khallouki *et al*. (2012)
Other plant products		
Coffee	27.9-52.0	Monteiro and Farah (2012)
Mate tea	4.8-24.9	Heck *et al*. (2008)
Potato	0.01-4.60	Deußer *et al*. (2012)
Sunflower seeds	29.9-45.5	Singh *et al*. (1999)

Variation for CGA content in eggplant

Eggplant presents a wide morphological and molecular diversity (Hurtado *et al.*, 2012; Prohens *et al.*, 2005), as well as a broad variation for composition traits, including total phenolics and CGA content (Arivalagan *et al.*, 2012; Hanson *et al.*, 2006; Okmen *et al.*, 2009; Prohens *et al.*, 2007; Raigón *et al.*, 2008; Stommel and Whitaker, 2003). Few studies have been performed in which the variation for CGA content has been studied in a relevant number of

eggplant accessions. The first and broadest study was performed by Stommel and Whitaker (2003), who found differences of up to 4.4-fold in the CGA content and a continuous range of variation in a collection of 97 accessions of cultivated eggplant from the core collection of the USDA-ARS collection. These same authors also studied seven commercial varieties and found differences in CGA content of up to 2.2-fold among them (Whitaker and Stommel, 2003). Another study was performed by Mennella *et al.* (2012), in which they studied the variation for CGA content in 10 accessions of eggplant at three ripening stages. These authors found differences of 2.8, 3.7, and 4.0-fold between accessions for the unripe, commercially mature, and physiologically ripe stages, respectively. Also, they found that the accessions of the non-Japanese type (containing the anthocyanin delphinidin-3-rutinoside in the fruit skin), on average, had a higher CGA content than the Japanese type (containing the anthocyanin nasunin in the fruit skin). However, a considerable diversity was found within each of these types (Mennella *et al*., 2012). Overall, the results of these studies show that there is a wide diversity for CGA content within cultivated eggplant germplasm.

There are a few more studies in which, although CGA has not been measured, the total phenolics content has been estimated. In this respect, Hanson *et al.* (2006) found differences of up to 1.7-fold for total phenolics in a study involving 35 accessions of eggplant, Prohens *et al.* (2007) of up to 3.0-fold in a collection of 69 *S. melongena* accessions from different origins, Raigón *et al.* (2008) of up to 1.8-fold in a collection of 31 commercial varieties, landraces, and experimental hybrids, and Okmen *et al.* (2009) of 2.2-fold in a collection of 26 Turkish accessions of eggplant. A recent study, in which diversity for both CGA content and total phenolics were estimated (Mennella *et al.*, 2012) shows that variation for total phenolics in a collection of 10 eggplant accessions is lower than variation for CGA content. In this respect, these authors found differences of 2.4, 2.0, and 2.2-fold between accessions for the unripe, commercially mature, and physiologically ripe stages, respectively, which are lower values for relative differences than those observed for CGA content (see above). These results confirm the wide variation for phenolic content, and therefore for CGA content, in eggplant.

Non-genetic sources of variation can also contribute to the wide range of variation observed for CGA content in eggplant. Mennella *et al.* (2012) found important differences among fruit developmental stages. These authors found that there is a sharp decrease in CGA content during the fruit development, so that average values in a collection of 10 eggplant cultivars for the unripe, commercially mature, and physiologically ripe stages were of 21.6, 12.9, and 7.1 $mg \cdot kg^{-1}$, respectively. Also, important differences, which are nutritionally relevant, have been found by Whitaker and Stommel (2003) among different parts of the eggplant fruit. These authors found that the fruit flesh from midsection and blossom end part of the fruit had much higher content in CGA (on average 93% and 76% higher, respectively) than the stem end of the fruit. Also, Gajewski *et al.* (2009) found an average decrease of 37% in the CGA content after storage of eggplant for one week at 16ºC. Contrarily, Concellón *et al.* (2012) found that storage for 14 days at 10ºC increased CGA content, while a reduction was observed when stored at 0ºC.

Not much information exists for variation among years or cultivation conditions for CGA in eggplant. Mennella *et al.* (2010) found small (5%), although statistically significant, differences between two years for CGA content in eggplant genotypes; however, yearly differences were much higher (46%) for eggplant lines with introgressions from *S. aethiopicum* L. Hanson *et al.* (2006) also found very large and significant differences in total phenolics with average differences of 50% between two years. Regarding cultivation conditions, Luthria *et al.* (2010) did not find differences in CGA content when comparing eggplant fruits grown in two farms, one using conventional growing conditions and the other using organic cultivation. However, Raigón *et al.* (2010) found that in eggplants grown in the same farm, organically produced eggplants had 30% more total phenolic content than conventionally grown eggplants. An additional source of variation, in particular for comparing results from different research groups, comes from the methodology used for extraction and measurement of CGA (Luthria, 2012; Luthria and Mukhopadhyay, 2006).

All these data suggest that genetic, as well as many environmental factors (including extraction procedures), can affect the estimations of CGA content in eggplant and can contribute to differences observed among

different works (Table 1). In particular, for an efficient breeding for CGA content it is important to include sufficient genetic diversity in the breeding programs as well as to reduce the non-genetic causes of variation and to standardize protocols for taking and processing samples.

Breeding strategies for increased CGA content in eggplant

Several strategies based on the exploitation of the naturally available variation can be applied for developing new cultivars of eggplant with increased chlorogenic content. A successful new commercial variety with improved concentrations of CGA will also require having good agronomic and commercial characteristics (i.e., good yield, lack of prickles, fruit shape and color adapted to consumer demands, etc.) (Daunay, 2008). Studies on variation for phenolic content, as well as new genomic information will be of great assistance for the development of these new improved cultivars.

The high intraspecific variation for CGA content and total phenolic content (Hanson *et al.*, 2006; Mennella *et al.*, 2012; Okmen *et al.*, 2009; Prohens *et al.*, 2007; Raigón *et al.*, 2008; Stommel and Whitaker, 2003; Whitaker and Stommel, 2003) can be used in several ways in conventional breeding programs. For example, selection among the accessions or varieties with highest CGA content can result in the identification of materials with higher content in CGA. However, very likely, landraces with high content in CGA will not present agronomic and commercial characteristics competitive with present modern varieties, and its practical utility as commercial varieties may be limited. An alternative is the development of hybrids between accessions or lines with high content in CGA and complementary for agronomic traits. Eggplant hybrids are known to be heterotic for yield (Rodriguez-Burrruezo *et al.*, 2008) and competitive with commercial hybrids in open field conditions (Muñoz-Falcón *et al.*, 2008). Prohens *et al.* (2007) and Raigón *et al.* (2008) studied the total phenolic content in eggplant landraces and hybrids among them. Some of these hybrids, in particular those involving one or both parents with high content in phenolics, had values close to those of the parent with the highest value. Also, these hybrids can be used, through several breeding methods (Acquaah, 2012), to select and develop inbred lines with higher content in CGA and improved agronomic and commercial characteristics or to introgress this trait in élite lines.

Cultivated eggplant can be hybridized, although with different degrees of success, with a group of related species, including wild species and the cultivated scarlet (*S. aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplants (Daunay, 2008). Some of these species have high contents in CGA, which could be introgressed into eggplant. For example, *S. incanum* presents high contents in CGA (Ma *et al.*, 2011; Prohens *et al.*, 2013; Stommel and Whitaker, 2003). *Solanum incanum* is considered as the putative ancestor of eggplant (Lester and Hasan, 1990) and interspecific hybrids and subsequent backcross generations to eggplant are fully fertile (Prohens *et al.*, 2013). The latter authors studied an interspecific family between *S. melongena* and *S. incanum* and found that even in the first backcross generation it was possible to select individuals with high content in CGA. This study also revealed that additive genetic effects were the most important in explaining CGA variation, suggesting that alleles from *S. incanum* should be placed in homozygous state to obtain a higher expression of the trait. Other species, like *S. sodomaeum* L. *(=S. linneanum* Hepper & Jaeger) also show a higher CGA content than that of *S. melongena* (Mennella *et al.*, 2010). However, eggplant lines with introgressions from *S. sodomaeum* did not present particularly high levels of CGA, very likely because these lines had not been selected for high CGA content (Mennella *et al.*, 2010).

Molecular breeding strategies can also be of great utility for developing eggplant cultivars with improved CGA content. The availability of genetic maps (Barchi *et al.*, 2010; Doganlar *et al.*, 2002; Fukuoka *et al.*, 2012; Wu *et al.*, 2009) can be useful for the detection of quantitative trait loci (QTLs) affecting CGA content, as has been done for other traits like anthocyanin content or parthenocarpy (Barchi *et al.*, 2012; Miyatake *et al.*, 2012). Also, the CGA synthesis pathway in Solanaceae (Figure 2) is known (Clé *et al.*, 2008; Niggeweg *et al.*, 2004) and the sequences of the six genes codifying for the enzymes involved in this pathway (phenylalanine ammonia lyase, PAL; cinnamate 4-hydroxilase, C4H; 4-hydroxycinnamoyl-CoA ligase, 4CL; hydroxycinnamoyl-coA shilimate/quinate hydroxycinnamoil transferase, HCT; *p*-coumaroyl ester 3'-hydroxilase, C3'H; and, hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase, HQT) are available (Comino *et al.*, 2007, 2009; Joët *et al.*, 2010; Mahesh *et al.*, 2007; Menin *et al.*, 2010; Niggeweg *et al.*, 2004). In consequence, it is possible to map these genes and to study their co-

segregation with QTLs for CGA content. Sequencing of these alleles in a collection of germplasm, as well as TILLING or EcoTILLING strategies, can be useful to identify allelic variants for these genes (Pérez-de-Castro *et al.*, 2012). A selection of the most favorable alleles for each of these, which could be pyramided in a single variety (Ishii and Yonezawa, 2007), could be done through analyses of gene expression, as has been done on coffee (Lepelley *et al.*, 2007).

Genetic transformation has been successfully applied for several traits in eggplant (Acciarri *et al.*, 2000; Donzella *et al.*, 2000; Pal *et al.*, 2009). Niggeweg *et al.* (2004) obtained transgenic plants of tomato overexpressing the HQT enzyme, which resulted in accumulation of higher levels of CGA. This opens the way to use similar approaches in eggplant. However, many sectors from the society, especially in Europe, reject genetically modified (GM) plants and regulations for getting approval of GM cultivars are long, complicated, and expensive (Raybould and Poppy, 2012).

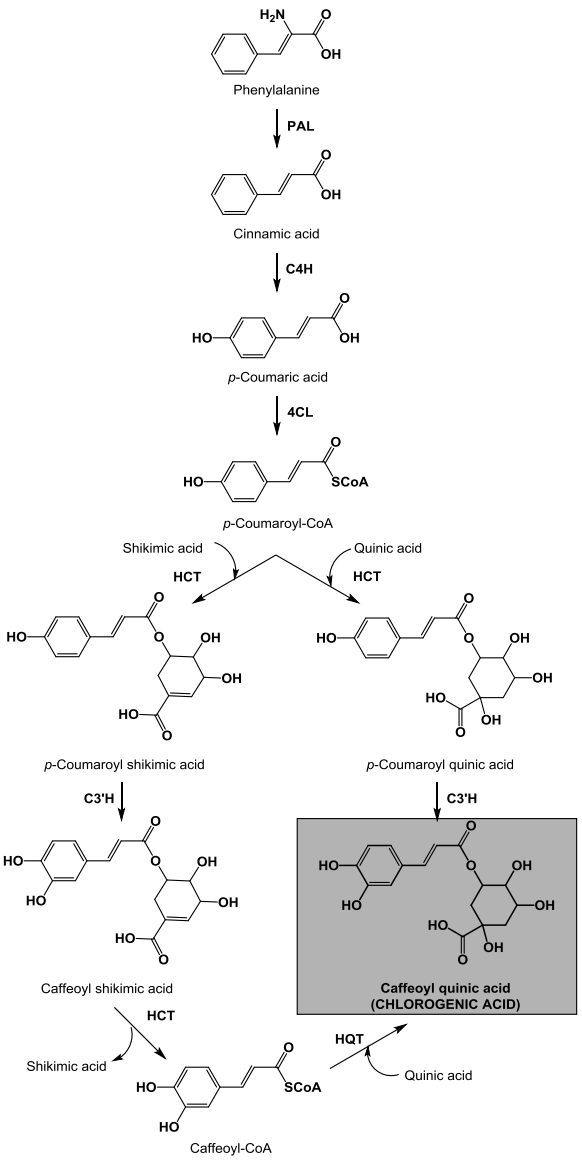

Figure 2. Biochemical pathway for the synthesis of chlorogenic acid in eggplant (Clé *et al.*, 2008; Comino *et al.*, 2007, 2009; Joët *et al.*, 2010; Mahesh *et al.*, 2007; Menin *et al.*, 2010; Niggeweg *et al.*, 2004). Enzymes involved in the pathway are indicated: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxilase; 4CL, 4-hydroxycinnamoyl-CoA ligase; HCT, hydroxycinnamoyl-coA shilimate/quinate hydroxycinnamoil transferase; C3'H, *p*-coumaroyl ester 3'-hydroxilase; HQT, hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase.

Fruit flesh browning as a side effect of CGA content improvement

As in other fruits and vegetables, like apple or artichoke, oxidation of phenolic compounds, including CGA, present in the eggplant flesh results in flesh browning, which reduces apparent quality (Adams and Brown, 2007; Macheix, 1990; Mishra *et al.*, 2012). When an eggplant fruit is cut open, the breakdown of the cellular compartments allows the orthophenolic compounds (hydroxycinnamic acid derivatives, like CGA) to be accessible to polyphenol oxidases (PPO), which catalyze their oxidation to quinones. Quinones then react non-enzymatically with O_2 and other molecules to produce compounds which cause the browning of the flesh in the cut area (Mishra *et al.*, 2012; Ramírez, 2002). Fujita and Tono (1988) found that CGA was the substrate for which eggplant PPO presented a greater affinity. However, Mishra *et al.* (2012) describe an intermediate specificity of eggplant PPO for CGA, showing only a 31% relative activity (using with 4-methylcatechol as 100% reference). In fact, eggplant PPO had a higher affinity for dihydrocaffeic acid or pyrocatachol, but a lower affinity for pyrogallol or gallic acid, than for CGA (Mishra *et al.*, 2012). In any case, under the same conditions, the higher the content in CGA in the fruit flesh, the higher the browning.

The fact that differences in PPO activity exist among eggplant cultivars (Dogan *et al.*, 2002; Mennella *et al.*, 2012) suggests that selection for low PPO activity could be carried out. In this way, simultaneous selection for low PPO activity and high content in CGA content could result in materials with greater functional quality and low browning. Also, other factors, like intracellular pH or ascorbic acid content, which affect the PPO activity (Concellón *et al.*, 2012; Doğan *et al.*, 2002; Mishra *et al.*, 2012), could play a role in the reduction of the degree of browning.

Prohens *et al.* (2007) studied the relationship between fruit flesh browning and the total phenolic content in eggplant. These authors found a wide variation for fruit browning among the cultivated germplasm, and a positive, but moderate, relationship between total phenolics content and fruit flesh browning (r=0.389), which indicates that it is possible to select varieties with high content in phenolics and low or moderate fruit flesh browning. More recently, the relationship between fruit flesh browning and total content in hydroxycinnamic conjugates (of which CGA was, by far, the most abundant)

has been studied in an interspecific family between *S. melongena* and *S. incanum* (Prohens *et al.*, 2013). In this study it has been found that the correlation coefficient was low (r=0.245 for F2 and r=0.116 for the first backcross to *S. melongena*).

The fact that PPO genes in eggplant display considerable variation and that seem to be situated in a cluster in chromosome 8 (Shetty *et al.*, 2011) suggests that it is possible to use marker assisted selection for low PPO activity. Therefore, the data suggest that it is possible to select eggplant varieties with high content in CGA and low fruit flesh browning.

Conclusions

Given the many beneficial properties of CGA for human health and the high contents of this phenolic compound present in the eggplant fruit, developing new eggplant cultivars with improved functional quality resulting from increased CGA contents is of interest. The high genetic diversity among eggplant cultivars will facilitate the selection of sources of variation for high CGA content for breeding programs. Also, the use of wild relatives like *S. incanum* can result in the introgression of genes for high CGA values from these species. In both cases, the use of molecular breeding techniques, including marker assisted selection and the identification of allelic variants, can make an effective contribution to reaching this goal. Also, the low or moderate relationship between CGA content and fruit flesh browning together with selection for low PPO activity suggests that, in eggplant, it is possible to develop new cultivars with a combination of high CGA content and low fruit flesh browning.

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Objetivos

El objetivo principal de esta Tesis Doctoral es la evaluación, selección y desarrollo de material vegetal para la mejora de la calidad nutraceútica, entendida como un aumento en el contenido en polifenoles, y en particular de ácido clorogénico, de la berenjena. Para ello utilizaremos material vegetal tanto de la berenjena común como de especies cultivadas y silvestres relacionadas. Pretendemos que la información y material vegetal obtenidos sean de utilidad en el desarrollo de nuevas variedades de berenjena con alto valor añadido.

Para cumplir este objetivo principal hemos dividido este estudio en bloques diferenciados:

1. Estudiar la diversidad en la berenjena común (*S. melongena*) para compuestos bioactivos y caracteres relacionados. Para ello se evaluarán el contenido en polifenoles, el ácido clorogénico, pardeamiento del fruto y otros caracteres relacionados, definiendo qué caracteres serán los más importantes y las interrelaciones entre ellos.

2. Estudiar la diversidad en berenjenas escarlata (*S. aethiopicum*) y gboma (*S. macrocarpon*) para una mejora integral. Se evaluarán los caracteres morfoagronómicos y los compuestos bioactivos.

3. Estudiar la factibilidad e interés de la hibridación interespecífica de la berenjena común con la berenjena escarlata y la especie silvestre *S. incanum* para la mejora de la calidad nutracéutica de la berenjena.

    3.1 Evaluar las características morfoagronómicas y el contenido en compuestos bioactivos, así como parámetros genéticos de interés en la mejora.
    3.2 Asimismo, se pretende evaluar el interés de los retrocruzamientos obtenidos para la introgresión en berenjena común de los caracteres de interés evaluados.

Resultados

3.1 Diversidad en berenjena común para compuestos bioactivos y caracteres relacionados

3.1.1 Diversity and Relationships in Key Traits for Functional and Apparent Quality in a Collection of Eggplant: Fruit Phenolics Content, Antioxidant Activity, Polyphenol Oxidase Activity, and Browning

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Abstract

Eggplant (*Solanum melongena*) fruits contain high levels of phenolic compounds, in particular of chlorogenic acid. Developing new varieties with increased levels of phenolics is of interest for enhancing the functional quality of this fruit. However, this improvement might result in greater fruit flesh browning caused by the oxidation of phenolics in the cut surfaces of the fruit, thereby reducing apparent quality. In order to study the relationships between phenolics content and browning, we evaluated 18 eggplant accessions with different morphological characteristics for fruit total phenolics content, chlorogenic acid content, DPPH scavenging activity, polyphenol oxidase (PPO) activity, liquid extract browning, and fruit flesh browning. For all the traits we found a high diversity, with differences in the range of variation among accessions between 1.82-fold for DPPH scavenging activity and 3.36-fold for fruit flesh browning. The ratio of chlorogenic acid content to total phenolics acid, which presented an average value of 21%, was also very variable. A high phenotypic correlation value (r=0.612) was found between chlorogenic acid content and DPPH scavenging activity, confirming that chlorogenic acid is a key factor in the functional activity of eggplant. Variation in total content in phenolics and in chlorogenic acid content accounted only for 18.9% and 6.0% in the variation in fruit flesh browning. PPO activity was not significantly correlated with fruit flesh browning. Liquid extract browning was not correlated with fruit flesh browning, but the former was highly correlated with chlorogenic acid content (r=0.852). Values for environmental correlations were in agreement with phenotypic correlations. Multivariate principal components (PCA) analysis allowed identifying four groups of accessions which presented different profiles for total phenolics, chlorogenic acid content, total phenolics to chlorogenic acid content ratio, DPPH scavenging activity and liquid extract browning. The results we have obtained suggest that it is possible to select and develop new eggplant varieties with improved functional and apparent quality.

Introduction

Fruits with a high content in phenolics have been reported to present increased antioxidant activity and to prevent some chronic and degenerative diseases, including several types of cancer (Sato *et al.,* 2011). Eggplant (*Solanum melongena* L.) is one of the vegetables with greatest antioxidant activity (Cao *et al.*, 1996), and presents anti-diabetic, hypotensive,

cardioprotective, and hepatoprotective effects (Kwon *et al.,* 2008; Akanitapichat *et al.,* 2010; Das *et al.,* 2011). These properties have been attributed to its high content in phenolics (Plazas *et al.,* 2013). Major phenolic compounds in eggplant include hydroxycinnamic acids (Okmen *et al.,* 2009; Menella *et al.,* 2010; Mennella *et al., 2012),* which are found both in the fruit flesh and in the skin, and anthocyanins, which are present only in the skin (Gajewski *et al.,* 2009). Given that most of the volume of the eggplant fruit is fruit flesh, hydroxycinnamic acids, of which chlorogenic acid (5-*O*-chlorogenic acid and its isomers) is the major representative in eggplant (Stommel and Whitaker, 2003), are the phenolic compounds that make a greater contribution to the functional quality of the eggplant fruit (Nisha *et al.,* 2003). Chlorogenic acid presents many properties beneficial for human health, such as anti-oxidant, anti-carcinogenic, anti-inflammatory, analgesic, anti-microbial, neuroprotective, and cardioprotective effects (Plazas *et al.,* 2013). In addition, chlorogenic acid also plays an important role in plant defence (López-Gresa *et al.,* 2011).

The interest in developing new eggplant cultivars with enhanced bioactive properties has led to the development of breeding programs specifically aimed at improving the content in total phenolics, in particular of chlorogenic acid (Plazas *et al.,* 2013; Stommel and Whitaker, 2003; Prohens *et al.,* 2013). A wide diversity has been found among eggplant cultivars for total phenolics and chlorogenic acid content. (Okmen *et al.,* 2009; Stommel and Whitaker, 2003; Hanson *et al.,* 2006; Prohens *et al.,* 2007). Broad-sense heritability values for total phenolics in eggplant are intermediate (Prohens *et al.,* 2007), which is an indication that selection and breeding for content in phenolics in eggplant will be efficient for the development of improved cultivars.

However, it is well known that in fruits and vegetables the oxidation of phenolics after the exposure of internal tissues to the air results in browning, which reduces the apparent quality (Toivonen and Brummell, 2008). The destruction of the cell compartments allows the ortodiphenolic substrates (chlorogenic acid and other hydroxycinnamic acid derivatives), mostly confined within the vacuoles, to be accessible to polyphenol oxidases (PPOs), which are found in the plastid membranes (Toivonen and Brummell, 2008; Mayer, 2006).

PPOs catalyze the oxidation of these phenolic compounds to quinones, which subsequentially react nonenzymatically with O_2, and other compounds, like sulfhydryl compounds, amines, amino acids, and proteins to give brown-colored compounds. PPO activity, together with phenolics levels, plays a major role in browning of cut tissues in a number of crops (Mayer, 2006). Eggplant PPOs have shown a great affinity for chlorogenic acid (Fujita and tono, 1988; Todaro *et al.,* 2011), which might suggest that increases in chlorogenic acid content, could result in increased fruit flesh browning. Also, several studies have shown that there are differences among eggplant varieties for PPO activity (Mennella *et al.,* 2010; Mennella *et al.,* 2012; Todaro *et al.,* 2011; Doğan*et al.,* 2002), which opens the way to selecting varieties with reduced PPO activity.

Measurement of browning in eggplant has been performed in the fruit flesh either visually (Polignano *et al.,* 2010) or with a chromameter (Prohens *et al.,* 2007; Maestrelli *et al.,* 2008; Barbagallo *et al.,* 2012; Concellón *et al.,*2012). Chromameter measurements are generally considered as better than visual observations, as they allow an objective and precise measurement of browning. Also, browning in eggplant can be estimated in juice or in extracts of lyophilized tissue with a chromameter or by spectrometry (Todaro *et al.,* 2011; Barbagallo *et al.,* 2012).

Knowledge of the relationships between content in phenolics, antioxidant activity, PPO activity, and fruit flesh browning in genetically diverse collections of eggplant may be of interest in order to develop strategies for the development of new cultivars with improved fruit quality. Massolo *et al*. (2011) and Mishra *et al*. (2013) studied the relationships between total phenolics, chlorogenic acid, PPO activity and browning in eggplant. However, these authors used a single cultivar. Both studies found low variation in total phenolics and chlorogenic acid content in the different samples measured, and that samples with greatest browning also presented highest levels of PPO activity (Massolo *et al.,* 2011; Mishra *et al.,* 2013). In a recent paper, Mishra *et al*. (2013) used eight Asian cultivars to study the evolution of phenolics content, PPO activity, and browning during postharvest storage These authors found that evolution of fruit flesh browning during storage for two weeks was positively correlated with phenolics content and PPO activity in one group of

four accessions, and positively correlated with phenolics content and negatively with PPO activity in another group of four accessions (Mishra *et al.,* 2013). However, the examination of the results of accessions before storage reveals that both the content in phenolic acids and PPO activity presented, respectively, low and moderate correlations with fruit flesh browning (Mishra *et al.,* 2013). In any case, the number of accessions used was quite limited (eight accessions) in order to draw generalizations. Also, given that oriental (Asian) and occidental (Mediterranean and African) eggplants are genetically differentiated (Vilanova *et al.,* 2012) it would be of interest to study these relationships in Occidental type materials, which are the most important in Europe, Middle East, Africa, and America.

In a study using a wide genetic diversity of eggplant (69 accessions), Prohens *et al*. (2007) found that the correlation between total phenolics and fruit flesh browning measured with a chromameter was moderate (r=0.388) and suggested that other factors other than the total phenolics compounds had a major role in fruit flesh browning in eggplant. Also, Prohens *et al*. (2013) in a study of segregating generations from an interspecific family between *S. melongena* and the wild relative *S. incanum* L. reached a similar conclusion.

Here, we evaluate the total phenolics content, chlorogenic acid content, antioxidant activity, PPO activity, liquid extract browning, and fruit flesh browning in a collection of 18 accessions with different morphological characteristics from the region of Valencia, which is situated in the Spanish secondary center of diversity for eggplant (Hurtado *et al.,* 2012). The objective is to study the variation and relationships between these traits in order to obtain information relevant for the selection and development of eggplant varieties with improved bioactive properties and reduced fruit flesh browning.

Materials and methods

Plant Material

Fruits from a total of 18 eggplant accessions originating from the provinces of Alicante and Valencia, situated in the Autonomous Community of Valencia (Spain), were used for the present study (Table 1).

Table 1. Accessions, code used in the present work, fruit size measures (length, width, and weight; mean±SD), fruit color class, and geographical origin of the eggplant materials used for the study of the diversity and relationships between phenolics content, antioxidant activity, polyphenol oxidase activity, and browning.

Accession	Code	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit color class	Origin
B-31	B31	10.0 ± 1.2	9.4 ± 1.4	225 ± 78	Black	Valencia province, Spain
B-32	B32	14.2 ± 2.2	8.1 ± 1.5	346 ± 148	White	Valencia province, Spain
B-33	B33	15.1 ± 1.8	5.2 ± 0.5	171 ± 36	White	Valencia province, Spain
B-36	B36	14.7 ± 2.2	5.0 ± 0.7	176 ± 74	Purple	Valencia province, Spain
V-S-4	V4	19.6 ± 3.4	5.1 ± 0.7	206 ± 80	Black	Gandía, Valencia, Spain
V-S-5	V5	21.5 ± 3.6	4.9 ± 0.6	232 ± 83	Black	Xeraco, Valencia, Spain
V-S-6	V6	20.8 ± 2.9	5.2 ± 0.7	223 ± 60	Black	Xeraco, Valencia, Spain
V-S-7	V7	13.3 ± 1.4	9.2 ± 1.5	346 ± 90	Striped purple	Xeraco, Valencia, Spain
V-S-9	V9	6.7 ± 0.9	8.2 ± 1.1	233 ± 81	Green with purple streaks	La Aparecida (Orihuela), Alicante, Spain
V-S-10	V10	10.2 ± 0.9	6.7 ± 1.1	169 ± 55	Purple	La Aparecida (Orihuela), Alicante, Spain
V-S-12	V12	25.2 ± 3.4	4.6 ± 0.4	200 ± 54	Black	Benijófar, Alicante
V-S-13	V13	7.6 ± 1.1	7.8 ± 0.9	180 ± 54	Purple	San Fulgencio, Alicante, Spain
V-S-14	V14	17.5 ± 1.8	6.1 ± 0.8	250 ± 73	Black	Rafal, Alicante, Spain
V-S-16	V16	17.9 ± 4.7	7.7 ± 1.6	290 ± 85	Black	Novelda, Alicante, Spain
V-S-17	V17	17.6 ± 4.2	5.9 ± 0.8	228 ± 91	Black	Elx, Alicante, Spain
V-S-18	V18	17.5 ± 2.9	5.1 ± 0.8	168 ± 62	Black	Elda, Alicante, Spain
V-S-19	V19	23.1 ± 2.9	5.2 ± 0.6	252 ± 67	Black	Mutxamel, Alicante, Spain
V-S-21	V21	23.6 ± 3.7	5.9 ± 1.0	301 ± 107	Black	Gandía, Valencia, Spain

The accessions used included different fruit sizes, shapes, and colors, reflecting the wide morphological and molecular diversity of eggplant accessions from this region (Hurtado *et al.,* 2012; Prohens *et al.,* 2005; Muñoz-Falcón *et al.,* 2008). Plants from which the fruits were harvested were grown in the open field at theAgricultural Experimental Farm of Carcaixent (Valencia, Spain) using the standard horticultural practices.

Preparation of samples

For each accession, fifteen commercially ripe fruits, evaluated by the size, color and glossiness of the fruit, were harvested between July 25 and September 5, 2011. A total of five samples, each consisting of three fruits, were considered for each accession. Fruits were washed and cut transversally with a well sharpened knife at the mid-point between the blossom and stem ends for the measurement of fruit flesh browning. After fruit flesh browning had been measured, a 1-cm wide longitudinal section was cut from the middle of the fruit, peeled, and immediately frozen in liquid N_2 and stored at -80 ºC until lyophilized. Powdered tissue of each sample was used for the analyses.

Traits measured

Total phenolics content was measured according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965). For each sample, 0.25 g of the lyophilized tissue were extracted with 10 mL methanol:water (80:20, v/v) for 24 h at room temperature. An aliquot of the 1.25 mL of the extracted phenolic sample was centrifuged at 8000 rpm for 5 min and 65 µL of the supernatant were mixed with 0.5 mL diluted (10%, v/v) Folin-Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany) and allowed to stand at room temperature for 5 min. Subsequently, 0.5 mL of a sodium carbonate solution (60 g/L) was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm in a Nanodrop ND-1000 (Nanodrop Technologies, Montchain, DE, USA) spectrophotometer. Chlorogenic acid (Sigma Aldrich) was used as a standard, and total phenolics content was expressed as chlorogenic acid equivalents in g/kg of dry weight.

Chlorogenic acid was extracted basically according to Naranjo *et al*. (2003) Lyophilized samples (0.1 g) were homogenized in 2.5 mL methanol. The total extract (2 mL) was vortexed vigorously, sonicated for 10 min, and then

centrifuged at 14000 rpm for 15 min using a refrigerated (4 ºC) centrifuge and 2 mL Eppendorf tubes to remove cellular debris. The pellet was re-extracted with 1 mL of methanol and centrifuged again as above. The combined supernatants were filtered through 0.45-µm Spartan 13/0.45RC filters (Schleicher & Schuell, Keene, NH, U.S.A.), nylon filters (Waters, Milford, MA, U.S.A.), and dried under nitrogen at 40 ºC using glass tubes of 5 mL. The dried residue was dissolved in 1 mL methanol containing 0.02% H_3PO_4, vortexed, and centrifuged as above for 5 min. The supernatant (1 mL) was filtered again, and aliquots (40 µL) were injected at room temperature with a Waters 717 (Waters) autosampler into a reverse-phase Symmetry 5-µm C18 (4.6 by 150 mm; Waters) column, previously equilibrated in 99% H_2O:1% acetic acid (solvent A). A lineal gradient starting with 100% solvent A and ending with 100% methanol (solvent B) was applied over 20 min at a flow rate of 1 mL/min. Then, the column was washed with solvent B for 10 min, and allowed to equilibrate again with solvent A, with a total run time of 40 min. Chlorogenic acid was detected photometrically (λ = 320 nm) with a Waters 996 photodiode array detector, and quantified with the Waters Millenium[32] (Waters) software using authentic chlorogenic standard (Bellés *et al.,* 2008).

The antioxidant capacity was evaluated according to Falchi *et al.* (2006) by measuring spectrophotometrically at 517 nm the ability to quench the stable radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$). Fifty µL of methanol-soluble phenolic fraction were diluted with 2 mL of ethanol 96º. An aliquot of 0.5 mL of the resulting solution was added to 1.5 mL of ethanol 96º, and 0.5 mL ethanolic solution containing $DPPH^{\cdot}$ 0.5 mmol/L. The blank sample was prepared using 2 mL ethanol and 0.5 mL of the same $DPPH^{\cdot}$ ethanolic solution in order to check the radical stability. After incubation of the mixture at 25 ºC for 10 min, the absorbance at 517 nm was measured using a Pharmacia Biotech 1000E UV-Vis (Pharmacia Biotech, Piscataway, NJ, USA) spectrophotometer. The radical scavenging activity (S) of each extract after 10 min was expressed in percentage and calculated as $S = 100-[(A_x/A_o)\times100]$, where A_x is the optical density of $DPPH^{\cdot}$ solution in presence of eggplant extract and A_o the optical density of $DPPH^{\cdot}$ solution in absence of the sample.

Polyphenol oxidase (PPO) activity was measured basically according to Bellés *et al*. (2006) Samples (0.1 g) of lyophilized material were reduced to a

fine powder with a pestle in a cooled mortar and homogenized in 4 mL of 0.1 M sodium phosphate buffer (pH 6.0) and centrifuged at 12.000 rpm for 15 min at 4 ºC. Supernatant was diluted 5-fold in buffer extraction solution, and PPO assay was carried out in a total volume of 2 mL containing 50 μL of diluted supernatant (enzyme extract), 150 μL of 0.1 M chlorogenic acid (dissolved in 50% methanol), and 1.8 mL of 0.1 M sodium phosphate buffer (pH 6.0). The reaction blank contained 50 μL of buffer instead of enzyme extract. The enzymatic reaction was followed colorimetrically at 420 nm in a Pharmacia Biotech 1000E UV-Vis spectrophotometer. PPO activity was measured as increments in absorbance at 420 nm per min and mg of dry weight during the first 1.5 min of the reaction, period in which the enzymatic activity was lineal for all substrate concentrations. One unit of enzyme activity was defined as the increase in 0.1 absorbance units per minute per mg of dry weight.

Browning in the liquid extract of lyophilized sample was determined by spectrophotometry at 420 nm (Sapers and Douglas, 1987). For each sample, 0.25 g of lyophilized tissue was homogenized with 2.5 mL of water and was incubated at room temperature for 10 min. Subsequently, 2.5 mL of a 4 % metaphosphoric solution was added to stop the oxidizing reaction (Luthria *et al.,* 2002). For each sample, a control was prepared in which 0.25 g of lyophilized tissue were homogenized with 2.5 mL of 4% metaphosphoric acid and incubated at room temperature for 10 min. After that 2.5 mL of water were added to the solution. Both the sample and its respective control solutions were centrifuged at 8000 rpm for 5 min. Absorbance was then determined at 420 nm in a Nanodrop ND-1000 spectrophotometer. One unit of extract browning was defined as a difference in 0.01 absorbance units between the sample and the control.

For fruit flesh browning measurement, the CIELAB 1976 color coordinates of the fruit flesh were measured with a Minolta CR-300 (Minolta, Osaka, Japan) chromameter in each of the three fruits that constitute a sample. Measurements were made in the central part of a transversal section of the fruit immediately after being cut (0 min) and 10 min later. Fruit flesh browning was measured as the difference in the degree of whiteness (DW), calculated as $DW[(100-L^*)^2+a^{*2}+b^{*2}]^{0.5}$ (Prohens *et al.,* 2007), between 10 min (DW_{10}) and 0 min (DW_0). For each sample, the fruit flesh browning was

obtained as the average of the three fruits. One unit of fruit flesh browning was defined as one unit in the difference between DW_{10} and DW_0.

Data analyses

For each trait, accession means were obtained and varieties were ranked from highest to lowest value. Average standard errors (SE) for the means and coefficient of variation (CV; %) were also calculated. Phenotypic and environmental correlations between traits were calculated from correlations between variety means (phenotypic correlations) and between the residual effects of individual samples (environmental correlations), respectively. Principal components analysis (PCA) was performed for standardized values using pairwise Euclidean distances among variety means. The results of the PCA analysis were used to establish four groups of accessions with different profile for the traits studied. Signification of differences among groups of accessions for the traits studied was evaluated by means of analyses of variance (ANOVA) using a fixed-effects model for the effect of accession. All statistics were conducted using specific software (Statgraphics Centurion XVI, StatPoint Technologies, Warrenton, VA, USA).

Results

Traits evaluated

Considerable variation among accessions was found for all traits (Table 2). Differences between the lowest and highest mean value for the accessions tested ranged from 1.82-fold for DPPH scavenging activity and 3.36-fold for flesh browning. The coefficient of variation did not present large differences among the traits studied, and ranged between 39.24% for total phenolics content and 54.35% for PPO activity (Table 2).

The total phenolics content ranged between 8.14 g/kg (V21) and 22.47 g/kg (B33), with an average value of 16.86 g/kg (Table 2). The chlorogenic acid content presented a mean value of 3.55 g/kg and varied between 2.47 g/kg (V21) and 6.27 g/kg (V17). This latter accession (V17) presented a remarkably high value of chlorogenic acid content, with a value 41% greater than the accession that ranked second (V9; 4.42 g/kg) (Table 2). Chlorogenic acid was measured at 320 nm wavelength, as this was the wavelength that provided better resolution after diode array detector scanning

from 240 to 400 nm. As observed in Figure 1 chlorogenic acid was the major UV-absorbing peak and had a retention time of 11.94 min. Chlorogenic acid represented, on average, 21.1% of the total phenolics content measured by the Folin-Ciocalteu method, although considerable differences were found among accessions for the chlorogenic acid content to total phenolics content ratio, so that the percentage of total phenolics content accounted by chlorogenic acid varied between 13.6% (B32) and 36.2% (V19) (Figure 2).

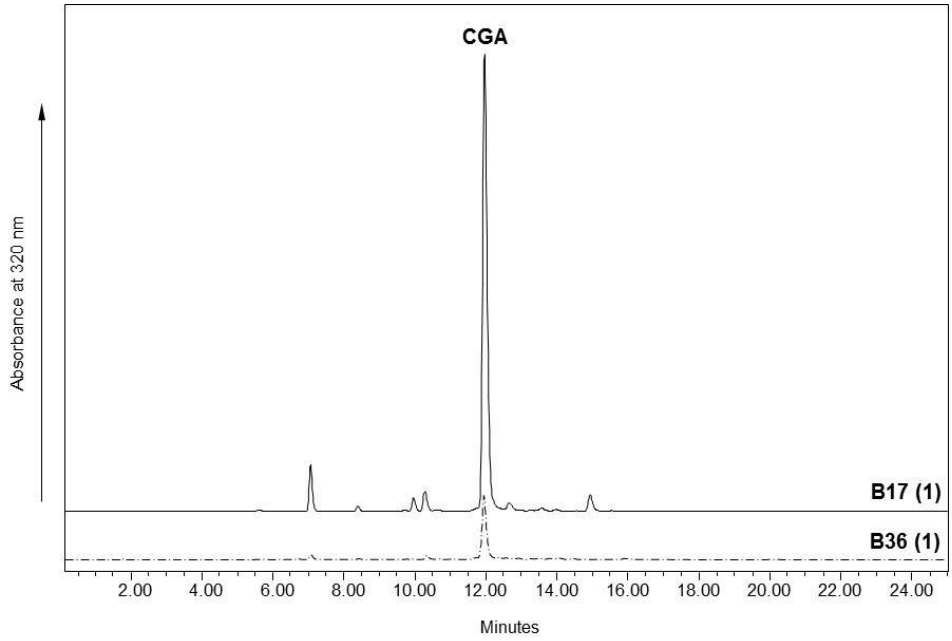

Figure 1. Representative C_{18} column HPLC chromatograms from methanolic extracts of two samples of eggplant with contrasting contents in chlorogenic acid (CGA) content. Absorbance as measured at 320 nm.

The DPPH scavenging activity ranged between 27.5% (B36) and 50.3% (V9), with an average value of 35.6% (Table 2). Five accessions presented DPPH scavenging activity values above 40%, while other five presented values below 30%. The PPO activity varied between 0.870 units/g (B31) and 2.490 units/g (V17), with a mean value of 1.552 units/g. Liquid extract browning ranged between 2.38 units (V7) and 7.06 units (V17), with an average value of 4.12 units. As occurred for chlorogenic acid content, accession V17 presented an

extract browning value much higher (26.1%) than that of the variety ranking second (V4; 5.60 units). Finally, the fruit flesh browning varied between 2.47 units (V16) and 8.31 units (B33), with the average value being 5.15 units.

Table 2. Mean values and rank (from highest to lowest; between brackets, italics) for each accession, average standard error (SE; obtained from the ANOVA analyses) and coefficient of variation (CV; %) for fruit traits in a collection of 18 eggplant accessions.

Accession code	Total phenolics (g/kg dw)		Chlorogenic acid (g/kg dw)		DPPH scavenging activity (%)		PPO activity (units/mg dw)		Liquid extract browning (units)		Fruit flesh browning (units)	
B31	18.52	(*8*)	3.11	(*13*)	34.3	(*8*)	0.870	(*18*)	2.65	(*17*)	4.28	(*13*)
B32	20.99	(*4*)	2.86	(*15*)	32.7	(*10*)	0.954	(*17*)	4.42	(*8*)	4.27	(*14*)
B33	22.47	(*1*)	3.35	(*9*)	31.3	(*12*)	1.120	(*15*)	3.71	(*12*)	8.31	(*1*)
B36	20.59	(*5*)	3.28	(*10*)	27.5	(*18*)	1.418	(*10*)	3.59	(*13*)	6.14	(*4*)
V4	16.69	(*11*)	4.24	(*4*)	29.3	(*15*)	1.818	(*7*)	5.60	(*2*)	5.57	(*8*)
V5	19.13	(*7*)	3.25	(*12*)	34.7	(*7*)	1.872	(*4*)	4.77	(*5*)	4.69	(*11*)
V6	16.35	(*12*)	3.84	(*5*)	32.6	(*11*)	1.698	(*8*)	5.22	(*3*)	4.51	(*12*)
V7	21.94	(*3*)	2.98	(*14*)	46.5	(*3*)	1.832	(*6*)	2.38	(*18*)	8.08	(*2*)
V9	17.53	(*10*)	4.42	(*2*)	50.3	(*1*)	2.154	(*3*)	4.81	(*4*)	3.49	(*16*)
V10	10.06	(*16*)	2.82	(*16*)	28.0	(*17*)	1.308	(*12*)	2.81	(*15*)	5.62	(*7*)
V12	20.06	(*6*)	3.53	(*7*)	36.2	(*6*)	1.392	(*11*)	3.99	(*10*)	5.68	(*6*)
V13	18.26	(*9*)	3.49	(*8*)	40.7	(*5*)	2.174	(*2*)	2.98	(*14*)	5.21	(*9*)
V14	16.08	(*13*)	4.34	(*3*)	34.2	(*9*)	1.474	(*9*)	4.77	(*6*)	4.88	(*10*)
V16	9.86	(*17*)	2.67	(*17*)	30.9	(*13*)	1.202	(*14*)	2.72	(*16*)	2.47	(*18*)
V17	22.00	(*2*)	6.27	(*1*)	46.8	(*2*)	2.490	(*1*)	7.06	(*1*)	6.53	(*3*)
V18	14.58	(*14*)	3.25	(*11*)	45.6	(*4*)	1.066	(*16*)	3.80	(*11*)	4.08	(*15*)
V19	10.23	(*15*)	3.71	(*6*)	29.5	(*14*)	1.860	(*5*)	4.64	(*7*)	5.93	(*5*)
V21	8.14	(*18*)	2.47	(*18*)	29.0	(*16*)	1.228	(*13*)	4.27	(*9*)	3.03	(*17*)
Mean	16.86		3.55		35.6		1.552		4.12		5.15	
Average SE	2.16		0.77		8.7		0.346		0.82		0.84	
CV (%)	39.24		50.47		52.51		54.35		50.34		46.10	

Correlations between traits

The pairwise coefficients of phenotypic correlation among the six traits studied presented positive values and were significant (P<0.05) for 11 out of the 15 correlations studied (Table 3). The highest value for the phenotypic correlation coefficient was between chlorogenic acid content and liquid extract browning (r=0.852) (Figure 3). The coefficient of determination (r^2) for this phenotypic correlation had a value of 72.5%. Values for the phenotypic coefficient of correlation above 0.6 were also found between chlorogenic acid on one side and total phenolics (r=0.633) and DPPH scavenging activity (r=0.612) on the other (Table 3). The phenotypic coefficients of determination for the correlation between these pairs of traits were of 40.0% and 37.5%, respectively (Table 3). The fruit flesh browning presented low, although significant, values for the phenotypic correlation with total phenolics (r=0.434) and with chlorogenic acid content (r=0.253). The coefficient of determination for the correlation between fruit flesh browning and total phenolics and chlorogenic acid contents, was of only 18.9% and 6.4%, respectively. Fruit flesh browning was not significantly correlated with PPO activity and liquid extract browning (Table 3).

Table 3. Phenotypic correlations (r; above the diagonal) and coefficients of determination (%, below the diagonal) for fruit traits (total phenolics, TP; cholorogenic acid, CGA; DPPH scavenging activity, DPPH; polyphenol oxidase activity, PPO; liquid extract browning, LEB; and, fruit flesh browning; FFB) in a collection of 18 eggplant accessions.

	TP	CGA	DPPH	PPO	LEB	FFB
TP		0.633***	0.461***	0.197ns	0.512***	0.434***
CGA	40.0		0.612***	0.522***	0.852***	0.253*
DPPH	21.2	37.5		0.395***	0.453***	0.135ns
PPO	3.9	27.2	15.6		0.464***	0.185ns
LEB	26.2	72.5	20.5	21.5		0.192ns
FFB	18.9	6.4	1.8	3.4	3.7	

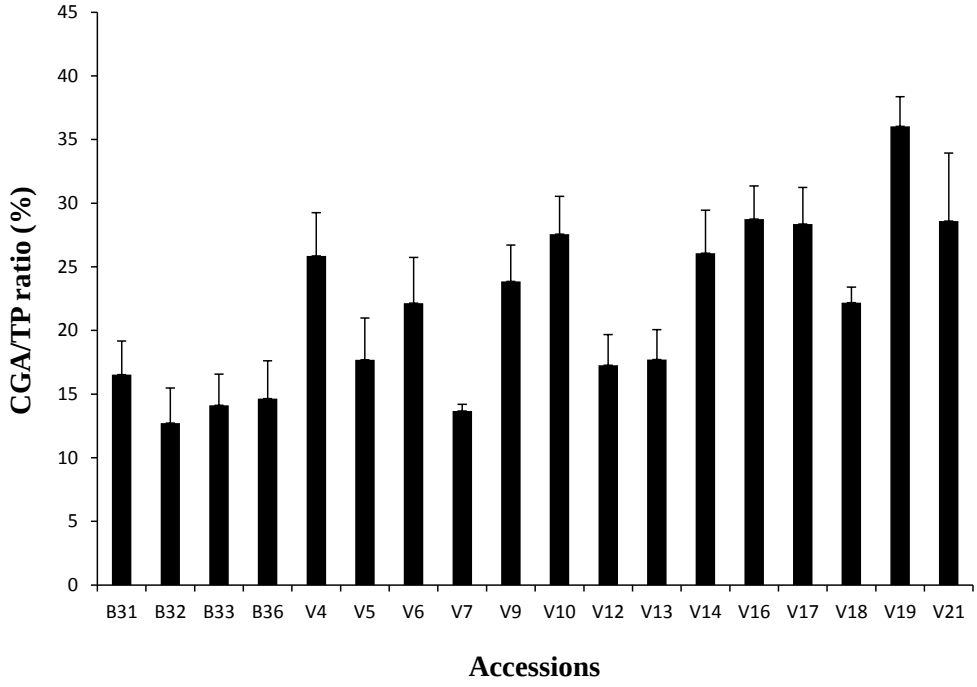

Figure 2. Mean values and standard error (SE) for the ratio between chlorogenic acid content and total phenolics content (GCA/TP ratio, %) in a collection of 18 eggplant accessions.

Environmental correlations values were mostly in agreement with the results obtained for phenotypic correlations. In this case, all correlations were also positive and the 11 pairwise environmental correlations that were significant for the phenotypic correlations, plus the correlation between liquid extract browning and fruit flesh browning were significant (Table 4). Also, as occurred for the phenotypic correlations, the highest value for the environmental correlation coefficient was between chlorogenic acid content and liquid extract browning (r=0.883) (Figure 3). The coefficient of determination (r^2) in this case reached a value of 78.0%. High values for the environmental coefficient of correlation were also found between total phenolics on one side and chlorogenic acid content (r=0.834) and extract browning (r=0.792) on the other, and also between cholorogenic acid content and DPPH scavenging activity (r=0.653). In these cases, the coefficients of determination for the correlation between these pairs of traits were of 69.6%, 62.7%, and 42.7%, respectively (Table 4). Similarly to what happened for the

phenotypic correlations, the fruit flesh browning presented low, although significant, values for the environmental correlation with total phenolics (r=0.304), chlorogenic acid content (r=0.253), and extract browning (r=0.319). Therefore, the coefficient of determination for the correlation between the degree of browning and the total phenolics, chlorogenic acid contents, and extract browning was, respectively, of 9.2%, 7.35, and 10.2%. As occurred with the phenotypic correlations, fruit flesh browning did not present a significant environmental correlation with PPO activity (Table 4).

Table 4. Environmental correlations (r; above the diagonal) and coefficients of determination (%, below the diagonal) for fruit traits (total phenolics, TP; cholorogenic acid,CGA; DPPH scavenging activity, DPPH; polyphenol oxidase activity, PPO; liquid extract browning, LEB; and, fruit flesh browning; FFB) in a collection of 18 eggplant accessions.

	TP	CGA	DPPH	PPO	LEB	FFB
TP		0.834^{***}	0.547^{***}	0.203^{ns}	0.792^{***}	0.304^{**}
CGA	69.6		0.653^{***}	0.458^{***}	0.883^{***}	0.270^{*}
DPPH	29.9	42.7		0.379^{***}	0.561^{***}	0.170^{ns}
PPO	4.1	21.0	14.3		0.439^{***}	0.159^{ns}
LEB	62.7	78.0	31.5	19.3		0.319^{**}
FFB	9.2	7.3	2.9	2.5	10.2	

Principal components analysis

The first and second components of the PCA accounted, respectively, for 41.1% and 31.2% of the total variation for the seven traits included in this study. The first component was positively correlated with all the traits included in the PCA analysis (Table 5). The highest values (>0.4) for the correlation of the first principal component were with chlorogenic acid content, PPO activity, and liquid extract browning. Also, moderate correlations (between 0.2 and 0.4) for this first component were found with DPPH scavenging activity, total phenolics, and fruit flesh browning. The positive correlation of this first component with CGA/TP ratio had a very low value (Table 5). The second component presented a high positive correlation with the CGA/TP ratio (0.640). A moderate positive correlation (0.291) was found with liquid extract browning. The positive correlations with chlorogenic acid content, and PPO activity were much lower (<0.2). This second component presented a high

negative correlation with total phenolics (-0.541), and also a moderate negative correlation with fruit flesh browning (-0.389) (Table 5). The negative correlation with DPPH scavenging activity was very low in absolute values (-0.129).

Table 5. Correlation coefficients for each fruit trait for the first and second principal components, eigenvalue, and relative and cumulative proportion of the total variance explained by these components, in a collection of 18 eggplant accessions.

	Common principal component coefficients	
Traits	Component 1	Component 2
Total phenolics (TP)	0.318	-0.541
Chlorogenic acid (CGA)	0.545	0.152
CGA/TP ratio	0.048	0.640
DPPH scavenging activity	0.361	-0.129
Polyphenol oxidase activity	0.485	0.148
Liquid extract browning	0.425	0.291
Fruit flesh browning	0.232	-0.389
Eigenvalue	3.397	1.505
Variance explained (%)	48.5	21.5
Cumulative variance explained (%)	48.5	70.0

The projection of the individual accessions in the PCA plot shows that they are widely spread over the graph area, with four to six accessions plotting in each of the quadrants (Figure 4). Accession V17, which presented very high values for the first component, is the accession that had the highest values for the three traits, i.e., chlorogenic acid content, PPO activity, and liquid extract browning (Table 2) that present a higher correlation with the first principal component. Also, accession V9, which presents the second highest value for the first component (Figure 4) is the accession that ranks second for chlorogenic acid content, third for PPO activity, and fourth for liquid extract browning (Table 2).

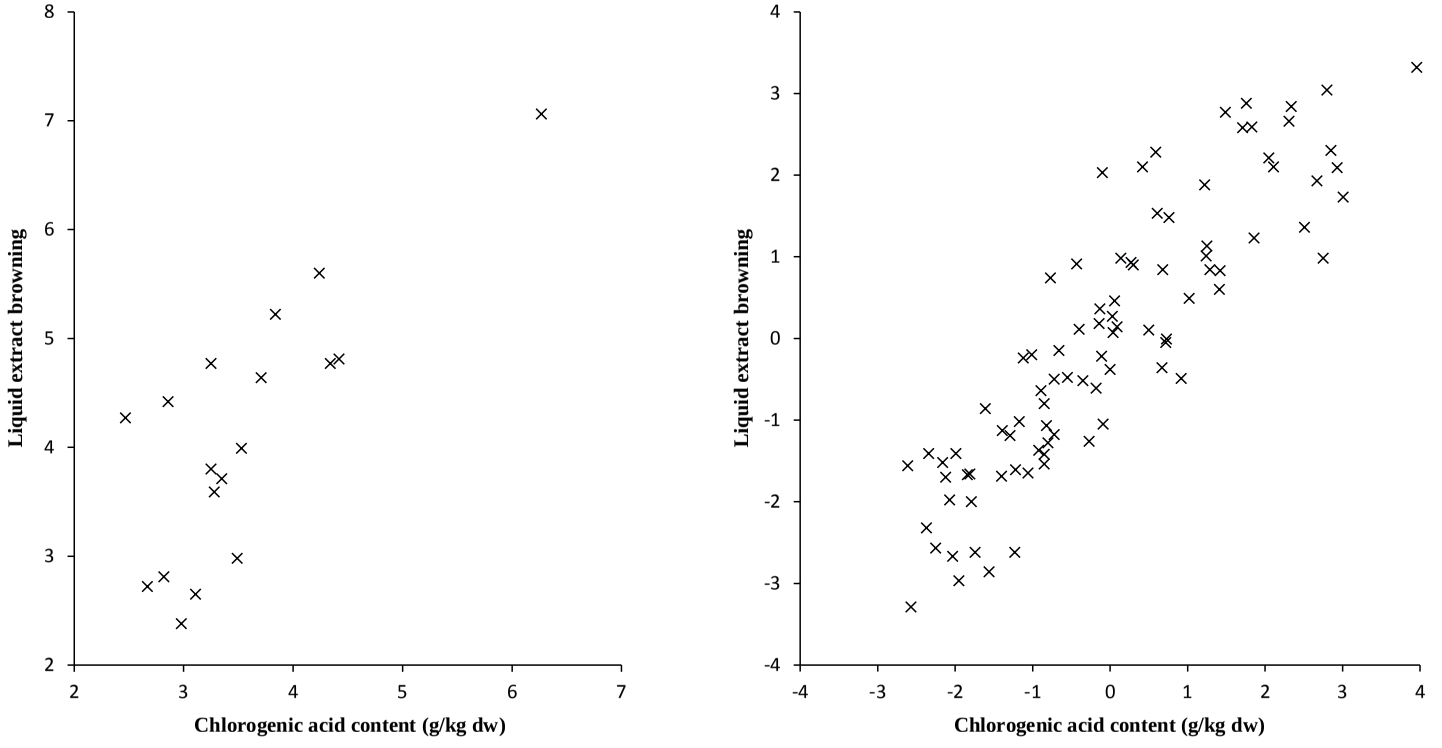

Figure 3. Phenotypic (left) and environmental (right) relationships between chlorogenic acid content (g/kg dw, *x*-axis) and liquid extract browning (*y*-axis) in a collection of 18 eggplant accessions with five samples per accession.

Similarly, the three accessions with lowest values for the first component (V16, V21, and V10) were also the ones with lowest values for chlorogenic acid content (Table 2). The two accessions with highest values for the second component (V19 and V21) were the ones with highest values for the CGA/TP ratio (highest positive correlation with this component) (Figure 2), and presented very low values for total phenolics (highest negative correlation with this component), ranking 15 and 18 respectively (Table 2). The two accessions with lowest values for the second component (V7 and B33) are among the lowest (rank 18 and 16, respectively) for the CGA/TP ratio (Figure 2), and presented high values (ranks 3 and 1, respectively) for the total phenolics (Table 2).

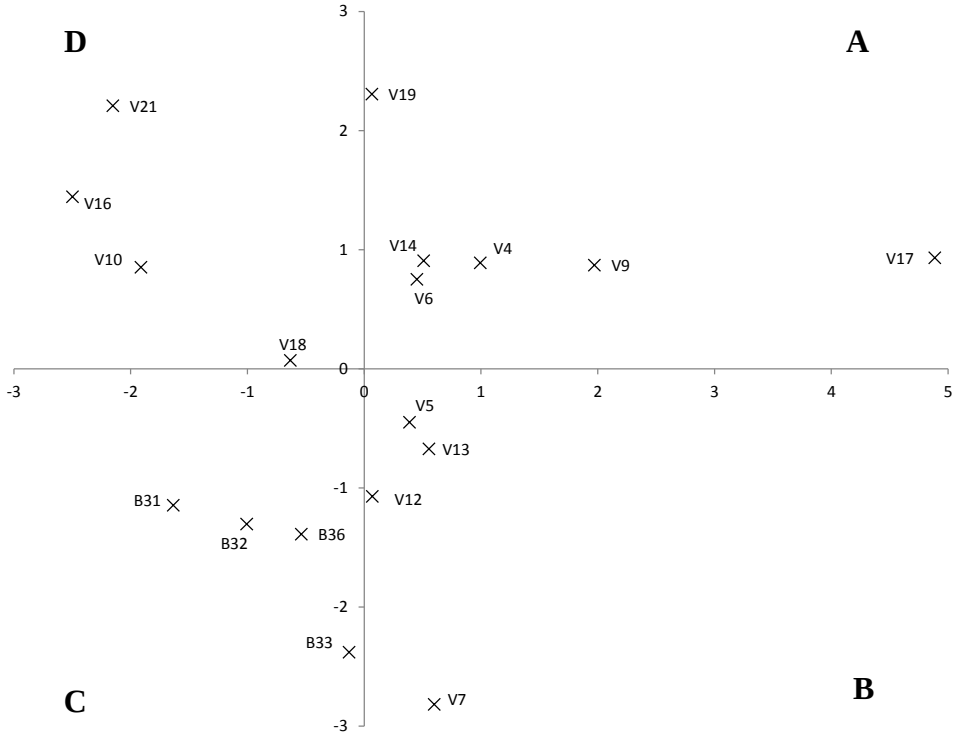

Figure 4. Similarities among the 18 eggplant accessions evaluated based on seven traits (total phenolics content (TP), chlorogenic acid content (CGA), CGA/TP ratio, DPPH scavenging activity, PPO activity, liquid extract browning, and fruit flesh browning) represented on the two first components of the principal components analysis (24.8% and 16.7% of the total variation, respectively). Accession group names (A, B, C, and D), which correspond to the accessions that plot in each quadrant, are indicated.

Differences between accession groups

The PCA analysis allows allocating the 18 accessions to four groups according to the PCA quadrant in which they plot. We have named these groups A (positive for both components), B (positive for the first component and negative for the second one), C (negative for both components), and D (negative for the first component and positive for the second one). Significant differences were detected among traits for total phenolics, chlorogenic acid content, chlorogenic/total phenolics ratio, PPO activity, and liquid extract browning (Table 6). No significant differences among groups were observed for DPPH scavenging activity and fruit flesh browning.

Table 6. Mean values for fruit traits (total phenolics, TP; cholorogenic acid, CGA; CGA/TP ratio, CGA/TP; DPPH scavenging activity, DPPH; polyphenol oxidase activity, PPO; liquid extract browning, LEB; and, fruit flesh browning; FFB) in the four groups of accessions established (A-D) by a multivariate principal components analysis in a collection of 18 eggplant accessions, and probability of the *F*-statistic, obtained from ANOVA analyses, for differences among means.

	Accession group				
	A	B	C	D	
n =	6	4	4	4	Prob. *F*
TP[a]	16.48 b	19.85 b	20.64 b	10.66 a	<0.001
CGA	4.47 b	3.31 a	3.15 a	2.80 a	0.003
CGA/TP	27.60 b	16.80 a	15.30 a	26.90 b	<0.001
DPPH	37.10 a	39.50 a	31.50 a	33.40 a	0.401
PPO	1.916 b	1.818 b	1.091 a	1.201 a	0.001
LEB	5.35 b	3.53 a	3.59 a	3.40 a	0.008
FFB	5.15 a	5.92 a	5.75 a	3.80 a	0.202

[a]Means separated by different letters within a row are significantly different according to the Student-Newman-Keuls multiple range test at P<0.05.

Assignation of low (-) or high (+) levels to each of the groups for the traits for which significant differences among groups were found allowed establishing a simplified profile for each of the groups (Table 7). The results obtained for individual accessions (Table 2) are largely in agreement with the results of the PCA classification. For example, group A, which is the only one

with high chlorogenic acid content and liquid extract browning values (Tables 6 and 7) includes the six accessions which rank first in chlorogenic acid content, and six of the seven accessions with highest values for liquid extract browning activity. Similarly, group D, which is the only one with low total phenolics content values (Tables 6 and 7) includes four of the accessions with lowest total phenolics content (Table 2). Groups B and C, which are classified as having high values for CGA/total phenolics (Tables 6 and 7) ratio include the 10 accessions with highest value for the CGA/total phenolics ratio (Figure 2), while groups C and D, which are characterized for high PPO activity include 10 out of the 11 accessions with highest PPO activity (Table 2).

Table 7. Simplified representation of the levels for the traits for which significant differences exist among groups of accessions, according to low (-) or high (+) levels, in the four groups of accessions established (A-D) by a multivariate principal components analysis in a collection of 18 eggplant accessions.

	Accession group			
Traits	A	B	C	D
Total phenolics (TP)	+	+	+	-
Chlorogenic acid (CGA)	+	-	-	-
CGA/TP ratio	+	-	-	+
PPO activity	+	+	-	-
Liquid extract browning	+	-	-	-

Discussion

Although the accessions we have studied come from a geographically limited region, a wide variation has been found for the traits studied. For the 18 accessions studied we have detected differences of up to 2.8-fold for total phenolics content and of up to 2.5-fold for chlorogenic acid content. These values are close to the maximum differences reported for germplasm collections for total phenolics (3-fold in a collection of 69 accessions) and chlorogenic acid content (4.4-fold in a collection of 96 accessions) (Stommel and Whitaker, 2003; Prohens *et al.,* 2007). This confirms the high diversity of eggplants from the Mediterranean region of Spain (Hurtado *et al.,* 2012; Prohens *et al.,* 2005; Muñoz-Falcón *et al.,* 2008).

Our results confirm that eggplant may represent a major dietary source of phenolic compounds (Plazas *et al.,* 2013). Development of new eggplant cultivars with enhanced content in phenolics, in particular of chlorogenic acid, is of interest. The wide range of variation for chlorogenic acid content may also have implications for breeding for improved resistance to fruit diseases and pests, as chlorogenic acid induces the expression of resistance genes (López-Gresa *et al.,* 2011). The inheritance of total phenolics content and chlorogenic acid content has not been studied in depth in eggplant. However, Prohens *et al*. (2007) found broad sense heritability values of 0.5 in a germplasm collection of eggplants. A subsequent study revealed a mostly additive mode of inheritance in an interspecific *S. melongena* × *S. aethiopicum* family (Prohens *et al.,* 2013). Mapping of genes and QTLs involved in the pathway of chlorogenic acid synthesis may provide tools (molecular markers) and relevant information for the breeding of eggplant cultivars with improved chlorogenic acid content (Plazas *et al.,* 2013).

We have found that the average value for the ratio between CGA and total phenolics is of 21.1%, which is somewhat lower than the values found by other authors (Mennella *et al.,* 2010; Gajewski *et al.,* 2009; Mishra *et al.,* 2013; Luthria *et al.,* 2010). Furthermore, the correlation coefficient between CGA and total phenolics found by us has been lower than that found in Luthria *et al*. (2010) and Mennella *et al*. (2012). Differences in the methodology of extraction, as well as among environmental conditions may account for these results (Mennella *et al.,* 2012; Luthria and Mukhopadhyay, 2006). Also, as found by Mennella *et al*. (2012) a considerable variation exists for the chlorogenic acid to total phenolics ratio among eggplant cultivars we have analyzed.

The wide variation for phenolics content has been matched by high values for variation in antioxidant activity. The moderate values obtained by us for the phenotypic correlation between total phenolics and CGA content on one side and DPPH scavenging activity on the other, are similar to those obtained by other authors between total phenolics and chlorogenic acid on one side and superoxide scavenging activity on the other (Mennella *et al.,* 2010; Mennella *et al.,* 2012; Hanson *et al.,* 2006), and between total phenolics and ABTS antioxidant activity (Okmen *et al.,* 2009). Singh *et al*. (2009) found

values much higher for the correlation between total phenolics and chlorogenic acid content on one side, and the inhibition of lipid conjugated diene formation on the other. However, these latter authors used only two varieties and their results are not comparable with the other studies.

Fruit flesh browning is a major issue to be taken into account when breeding for high content in phenolics in eggplant (Plazas *et al.,* 2013). PPO activity, liquid extract browning, and fruit flesh browning also presented a high degree of variation suggesting that eggplant materials are amenable to selection for these traits. The low phenotypic correlation values between fruit flesh browning on one side and total phenolics, chlorogenic acid content, DPPH scavenging activity, PPO activity, and liquid extract browning on the other may suggest that, although eggplant PPOs show a great affinity for chlorogenic acid (Fujita and Tono, 1988; Todaro *et al.,* 2011), several factors other than total phenolics and/or chlorogenic acid contents and PPO activity may have an influence in fruit flesh browning after the fruit has been cut (Mishra *et al.,* 2013). Cell and cell compartments size, morphology, and composition have been reported to have an important influence in fruit flesh browning (Toivonen and Brummell, 2008; Mishra *et al.,* 2013). In this respect, PPO enzymes are present in the plastids and phenolics in the vacuoles (Toivonen and Brummell, 2008; Mayer, 2006). Therefore, differences in: the amount of PPO and phenolics released from these cell compartments, their diffusion through the tissue, the accessibility of O_2 to phenolic compounds, the amounts of cell compounds that are able to react with the quinones resulting from the oxidation of phenolics to produce brown compounds, intracellular pH, or the concentration of other antioxidants that are able to prevent oxidation of phenolics, like ascorbic acid, may also have a main role in fruit flesh browning in eggplant (Todaro *et al.,* 2011; Doğan *et al.,* 2002; Concellón *et al.,* 2012; Barbagallo *et al.,* 2012; Mishra *et al.,* 2013). These factors would have less relevance for liquid extract browning, in which the whole tissue is disintegrated and phenolics and PPO oxidases are released in the solution.

The low phenotypic correlations of fruit flesh browning with total phenolics, chlorogenic acid content, DPPH scavenging activity, and PPO activity are of interest for the development of cultivars that have a high content in total phenolics and/or chlorogenic acid and that at the same time present a

low or moderate fruit flesh browning. In this respect, only 18.9% of the total variation for fruit flesh browning is caused by the differences in total phenolics. The results that we have found are in agreement with those of Prohens *et al*. (2007), who in a collection of 69 *S. melongena* accessions found that the variation in fruit flesh browning accounted by the content in total phenolics was of 15.1%. Similarly, Prohens *et al*. (2013) in an interspecific family between *S. melongena* and *S. incanum* found that only 6.0% of the variation in fruit flesh browning found in the F2 generation was accounted by variation in chlorogenic acid content. These results are largely in agreement with those of Mishra *et al*. (2013) in which when eight eggplant accessions were compared before being subjected to storage, there was a moderate correlation between PPO activity and fruit flesh browning. Low or moderate values of correlation between fruit flesh browning and PPO activity have also been reported in other crops (CoSeteng and Lee, 1987; Radi *et al.,* 1997). In any case, several studies have shown that inhibition of PPO activity in eggplant reduces browning (Barbagallo *et al.,* 2012; Barbagallo *et al.,* 2012; Massolo *et al.,* 2011; Mishra *et al.,* 2013; Hu *et al.,* 2010). However, lack of correlation of PPO with fruit flesh browning may suggest that PPO activity levels in the materials we have studied are not a limiting factor to produce fruit flesh browning. This does not preclude that selection of eggplant varieties with highly reduced PPO activity might have decreased levels of fruit flesh browning.

The high phenotypic correlation value between chlorogenic acid content and DPPH scavenging activity (r=0.612) confirms that chlorogenic acid is a key compound for the functional activity of eggplant fruit (Plazas *et al.,* 2013). Also, a high correlation value (r=0.852) was found between chlorogenic acid content and liquid extract browning, measured at 420 nm, which suggests that the measurement of liquid extract browning could be a rapid method for the evaluation for chlorogenic acid content in large samples of material, like collections of germplasm or segregating generations in breeding programs. Luthria (2012) suggested the use of a simplified UV spectral scan method, in which measurements of methanolic extracts obtained from lyophilized tissue were made at 330 nm, for the rapid determination of chlorogenic acid content in eggplant. Measurement of liquid extract browning may be an even simpler method as it does not require extraction with methanol.

Few studies have been made to evaluate the environmental effects on the traits measured by us (Mennella *et al.,* 2010; Hanson *et al.,* 2006; Raigón *et al.,* 2010). These works show that the environment has considerable effect on the content in phenolics and on antioxidant activity. However, no reports are known to us on the study of environmental correlations between these traits in eggplant. Here, we have shown that there are important environmental correlations between several traits, suggesting that environment may have a relevant role in the observed phenotypic correlations. Some of these environmental correlations were expected, as those between total phenolics, chlorogenic acid, DPPH activity, and liquid extract browning, as these traits may have a common physiological and genetic basis. However, it is remarkable that positive environmental correlations also exist between PPO and CGA, suggesting that environmentally induced increases in CGA content may activate the expression of PPO genes in eggplant fruit (López-Gresa *et al.,* 2011; Mayer, 2006).

The multivariate PCA analysis has allowed the identification of four groups of accessions with different profiles for the traits studied. These four groups present significant differences for five out of seven traits studied, which shows that multivariate studies may be of great utility for classification of eggplant accessions according to their phenolics, antioxidant activity and browning profile (Raigón *et al.,* 2010). Mishra *et al*. (2013) found two different profiles in the postharvest evolution of PPO activity in a set of eight eggplant accessions. In one of the groups, PPO activity increased during postharvest storage, while in the other it decreased (Mishra *et al.,* 2013). Each of the four groups established by us included accessions with different morphological characteristics showing that a wide diversity for the traits studied exists within cultivar groups (Okmen *et al.,* 2009; Mennella *et al.,* 2012). This suggests that it is possible to find, within a single cultivar group, accessions with different profile for phenolics content, PPO activity, and liquid extract browning. The identification of these groups may be of interest for the study of the genetic regulation of phenolics content, PPO activity, and browning, for physiology studies, and for identifying adequate sources of variation for breeding in a specific cultivar group of eggplant.

Within the collection studied, we have identified some accessions with interest for selection and breeding for high content in chlorogenic acid and antioxidant activity, and low fruit flesh browning. For example, among the 18 accessions evaluated, accession V17 presents very high values for chlorogenic acid content (ranks first, with values 41% greater than the variety ranking second) and also presents high values for total phenolics (ranks second), and DPPH scavenging activity (ranks second); however, it also has a high fruit flesh browning (ranks first). Also, accession V9 presents a high content in chlorogenic acid (ranks second), has a high DPPH scavenging activity (ranks first), and a low fruit flesh browning (ranks sixteenth). Inclusion of accessions presenting high values for chlorogenic acid content or an adequate combination of functional activity and apparent quality traits of interest in breeding programs could be of great interest for developing new cultivars with high added value (Plazas *et al.,* 2013).

In conclusion, we have found that an important variation exists among eggplant accessions for traits related to phenolics content and browning. In the set of accessions studied, PPO activity does not seem to be a limiting factor for fruit flesh browning. Low correlation values between phenolics content and fruit flesh browning suggests that it is feasible to identify eggplant materials with improved bioactive properties and low fruit flesh browning. The identification of four different groups of accessions according to the profile for phenolics content, antioxidant activity, and liquid extract browning allows the classification of eggplant materials for specific combinations of these characteristics. Overall, our results indicate that there are good prospects for the selection and genetic improvement of eggplant in order to obtain new varieties with improved functional (phenolics content, in particular chlorogenic acid) and apparent (low fruit flesh browning) quality.

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3.2 Diversidad en berenjenas escarlata y gboma para una mejora integral: caracteres morfoagronómicos y compuestos bioactivos

3.2.1 Conventional and phenomics characterization provides insight into the diversity and relationships of hypervariable scarlet (*Solanum aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplant complexes

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crop complexes, cultivar groups, descriptors, phenomics, *Solanum aethiopicum*, *Solanum marcrocarpon*, Tomato Analyzer

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Abstract

Scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplants are major vegetable crops in sub-Saharan Africa. Together with their respective wild ancestors (*S. anguivi* and *S. dasyphyllum*) and intermediate cultivated-wild forms they constitute the so-called scarlet and gboma eggplant complexes. We used conventional descriptors and the high-throughput phenomics tool Tomato Analyzer for characterizing 63 accessions of the scarlet eggplant complex, including the four *S. aethiopicum* cultivar groups (Aculeatum, Gilo, Kumba, and Shum), Intermediate *S. aethiopicum*-*S. anguivi* forms, and *S. anguivi*, and 12 cultivated and wild accessions of the gboma eggplant complex. A large diversity was found between both complexes, showing that they are very well differentiated from each other. Within the scarlet eggplant complex, many significant differences were also found among cultivar groups, but more differences were found for fruit traits evaluated with Tomato Analyzer than with conventional descriptors. In particular, Tomato Analyzer phenomics characterization was useful for distinguishing small fruited groups (Shum, Intermediate, and *S. anguivi*), as well as groups for which few or no significant differences were observed for plant traits. Multivariate principal components analysis (PCA) separated well all groups, except the Intermediate group which plotted between *S. anguivi* and small fruited *S. aethiopicum* accessions. For the gboma eggplant complex, *S. dasyphyllum* was clearly distinguished from *S. macrocarpon* and an important diversity was found in the latter. The results have shown that both complexes are hypervariable and have provided insight into their diversity and relationships. The information obtained has important implications for the conservation and management of genetic resources as well as for the selection and breeding of both scarlet and gboma eggplants.

Introduction

The scarlet (*Solanum aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplants are two cultivated African vegetable crops locally important in its region of origin in tropical sub-Saharan Africa (Lester *et al*., 1990; Schippers, 2000; Lester and Daunay, 2003; Maundu *et al*., 2009). Scarlet eggplant is, together with tomato, onion, pepper and okra, one of the five most important vegetables in Central and West Africa (Schippers, 2000; Maundu *et al*., 2009). Gboma eggplant is in general less important than scarlet eggplant, although in

some areas like in Benin and in the rain forest regions of Coastal Africa and Congo River, is one of the major vegetables (Lester *et al*., 1990; Dansi *et al*., 2008). Cultivation of both species is mostly restricted to Africa, but *S. aethiopicum* is also cultivated in the Caribbean and Brazil (Schippers, 2000), where it was probably brought by slaves, as well as in some areas of the south of Italy (Sunseri *et al*., 2010). Both scarlet and gboma eggplants are also important genetic resources for common eggplant (*S. melongena* L.) breeding, as the three species can be intercrossed giving hybrids with intermediate fertility (Daunay *et al*., 1991; Oyelana and Ugborogho, 2008; Prohens *et al*., 2012; Khan *et al*., 2013). Scarlet eggplant and its interspecific hybrids with *S. melongena* are also used as rootstocks for eggplant cultivation (Gisbert *et al*., 2011).

Within the genus *Solanum*, *S. aethiopicum* belongs to section Oliganthes (Lester, 1986; Lester and Niakan, 1986), while *S. macrocarpon* to section Melongena (Lester *et al*., 1990; Lester and Daunay, 2003; Lester *et al*., 2011). *Solanum aethiopicum* is a hypervariable species (i.e., characterized by many types and forms morphologically different), with hundreds of local varieties (Lester *et al*., 1986). Such morphological variability has resulted in about 20 different scientific names through the taxonomic history of this crop (Lester, 1986). Lester (1986) recognized four cultivar groups, namely Aculeatum, Gilo, Kumba, and Shum. The four cultivar groups of *S. aethiopicum* are completely interfertile (Lester and Niakan, 1986) and, although historically they have been treated as distinct species by several authors, it is generally accepted that they form part of a single species (Lester *et al*., 1986, 2011; Edmonds, 2012). Classification of accessions to their cultivar group can be made using a simple classification key (Lester *et al*., 1986). Regarding the utilization of each cultivar group, Aculeatum is used as an ornamental, Gilo for its fruits, Kumba for both fruits and leaves, and Shum for its leaves (Lester, 1986; Schippers, 2000; Lester and Daunay, 2003). The wild ancestor of *S. aethiopicum* is *S. anguivi* (Lester and Niakan, 1986). Hybrids between *S. aethiopicum* and *S. anguivi* Lam. are fully fertile (Lester and Niakan, 1986; Lester and Thitai, 1989). *Solanum aethiopicum* together with *S. anguivi* and their intermediate forms constitute the scarlet eggplant complex.

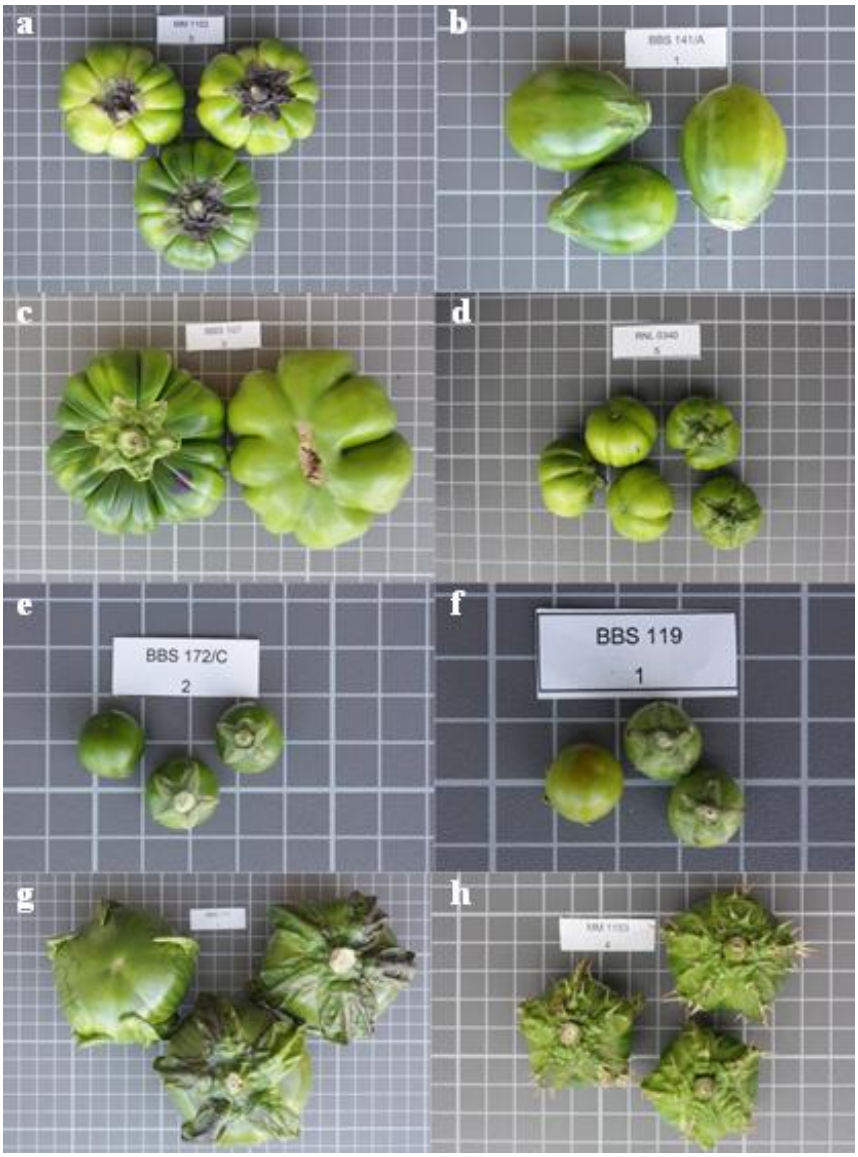

Figure 1. Representative fruits of each of the scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*) and gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*) groups evaluated. Groups include *S. aethiopicum* groups Aculeatum (A), Gilo (B), Kumba (C), Shum (D), Intermediate beween *S. aethiopicum* and *S. anguivi* (E), *S. anguivi* (F), *S. macrocarpon* (G), and *S. dasyphyllum* (H). Fruits are not depicted at the same scale; the size of the grid cells is 1 cm x 1 cm.

S. macrocarpon is also hypervariable in morphology, although to a lesser extent than *S. aethiopicum* (Lester and Daunay, 2003). Depending on the

cultivar, *S. macrocarpon* is cultivated for its fruits, leaves or both (Schippers, 2000; Lester and Daunay, 2003; Maundu *et al*., 2009). *Solanum marcrocarpon* was domesticated from the wild *S. dasyphyllum* Schum. and Thonn. (Bukenya and Carasco, 1994). Both species are fully interfertile and together with their weedy intermediate forms for the gboma eggplant complex (Bukenya and Carasco, 1994).

Morphological characterization using conventional descriptors has proved useful for describing and establishing relationships among cultivar groups and accessions in scarlet and gboma eggplants (Lester *et al*., 1986; Polignano *et al*., 2010; Sunseri *et al*., 2010; Adeniji *et al*., 2012, 2013). These works have mostly focused on scarlet eggplant, revealing that it is a highly variable crop. The most comprehensive study was performed by Lester *et al*. (1986) who characterized 108 accessions of the scarlet eggplant complex using morphological and taxonomically relevant traits and found that the four cultivar groups could be distinguished by a syndrome of characteristics (i.e., a set of characteristics that are observed in a single group). These authors also found some accessions which were intermediate between *S. anguivi* and *S. aethiopicum* (Lester *et al*., 1986). The rest of characterization works (Polignano *et al*., 2010; Sunseri *et al*., 2010; Adeniji *et al*., 2012, 2013) involved fewer accessions and were based on morphological and agronomic descriptors. These latter studies found some degree of differentiation among the four *S. aethiopicum* groups, but considerable overlapping among groups was found. Many fewer studies have been devoted to the diversity of gboma eggplant. Polignano *et al*. (2010) evaluated 16 accessions of *S. macrocarpon* and found that it was a variable crop, which presented a continuous variation for the morphological diversity.

Modern phenomics tools may also be useful for precise characterization and for studying the diversity and relationships in collections of genetic resources (Furbank and Tester, 2011). In this respect, the high-throughput phenomics software tool Tomato Analyzer, which was initially developed for fruit shape analysis in tomato (Brewer *et al*. 2006, 2007; Gonzalo and van der Knaap 2008, Rodríguez *et al*. 2010), has also proved useful for the detailed and accurate characterization of eggplant accessions (Hurtado *et al*., 2013) as well as for segregating generations between *S.*

melongena and *S. aethiopicum* (Prohens *et al*., 2012). Tomato Analyzer allows scoring a large number of fruit shape traits from scanned images of fruit sections and is a powerful tool for precise description of fruit morphology. Therefore, Tomato Analyzer may be useful for the fruit shape characterization of germplasm collections of scarlet and gboma eggplants. Furthermore, fruit shape is considered as a very important trait in the preferences of farmers in selecting a variety of scarlet or gboma eggplant (Adeniji and Aloyce, 2012) and in consequence is a trait of major importance in these two crops, especially in varieties used for their fruits.

In this work, we characterize a collection of accessions of the scarlet and gboma eggplants complexes using conventional and phenomics (Tomato Analyzer) descriptors. The objective is to provide phenotypic information of relevance on the diversity and relationships of the two crops and their cultivar groups. This information will be useful for the classification, management of genetic resources, selection and breeding of both crops.

Materials and methods

Plant material

Sixty-three accessions of the scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*) and 12 accessions of the gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*) from the germplasm bank of the Universitat Politècnica de València (València, Spain) were used for the present study. The scarlet eggplant complex accessions were classified according to the key to taxa of *S. aethiopicum* and *S. anguivi* established by Lester *et al*. (1986), which includes *S. aethiopicum* groups Aculeatum, Gilo, Kumba, and Shum, and *S. anguivi* (Figure 1). Accessions that could not be allocated to any of the groups, as they shared intermediate characteristics between *S. aethiopicum* and *S. anguivi* were assigned to a group denominated Intermediate. Gboma eggplant complex accessions were classified as *S. macrocarpon* or *S. dasyphyllum* according to the key of Lester *et al*. (2011) (Figure 1).

Characterization

Individual plants were characterized using 18 plant descriptors commonly used for cultivated eggplant species and wild relatives

characterization (IBPGR, 1990; Prohens *et al*., 2005; van der Weerden and Barendse, 2007; Polignano *et al*., 2010; Prohens *et al*., 2012). Plant descriptors include traits related to whole plant (5), leaves (7), and inflorescences and flowers (6) (Table 1). Seven plant descriptors are metric, four are meristic (traits in which the number of parts or components are counted), and seven are measured in a quantitative scale. For each individual plant, several commercially ripe (i.e., physiologically immature, see Figure 1) fruits were weighted, manually measured for length and breadth, and longitudinally cut and scanned with an HP Scanjet G4010 photo scanner (Hewlett-Packard, Palo Alto, CA, USA) at a resolution of 300 dpi and subjected to morphometric analysis with Tomato Analyzer version 3 software (Rodríguez *et al*. 2010). Data were recorded for a total of 27 fruit descriptors (Table 1), of which three were manually measured (Weight, Length, Breadth) and 24 were automatically obtained with Tomato Analyzer, including basic (6), fruit shape index (2), blockiness (3), homogeneity (3), proximal fruit end shape (1), distal fruit end shape (1), asymmetry (3), and internal eccentricity (5) descriptors. Nine fruit traits had units and 18 were unitless. All fruit descriptors were metric. Default settings were used for blockiness and proximal fruit end shape and distal fruit end shape descriptors (Rodríguez *et al*. 2010). A complete description of these traits can be found elsewhere (Rodríguez *et al*. 2010, Prohens *et al*. 2012; Hurtado *et al*., 2013).

Data analyses

The mean, range, and coefficient of variation (CV) values for plant and fruit descriptors were calculated for each of the scarlet eggplant and gboma eggplant complexes. Two-tailed *t* tests were performed on mean values for each descriptor in order to study signification of differences between means of scarlet and gboma eggplant complexes (Little and Hills, 1978). Analyses of variance (ANOVA) tests for each of the scarlet eggplant and gboma eggplant complexes were performed on plant and fruit values to detect differences among groups within each complex. For descriptors in which the mean was proportional to standard deviation, log transformed data were used for the ANOVA tests in order to avoid scaling effects (Little and Hills, 1978). Significant ($P<0.05$) differences among group means were detected using the Student-Newman-Keuls multiple range test. No corrections were performed for controlling type I error (false positives) derived from multiple testing (Snedecor

and Cochran, 1989). The number of significant differences between pairs of both scarlet eggplant and gboma eggplant groups means for plant and fruit descriptors were calculated. Principal components analysis (PCA) were performed using pairwise Euclidean distances among accession means.

Results

Diversity and differences between scarlet and gboma eggplant complexes

The morphological characterization of scarlet and gboma eggplant complexes revealed that the collection studied was phenotypically very diverse (Figure 2 and Figure 3). Both scarlet and gboma eggplant complexes displayed considerable diversity for most plant and fruit descriptors (Table 2). For plant traits measured in a scale, in both complexes the range of variation covers all or most of the scale range, with the exception of Corolla Colour, which in the scarlet eggplant complex presents low values of the scale, while in the gboma eggplant complex it presents intermediate-high values (Table 2). For the rest of plant traits, the range of variation was broad in both complexes. For both complexes, the largest values of CV were found for the two anthocyanin intensity and the two prickliness traits, with values always above 100% and up to 328.6% for Length of Largest Prickle in the scarlet eggplant complex (Table 2). Also, in both complexes the traits with lowest CV values were the Number of Sepals, Number of Petals, and Number of Stamens, with CV values always below 15%. For 12 out of the 18 plant traits, the CV value was higher in the scarlet eggplant complex than in the gboma eggplant complex (Table 2).

Table 1. Plant and fruit descriptors used for the characterization of a collection of scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*) and gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*) accessions.Full details of the descriptors can be consulted elsewhere (IBPGR, 1990; Prohens *et al*., 2005; Brewer *et al*., 2006, 2007; van der Weerden and Barendse, 2007; Darrigues *et al*., 2008; Gonzalo and van der Knaap, 2008; Rodríguez *et al*., 2010). Thirty-four descriptors are metric, four are meristic, and seven are measured in a quantitative scale. The two latter are indicated by an "M" and a "Q", respectively in their description.

Descriptors	Units/scale/description
Plant descriptors	
Plant Height	cm
Hypocotyl Anthocyanins Intensity	(S) 0=Absent; 9=Very strong
Shoot Tip Anthocyanins Intensity	(S) 0=Absent; 9=Very strong
Stem Diameter	cm
Angle Between Main Branches	(S) 1=<40º; 5>50º
Leaf Pedicel Length	cm
Leaf Blade Length	cm
Leaf Blade Breadth	cm
Leaf Blade Lobing	(S) 1=Very weak; 9=Very strong
Leaf Surface Shape	(S) 1=Flat; 9=Very convex or bullate
Leaf Prickles	(S) 0=None; 9=Very many (>20)
Length of Largest Leaf Prickle	cm
Number of Flowers per Inflorescence	(M) ---
Corolla Colour	(S)1=Greenish white; 9=Bluish violet
Number of Sepals	(M) ---
Number of Petals	(M) ---
Number of Stamens	(M) ---
Corolla Diameter	mm
Fruit descriptors	
Weight	g
Length	cm
Breadth	cm
Perimeter	cm
Area	cm^2
Width Mid-height	The width measured at ½ of the fruit's height (cm)
Maximum Width	The maximum horizontal distance of the fruit (cm)
Height Mid-width	The height measured at ½ of the fruit's width (cm)
Maximum Height	The maximum vertical distance of the fruit (cm)
Fruit Shape Index External I	The ratio of the Maximum Height to Maximum Width

Fruit Shape Index External II	The ratio of the Height Mid-width to Width Mid-height
Proximal Fruit Blockiness	Ratio of the width at the upper blockiness position to Width_MH
Distal Fruit Blockiness	Ratio of the width at the lower blockiness position to Width_MH
Fruit Shape Triangle	Ratio of the width at the upper blockiness position to the lower blockiness position
Ellipsoid	The ratio of the error resulting from a best-fit ellipse to the area of the fruit; smaller values indicate that the fruit is more ellipsoid
Circular	The ratio of the error resulting from a best-fit circle to the area of the fruit; smaller values indicate that the fruit is more circular
Rectangular	The ratio of the rectangle bounding the fruit to the rectangle bounded by the fruit
Shoulder Height	The ratio of the average height of the shoulder points above the proximal end point to Maximum Height
Distal End Protrusion	Ratio of the area of the distal protrusion to the total area of the fruit, multiplied by 10
Obovoid	Calculated according to the formula provided in the tomato Analyzer Manual (Rodríguez *et al*. 2010). The higher the value, the greater is the area of the fruit below mid height
Ovoid	Calculated according to the formula provided in the tomato Analyzer Manual (Rodríguez *et al*. 2010). The higher the value, the greater is the area of the fruit above mid height
Width Widest Pos	The ratio of the height at which the Max_Width occurs to the Max_Height
Eccentricity	The ratio of the height of the internal ellipse to the Maximum Height
Proximal Eccentricity	The ratio of the area of the height of the internal ellipse to the distance between the bottom of the ellipse and the top of the fruit
Distal Eccentricity	The ratio of the area of the height of the internal ellipse to the distance between the bottom of the ellipse and the bottom of the fruit
Fruit Shape Index Internal	The ratio of the internal ellipse's height to its width
Eccentricity Area Index	The ratio of the area of the fruit outside the ellipse to the total area of the fruit

As occurred for the plant descriptors, a wide diversity was found for most fruit traits within each of the scarlet and gboma eggplant complexes (Table 2). In particular, for the nine fruit size traits evaluated (Weight to Maximum Height) the ranges of variation were very large. For example Weight, ranged between 1 g and 351 g in the scarlet eggplant complex and between 22 g and 177 g in the gboma eggplant complex. For the unitless fruit shape traits in most cases an important variation was found in both complexes, although in some cases (e.g., Proximal Eccentricity and Distal Eccentricity in the gboma eggplant complex) the range of variation was very limited (Table 2). CV values of 100% or larger were found for Weight, Ellipsoid, Shoulder Height, and Distal End Protrusion in the scarlet group and for Distal End Protrusion and Obovoid for the gboma eggplant complex. In both complexes, the lowest CV values were found for Proximal Eccentricity and Distal Eccentricity, with values for both traits of 0.0% in the gboma eggplant complex and as low as 1.2% and 2.3%, respectively, in the scarlet eggplant complex. For all fruit traits, with the exception of Obovoid, the CV was larger for the scarlet eggplant complex than for the gboma eggplant complex (Table 2).

Table 2. Mean, range and coefficient of variation (CV; %) for the plant and fruit descriptors studied in the scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*) and gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*) accessions, and significance of the differences between complex means.

	Scarlet eggplant (n=63)			Gboma eggplant (n=12)		
Descriptors[a]	Mean	Range	CV	Mean[b]	Range	CV
Plant descriptors						
Plant Height (cm)	148	74-208	20.3	95***	50-129	26.3
Hypocotyl Anthocyanins Intensity	1.44	0.00-9.00	195.8	1.00^{ns}	0.00-7.00	209.0
Shoot Tip Anthocyanins Intensity	1.52	0.00-9.00	180.3	1.17^{ns}	0.00-7.00	199.1
Stem Diameter (cm)	2.77	1.50-4.20	23.1	2.66^{ns}	2.00-3.25	15.0
Angle Between Main Branches	2.65	1.00-5.00	35.8	3.84***	1.00-5.00	34.9
Leaf Pedicel Length (cm)	5.66	2.33-12.80	40.3	1.59***	0.53-2.67	56.0
Leaf Blade Length (mm)	21.9	9.67-34.2	20.0	33.3***	26.2-40.0	14.2
Leaf Blade Breadth (cm)	16.7	7.0-31.0	24.6	20.0*	14.8-25.0	19.5
Leaf Blade Lobing	4.40	1.00-7.40	25.0	6.13***	4.20-9.00	26.3
Leaf Surface Shape	2.59	1.00-5.80	74.9	5.00***	1.00-9.00	34.2

Leaf Prickles	0.48	0.00-6.60	322.9	1.66*	0.00-9.00	163.3
Length of Largest Leaf Prickle (cm)	0.14	0.00-2.06	328.6	0.42^{ns}	0.00-1.45	131.0
Number of Flowers per Inflorescence	3.34	1.00-12.6	83.5	3.66^{ns}	1.20-5.40	36.9
Corolla Colour	2.81	1.00-3.50	25.6	6.00***	5.00-7.00	17.3
Number of Sepals	5.66	5.00-7.00	9.5	5.27*	5.00-6.00	7.2
Number of Petals	5.62	5.00-8.00	10.7	5.19*	5.00-6.00	7.3
Number of Stamens	5.98	5.00-8.00	14.9	5.29*	5.00-6.00	8.1
Corolla Diameter (mm)	19.2	11.1-33.3	21.4	38.0***	23.5-55.3	23.9
		Fruit descriptors				
Weight (g)	48	1-351	147.9	111**	22-177	43.2
Length (cm)	3.59	1.10-7.65	36.2	4.99***	2.97-6.70	19.6
Breadth (cm)	4.42	1.16-11.14	50.0	6.66**	3.83-8.46	20.6
Perimeter (cm)	13.9	3.9-30.0	41.0	20.2***	11.9-25.2	20.8
Area (cm^2)	13.0	1.1-40.3	66.9	27.1***	9.3-41.4	37.6
Width Mid-height (cm)	4.20	1.21-9.72	45.7	6.55***	3.79-8.44	23.1
Maximum Width (cm)	4.24	1.21-9.90	46.2	6.60***	3.81-8.49	23.0
Height Mid-width (cm)	3.18	1.07-7.06	39.6	4.58***	2.94-6.79	23.6
Maximum Height (cm)	3.58	1.10-7.17	36.0	4.96***	3.03-6.92	23.0
Fruit Shape Index External I	0.93	0.54-1.89	36.6	0.77^{ns}	0.68-1.34	23.4
Fruit Shape Index External II	0.87	0.32-1.92	46.0	0.72^{ns}	0.60-1.32	26.4
Proximal Fruit Blockiness	0.66	0.46-0.79	12.1	0.74***	0.68-0.80	5.4
Distal Fruit Blockiness	0.64	0.39-0.76	12.5	0.62^{ns}	0.57-0.65	3.2
Fruit Shape Triangle	1.07	0.69-1.76	18.7	1.20*	1.07-1.32	6.7
Ellipsoid	0.07	0.02-0.33	100.0	0.05^{ns}	0.03-0.07	20.0
Circular	0.14	0.02-0.38	64.3	0.15^{ns}	0.08-0.28	33.3
Rectangular	0.52	0.44-0.61	7.7	0.52^{ns}	0.46-0.55	5.8
Shoulder Height	0.03	0.00-0.14	133.3	0.03^{ns}	0.01-0.05	33.3
Distal End Protrusion	0.02	0.00-0.17	150.0	0.01^{ns}	0.00-0.02	100.0
Obovoid	0.07	0.00-0.21	71.4	0.01***	0.00-0.05	100.0
Ovoid	0.08	0.00-0.23	75.0	0.15***	0.08-0.19	20.0
Width Widest Pos	0.49	0.39-0.59	8.2	0.45**	0.42-0.49	4.4
Eccentricity	0.71	0.44-0.79	14.1	0.74^{ns}	0.69-0.78	4.1
Proximal Eccentricity	0.89	0.81-0.96	1.2	0.89^{ns}	0.89-0.89	0.0
Distal Eccentricity	0.88	0.77-0.90	2.3	0.89^{ns}	0.88-0.89	0.0
Fruit Shape Index Internal	0.86	0.31-1.60	44.2	0.72^{ns}	0.60-1.32	26.4
Eccentricity Area Index	0.44	0.34-0.61	15.9	0.43^{ns}	0.38-0.46	4.7

[a]See Table 1 for definition of descriptors.[b] ***, **, *, ^{ns} indicate, respectively, significant at P<0.001, P<0.01, P<0.05, or non-significant differences between scarlet and gboma eggplant complexes means, according to a two-tailed *t* test.

Figure 2. Diversity among accessions of scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*) in the evaluated collection. Red fruits are physiologically mature.

Despite the wide diversity found within each of the scarlet eggplant and gboma eggplant complexes, many morphological significant ($P<0.05$) differences existed for mean values between both complexes (Table 2). In this respect, when considering plant traits, on average, scarlet eggplants had plants that were taller (Plant Height), less erect (Angle Between Main Branches), with smaller leaf blade (Leaf Blade Length and Leaf Blade Breadth), less lobed leaves (Leaf Blade Loging), flatter leaf surface (Leaf Surface Shape), less prickly leaves (Leaf Prickles), greater number of flower parts (Number of Sepals, Number of Petals, and Number of Stamens), smaller flowers (Corolla Diameter), and longer leaf pedicel (Leaf Pedicel Length) than gboma eggplants (Table 2). Regarding fruit traits, the scarlet eggplant complex fruits were, on average, smaller (lower values for the nine fruit size traits), less blocky in the proximal part (Proximal Fruit Blockiness), less triangular (Fruit Shape Triangle), more obovoid (and less ovoid), and with highest values for the ratio of the height at which the maximum width occurs (Width Widest Pos) than the gboma eggplant complex fruits (Table 2).

Figure 3. Diversity among accessions of gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*) eggplant in the evaluated collection. Yellow and brown fruits are physiologically mature.

Differences and relationships among scarlet eggplant groups

Significant ($P<0.05$) differences were found among the six scarlet eggplant complex groups for 15 out of the 18 plant traits evaluated (Table 3). The only exceptions were Stem Diameter, Leaf Blade Lobing, and Corolla Colour. The number of significant differences between means of the scarlet eggplant complex groups for the 18 plant morphological traits evaluated range from 0 (between Gilo and Shum on one side and Intermediate and *S. anguivi* on the other) and 8 (between Aculeatum and Shum) (Table 4). Few differences in plant traits were also found between group Gilo on one side and groups Kumba, Intermediate and *S. anguivi* on the other, as well as between groups Shum, Intermediate and *S. anguivi* (Table 4). Among the most relevant differences found among scarlet eggplant complex groups for plant traits average values, plants of group Gilo were taller than those of group Kumba, group Aculeatum had higher anthocyanin content and prickliness than the

other groups, groups Aculeatum, Intermediate and *S. anguivi* had more flowers per inflorescence than groups Gilo, Kumba and Shum, group Kumba had larger flowers and higher number of flower parts than groups Shum, Intermediate and *S. anguivi*, and larger leaves than groups Shum and *S. anguivi* (Table 3).

For fruit traits, significant ($P<0.05$) differences were found among the six scarlet eggplant complex groups for 24 out of the 27 fruit traits evaluated (Table 5). The number of significant differences among groups for fruit traits ranged from zero (between groups Shum and Intermediate) to 20 (between groups Kumba and *S. anguivi*) (Table 4). As occurred for plant traits, groups Shum, Intermediate and *S. anguivi* presented few differences (between 0 and 6). The rest of pairwise comparisons between groups presented at least 7 differences (Table 4). For the nine fruit size traits, in general the Kumba group presented the largest values, followed by groups Aculeatum and Gilo, then the groups Shum and Intermediate, and finally by *S. anguivi*, which presented the smallest fruits (Table 5).

When considering fruit shape traits, the most relevant differences were that groups Aculeatum and Kumba had fruits more flattened (Fruit shape Index External I and II) than groups Gilo and Intermediate, group Aculeatum presented higher values for Proximal Fruit Blockiness than *S. anguivi* and of Distal Fruit Blockiness than groups Kumba and Intermediate, group Kumba was characterized by higher values of Triangular than *S. anguivi* and was less ellipsoid (i.e., higher Ellipsoid values) than the rest of groups, groups Aculeatum and Kumba were less circular (i.e., higher Circular values), had higher Shoulder Height, Eccentricity, Fruit Shape Index Internal and Eccentricity Area Index than the rest of groups, and *S. anguivi* was more Obovoid than groups Kumba and Shum (Table 5).

Table 3. Mean values for scarlet eggplant complex groups (*S. aethiopicum* groups Aculeatum, Gilo, Kumba, and Shum, Intermediate between *S. aethiopicum* and *S. anguivi*, and *S. anguivi*) for the plant descriptors for which significant (P<0.05) differences have been found among group means.

Descriptors	*S. aethiopicum*				Intermediate	*S. anguivi*	Prob. F
	Aculeatum	Gilo	Kumba	Shum			
n	5	37	10	2	8	1	
Plant Height (cm)	151 ab	156 b	114 a	135 ab	153 ab	150 ab	0.0033
Hypocotyl Anthocyanins Intensity	7.80 b	0.31 a	2.74 a	0.50 a	1.49 a	0.00 a	<0.0001
Shoot Tip Anthocyanins Intensity	7.80 b	0.23 a	2.98 a	2.50 a	1.68 a	0.00 a	<0.0001
Angle Between Main Branches	1.72 ab	2.77 ab	3.21 b	2.00 ab	2.35 ab	1.00 a	0.0134
Leaf Pedicel Length (cm)	7.88 b	5.23 ab	6.74 ab	3.47 a	5.82 ab	3.00 a	0.0342
Leaf Blade Length (mm)	23.8 bc	21.5 abc	25.5 c	14.3 a	20.9 abc	15.5 ab	0.0031
Leaf Blade Breadth (cm)	17.8 ab	16.4 ab	19.5 b	10.5 a	16.1 ab	12.0 ab	0.0412
Leaf Surface Shape	1.00 a	2.54 ab	3.88 ab	4.33 b	2.00 ab	1.00 a	0.0469
Leaf Prickles	5.32 b	0.00 a	0.00 a	0.00 a	0.47 a	0.00 a	<0.0001
Length of Largest Leaf Prickle (cm)	1.62 b	0.00 a	0.00 a	0.00 a	0.07 a	0.00 a	<0.0001
Number of Flowers per Inflorescence	7.18 b	2.18 a	1.97 a	3.33 a	7.51 b	7.50 b	<0.0001
Number of Sepals	5.97 ab	5.60 ab	6.33 b	5.27 a	5.06 a	5.00 a	<0.0001
Number of Petals	5.92 ab	5.50 ab	6.39 b	5.33 ab	5.16 a	5.00 a	<0.0001
Number of Stamens	6.01 ab	5.91 ab	7.18 b	5.31 a	5.06 a	5.00 a	<0.0001
Corolla Diameter (mm)	19.1 ab	19.3 ab	22.7 b	12.5 a	16.8 ab	13.2 a	0.0017

[a]Means within rows separated by different letters are significantly different at P<0.05, according to the Student-Newman-Keuls test.

Table 4. Number of significant (P<0.05) differences among means for scarlet eggplant complex groups (*S. aethiopicum* groups Aculeatum, Gilo, Kumba, and Shum, Intermediate between *S. aethiopicum* and *S. anguivi*, and *S. anguivi*) for 18 conventional descriptors (above the diagonal) and for 27 Tomato Analyzer descriptors (below the diagonal).

	S. aethiopicum				Intermediate	*S. anguivi*
	Aculeatum	Gilo	Kumba	Shum		
S. aethiopicum						
Aculeatum		5	5	8	4	5
Gilo	8		1	0	1	1
Kumba	7	13		5	4	7
Shum	12	9	14		1	2
Intermediate	15	9	16	0		0
S. anguivi	14	14	20	3	6	

The first and second components of the PCA accounted, respectively, for 33.7% and 16.6% of the total variation among accession means (Table 6). The first component was positively correlated to elongated fruits (Fruit Shape Index External I and II, and Fruit Shape Index Internal), Width Widest Pos, and Eccentricity and negatively to number of flower parts (sepals, petals, stamens), fruit size (except for the fruit length traits), and fruits less ellipsoid and circular (i.e., higher Ellipsoid and Circular values), and with high values for Shoulder Height, Ovoid, and Eccentricity Area Index (Table 6). The second component was positively correlated with anthocyanin intensity traits, prickliness traits, Number of Flowers per Inflorescence, and Distal Fruit Blockiness and negatively with Angle between Main Branches and with traits related to elongated (Length, Height Mid-width, Maximum Height, Fruit Shape Index External I and II, and Fruit Shape Internal) and large fruits (Perimeter and Area) (Table 6).

The projection of the accessions on a two-dimensional PCA plot showed that accessions of the different scarlet eggplant complex groups plotted in different areas of the graph, although the Intermediate group overlapped with several of the other groups (Figure 4). The Aculeatum group had a low dispersion and all accessions presented negative values for the first component and highly positive values for the second component. The Gilo

group presented the largest dispersion; however, despite this wide dispersion it overlapped only with some accessions of the Intermediate group. Kumba group accessions presented intermediate values for the first component and high negative values for the second component and display an intermediate level of dispersion compared to Aculateum and Gilo groups in the PCA graph. The small-fruited Shum, Intermediate and *S. anguivi* groups presented a combination of high values for the first component (in particular *S. anguivi*) with moderate, generally positive, values for the second component. The Shum group and *S. anguivi* were separated from each other and from the Gilo group, but the Intermediate group overlapped with part of the areas where the Shum group accessions and small fruited accessions of the Gilo group plot and is also situated in the area intermediate between *S. anguivi*, Shum and Gilo groups (Figure 4).

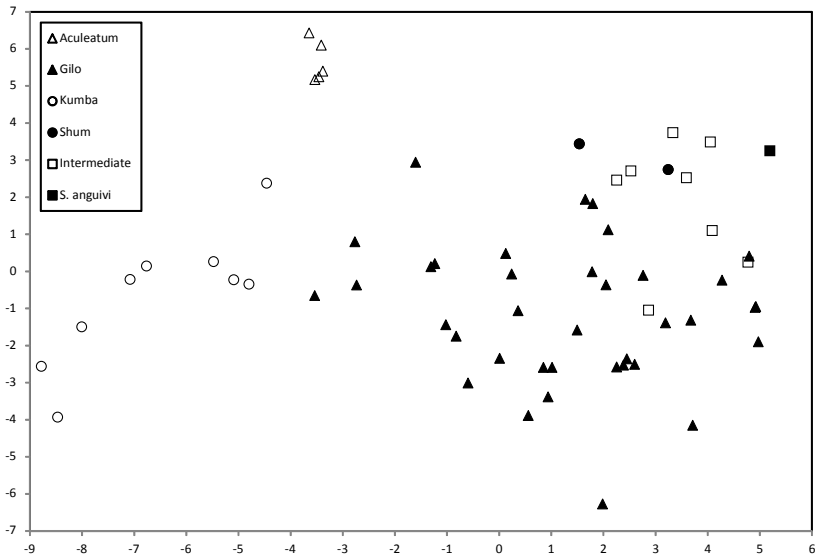

Figure 4. Similarities based on 18 plant and 27 fruit descriptors among 63 scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*) accessions represented on the two first principal components of PCA. First and second components account for 33.7% and 16.6% of the total variation, respectively. The six groups considered are represented by different symbols: *S. aethiopicum* groups Aculeatum (open triangle), Gilo (filled triangle), Kumba (open circle), and Shum (filled circle); Intermediate between *S. aethiopicum* and *S.anguivi* (open square); and, *S. anguivi* (filled square).

Table 5. Mean values for scarlet eggplant complex groups (*S. aethiopicum* groups Aculeatum, Gilo, Kumba, and Shum, Intermediate between *S. aethiopicum* and *S. anguivi*, and *S. anguivi*) for the fruit descriptors for which significant (P<0.05) differences have been found among group means.

Descriptors[a]	*S. aethiopicum*				Intermediate	*S. anguivi*	Prob. F
	Aculeatum	Gilo	Kumba	Shum			
n	5	37	10	2	8	1	
Weight (g)[b]	28.4 c	32.4 c	166.6 d	3.9 b	4.8 b	1.0 a	<0.0001
Length (cm)[b]	2.62 cd	4.03 d	4.21 d	1.43 ab	2.20 bc	1.10 a	<0.0001
Breadth (cm)[b]	4.63 c	4.09 c	8.22 d	2.02 b	2.06 b	1.16 a	<0.0001
Perimeter (cm)[b]	13.5 c	13.8 c	22.5 d	5.9 ab	6.9 b	4.0 a	<0.0001
Area (cm^2)[b]	9.7 c	13.1 c	25.2 c	2.5 ab	3.3 b	1.2 a	<0.0001
Width Mid-height (cm)[b]	4.59 b	3.96 b	7.41 c	1.97 a	2.00 a	1.24 a	<0.0001
Maximum Width (cm)[b]	4.61 b	3.98 b	7.54 c	1.98 a	2.02 a	1.25 a	<0.0001
Height Mid-width (cm)[b]	1.86 ab	3.82 c	2.92 bc	1.39 a	2.03 ab	1.15 a	<0.0001
Maximum Height (cm)[b]	2.71 cd	4.00 d	4.32 d	1.48 ab	2.07 bc	1.17 a	<0.0001
Fruit Shape Index External I	0.59 a	1.06 b	0.57 a	0.76 ab	1.04 b	0.93 ab	<0.0001
Fruit Shape Index External II	0.41 a	1.03 b	0.40 a	0.73 ab	1.03 b	0.92 ab	<0.0001
Proximal Fruit Blockiness	0.73 b	0.65 ab	0.71 b	0.69 ab	0.62 ab	0.57 a	0.0235
Distal Fruit Blockiness	0.72 b	0.65 ab	0.57 a	0.64 ab	0.60 a	0.64 ab	0.0035
Fruit Shape Triangle	1.01 ab	1.01 ab	1.33 b	1.07 ab	1.10 ab	0.90 a	0.0001
Ellipsoid[b]	0.09 a	0.04 a	0.18 b	0.04 a	0.04 a	0.02 a	<0.0001
Circular[b]	0.29 b	0.10 a	0.25 b	0.10 a	0.08 a	0.02 a	<0.0001
Rectangular	0.57 b	0.51 ab	0.54 ab	0.50 ab	0.48 a	0.50 ab	0.0007
Shoulder Height[b]	0.11 b	0.02 a	0.08 b	0.02 a	0.01 a	0.00 a	<0.0001
Obovoid	0.05 ab	0.08 ab	0.03 a	0.03 a	0.07 ab	0.13 b	0.0073
Ovoid	0.08 ab	0.06 ab	0.16 b	0.10 ab	0.08 ab	0.01 a	<0.0001
Width Widest Pos	0.47 ab	0.50 b	0.43 a	0.46 ab	0.49 ab	0.49 b	<0.0001
Eccentricity	0.55 a	0.75 b	0.55 a	0.76 b	0.78 b	0.78 b	<0.0001
Fruit Shape Index Internal	0.41 a	1.02 b	0.41 a	0.72 ab	1.03 b	0.92 b	<0.0001
Eccentricity Area Index	0.56 b	0.41 a	0.54 b	0.42 a	0.39 a	0.39 a	<0.0001

[a]Means within rows separated by different letters are significantly different at P<0.05, according to the Student-Newman-Keuls test.

[b]In order to avoid scaling effects caused by accession means being proportional to standard deviations, ANOVAs were performed on log transformed data.

Differences and relationships among gboma eggplant groups

Significant (P<0.05) differences between *S. macrocarpon* and *S. dasyphyllum* were found only for three morphological traits (Table 7). In this respect, *S. macrocarpon* presented significantly less bullate leaves (Leaf Surface Shape) and lower prickliness (Leaf Prickless and Length of Largest Leaf Prickle) than *S. dasyphyllum*. Regarding fruit traits, significant differences between the two gboma eggplant groups were found for eleven traits. *Solanum marcrocarpon* fruits presented significantly larger fruits (higher values for seven out of the eight fruit size traits, the exception being Height Mid-width), more ovoid (lower values for Obovoid and higher for Ovoid), and with lowest values for the ratio of the height at which the maximum width occurs (Width Widest Pos) than those of *S. dasyphyllum* (Table 7). The first and second components of the PCA accounted, respectively, for 31.3% and 22.5% of the total variation among accession means (Table 8).

The first component was positively correlated to prickliness (Leaf Prickles), elongated fruits (Fruit Shape Index External II), Width Widest Pos, and Eccentricity, and negatively to number of flower parts (Number of Petals, Number of Stamens), Corolla Diameter, leaf blade size, fruit size (except for the fruit length traits), and fruits more triangular, less ellipsoid and circular (i.e., higher Ellipsoid and Circular values), and with high values for Ovoid, and Eccentricity Area Index (Table 8). The second component was positively correlated with Plant Height, Stem Diameter, Number of Flowers per Inflorescence, Distal Fruit Blockiness and Proximal Eccentricity and negatively with anthocyanins intensity, traits related to elongated (Length, Height Mid-width, Maximum Height, Fruit Shape Index External I and II) and large fruits (Perimeter and Area) (Table 8).

The projection of the accessions on a two-dimensional PCA plot clearly shows that accessions of *S. macrocarpon* and *S. dasyphyllum* groups plot in different areas of the graph (Figure 5). The single accession of *S. dasyphyllum* presents the highest values for the first and second components. With the exception of one odd accession, all the *S. macrocarpon* accessions present intermediate values for the first component. The odd *S. macrocarpon* accession, with an extremely low value for the second component is distinct from the others in having elongated fruit shape.

Table 6. Correlation coefficients between plant and fruit descriptors and the two first principal components for the scarlet eggplant complex (*S. aethiopicum* and *S. anguivi*). Only those correlations with absolute values ≥0.15 have been listed.

Descriptor	First principal component	Second principal component
Plant descriptors		
Hypocotyl Anthocyanins Intensity		0.198
Shoot Tip Anthocyanins Intensity		0.218
Angle Between Main Branches		-0.160
Leaf Prickles		0.230
Length of Largest Leaf Prickle		0.230
Number of Flowers per Inflorescence		0.233
Number of Sepals	-0.193	
Number of Petals	-0.206	
Number of Stamens	-0.203	
Fruit descriptors		
Weight	-0.194	
Length		-0.312
Breadth	-0.231	
Perimeter	-0.205	-0.191
Area	-0.186	-0.218
Width Mid-height	-0.232	
Maximum Width	-0.233	
Height Mid-width		-0.315
Maximum Height		-0.309
Fruit Shape Index External I	0.182	-0.185
Fruit Shape Index External II	0.197	-0.177
Proximal Fruit Blockiness	-0.160	
Distal Fruit Blockiness		0.167
Ellipsoid	-0.197	
Circular	-0.200	
Shoulder Height	-0.195	
Ovoid	-0.172	
Width Widest Pos	0.169	
Eccentricity	0.233	
Fruit Shape Index Internal	0.202	-0.178
Eccentricity Area Index	-0.231	
Variance explained (%)	33.700	16.600

Table 7. Mean values for gboma eggplant complex groups (*S. macrocarpon* and *S. dasyphyllum*) for the plant and fruit descriptors for which significant (P<0.05) differences have been found among group means.

Descriptors[a]	Gboma eggplant		
	S. macrocarpon	*S. dasyphyllum*	Prob. F
n	11	1	
Leaf Surface Shape	4.64 a	9.00 b	0.0061
Leaf Prickles	0.99 a	9.00 b	0.0004
Length of Largest Leaf Prickle (cm)	0.33 a	1.45 b	0.0442
Weight (g)[b]	119.10 b	21.90 a	0.0026
Length (cm)[b]	5.18 b	2.97 a	0.0068
Breadth (cm)[b]	6.92 b	3.83 a	0.0075
Perimeter (cm)[b]	21.00 b	11.90 a	0.0136
Area (cm^2)[b]	28.80 b	9.30 a	0.0140
Width Mid-height (cm)[b]	6.80 b	3.79 a	0.0206
Maximum Width (cm)[b]	6.85 b	3.81 a	0.0199
Maximum Width (cm)[b]	5.14 b	3.03 a	0.0371
Obovoid	0.01 a	0.05 b	0.0093
Ovoid	0.16 b	0.08 a	0.0064
Width Widest Pos	0.44 a	0.49 b	0.0488

[a]Means within rows separated by different letters are significantly different at P<0.05, according to the Student-Newman-Keuls test. [b]In order to avoid scaling effects caused by accession means being proportional to standard deviations, ANOVAs were performed on log transformed data.

Discussion

Scarlet and gboma eggplants are important vegetables in tropical sub-Saharan Africa but have received little attention from the formal breeding sector (Lester and Thitai, 1989; Schippers, 2000; Seck, 2000; Adeniji and Aloyce, 2012; Prohens *et al*., 2012). This has allowed the on-site conservation of a large number of local varieties which, together with accessions conserved in germplasm banks, represent genetic resources for the enhancement of both crops (Lester *et al*., 1990; Bukenya and Carasco, 1994; Schippers, 2000; Sekara *et al*., 2007). The detailed morphological characterization of germplasm collections will allow studying the diversity and identification of potentially interesting accessions for selection and breeding, as well as devising strategies for conservation and management of germplasm (Furbank and Tester, 2011).

Also, given that both crops and their wild relatives form part of the secondary genepool of common eggplant, information on the phenotypic diversity of scarlet and gboma eggplants may be of interest for common eggplant breeding (Daunay *et al*., 1991; Oyelana and Ugborogho, 2008; Prohens *et al*., 2012; Khan *et al*., 2013).

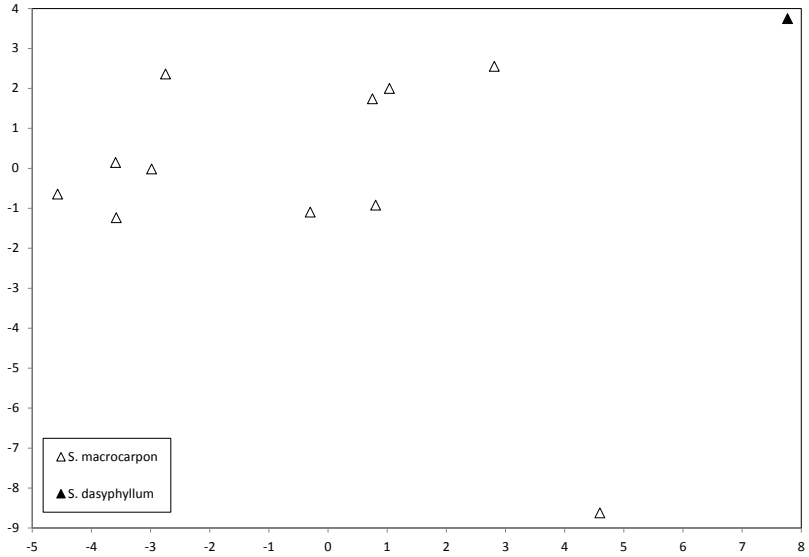

Figure 5. Similarities based on 18 plant and 27 fruit descriptors among 12 gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*) accessions represented on the two first principal components of PCA. First and second components account for 31.3% and 22.5% of the total variation, respectively. The two species are represented by different symbols: *S. macrocarpon* (open triangle), and *S. dasyphyllum* (filled triangle).

Morphological characterization is essential for the identification of valuable germplasm accessions as well as for typification and classification of accessions in cultivar groups (Spooner *et al*., 2003). Characterization of cultivated eggplants and wild relatives has usually been performed with conventional morphological descriptors highly heritable and simple to evaluate (IBPGR, 1990; Prohens *et al*., 2005; van der Weerden and Barendse, 2007; Polignano *et al*., 2010; Prohens *et al*., 2012). These descriptors are very useful but have some limitations especially for fruit shape characterization, which is one of the most important traits in a variety of any of the cultivated eggplant

species and for which great diversity exists (Adeniji and Aloyce, 2012; Hurtado *et al*., 2013). Here we have complemented a standard morphological characterization with a fruit shape phenomics characterization using the high-throughput phenomics Tomato Analyzer (Brewer *et al*., 2006, 2007; Gonzalo and van der Knaap, 2008, Rodríguez *et al*., 2010), which has allowed the automated acquisition of multiple data of different fruit shape characteristics in both scarlet and gboma eggplants complexes. Combination of both types of data has allowed identification of multiple traits which distinguish clearly not only both crops and the cultivated species from the wild relatives, but also cultivar groups, which is not always possible using conventional descriptors (Polignano *et al*., 2010; Adeniji *et al*., 2012, 2013), as well as to describe the diversity present for traits of interest for selecting and developing improved materials in both crops. Descriptors presenting highly significant differences among groups and which plot in different parts of the PCA graph (i.e., descriptors that do not present high correlation values) would be the most informative for distinguishing between cultivar groups.

Scarlet and gboma eggplants are classified in different botanical sections within *Solanum* subgenus *Leptostemonun* (Lester, 1986; Lester and Daunay, 2003; Lester *et al*., 2011; Edmonds, 2012). Our results confirm that the scarlet and gboma eggplant complexes differ in many morphological differences, both for plant and fruit traits of agronomic interest. Polignano *et al*. (2010) also found that the cultivated *S. aethiopicum* and *S. macrocarpon* presented considerable differences for agronomic descriptors. Although some differences considered as significant ($P<0.05$) might have resulted from false positives derived from multiple testing (Snedecor and Cochran, 1989), most of them have been highly significant ($P<0.001$), indicating that even with highly stringent tests they would have been significant, revealing that very likely they correspond to real differences. This differentiation is also confirmed at the molecular and chemical composition levels (Furini and Wunder, 2004; Polignano *et al*., 2010; Sánchez-Mata *et al*., 2010; Vorontsova *et al*., 2013). Given that both crops can be intercrossed and hybrids have intermediate fertility (Daunay *et al*., 1991; Oyelana and Ugborogho, 2008), scarlet and gboma eggplants could be used for reciprocal breeding in order to introgress traits of interest from one species into the other (Prohens *et al*., 2012).

Table 8. Correlation coefficients between plant and fruit descriptors and the two first principal components for the gboma eggplant complex (*S. macrocarpon* and *S. dasyphyllum*). Only those correlations with absolute values ≥0.15 have been listed.

Descriptor	First principal component	Second principal component
Plant descriptors		
Plant Height		0.219
Hypocotyl Anthocyanins Intensity		-0.266
Shoot Tip Anthocyanins Intensity		-0.247
Stem Diameter		0.206
Leaf Blade Length	-0.212	
Leaf Blade Breadth	-0.156	
Leaf Prickles	0.150	
Number of Flowers per Inflorescence		0.207
Number of Petals	-0.172	
Number of Stamens	-0.161	
Corolla Diameter	-0.217	
Fruit descriptors		
Weight	-0.216	
Length		-0.265
Breadth	-0.243	
Perimeter	-0.212	-0.154
Area	-0.201	-0.154
Width Mid-height	-0.238	
Maximum Width	-0.239	
Height Mid-width		-0.288
Maximum Height		-0.271
Fruit Shape Index External I		-0.255
Fruit Shape Index External II	0.152	-0.245
Proximal Fruit Blockiness		
Distal Fruit Blockiness		0.245
Fruit Shape Triangle	-0.154	
Ellipsoid	-0.169	
Circular	-0.168	
Ovoid	-0.197	
Width Widest Pos	0.224	
Eccentricity	0.230	
Proximal Eccentricity		0.194
Fruit Shape Index Internal	0.152	
Eccentricity Area Index	-0.220	
Variance explained (%)	31.300	22.500

We have found a large diversity in both scarlet and gboma eggplants complexes for plant and fruit traits, with wide ranges of variations for most descriptors, confirming that they are hypervariable (Lester and Niakan, 1986; Lester *et al*., 1986; Bukenya and Carasco, 1994; Schippers, 2000). The variation of *Solanum aethiopicum* is so high that the different cultivar groups have, in the past, been considered as different species (Lester, 1986; Lester *et al*., 1986, 2011). Our combined study of conventional and Tomato Analyzer descriptors, together with multivariate PCA results, shows that each of the *S. aethiopicum* cultivar groups as well as *S. anguivi* are distinguished by many traits, which supports Lester's (1986) view that each of the *S. aethiopicum* cultivar groups and the wild ancestor *S. anguivi* are characterized by a specific syndrome of characteristics. As expected, the wild *S. anguivi*, the intermediate forms between *S. aethiopicum* and *S. anguivi*, as well as the *S. aethiopicum* Shum group, which is only used for the leaves, have small fruits (Lester and Niakan, 1986; Lester *et al*., 1986, 1990; Schippers, 2000). Also, the gboma eggplant complex has proved to be highly diverse (Bukenya and Carasco, 1994; Lester *et al*., 1990; Polignano *et al*., 2010). Apart from leaf surface shape and prickliness, the differences observed between *S. macrocarpon* and *S. dasyphyllum* correspond to fruit traits evaluated with Tomato Analyzer. Most of the traits for which differences have been found among scarlet eggplant complex groups (including *S. anguivi*) as well as between *S. macrocarpon* and *S. dasyphyllum* correspond to fruit shape traits identified using the Tomato Analyzer tool, showing the potential of this phenomics tool for fruit shape characterization in eggplants (Prohens *et al*., 2012; Hurtado *et al*., 2013). In fact, while no significant differences were found for conventional plant descriptors between *S. aethiopicum* Gilo and Shum groups on one hand, as well as between the Intermediate group and *S. anguivi* on the other, the Tomato Analyzer characterization of fruit shape has allowed the detection of significant differences for fruit shape traits among them.

Apart from the differences among *S. aethiopicum* groups, a wide diversity has been found within each of them, as well as within *S. macrocarpon*. Within *S. aethiopicum*, the largest diversity has been found in the Gilo group, which is in agreement with previous observations and also with the fact that it is the most spread and important cultivar group (Lester *et al*., 1986, 1990; Schippers, 2000; Polignano *et al*., 2010; Sunseri *et al*., 2010). The

Kumba group has been found to be less diverse that the Gilo group. The fact that the characteristic highly furrowed and flattened fruits of the Kumba group may be the result of a mutation similar to that of the tomato *FASCIATED* mutation, which is responsible for a high degree of fasciation in this crop (Monforte *et al*., 2014), may account for the lower degree of diversity compared to the Gilo group (Lester *et al*., 1986). Amazingly, a low diversity has been found within the Aculeatum group. This group is not commonly found in Africa and it has been hypothesized that it was created in Europe for ornamental purposes after crossing *S. anguivi* and *S. aethiopicum* group Kumba (Lester *et al*., 1986; Schippers, 2000), which would explain its low diversity.

Lester *et al*. (1986, 1990) reported that some accessions were intermediate in characteristics between *S. anguivi* and *S. aethiopicum*. In our case, we have found several of them, which presented some key traits used for classification that were typical of *S. aethiopicum* while others were characteristic of *S. anguivi*. These materials plotted between *S. anguivi* and *S. aethiopicum*, having some overlap with the latter. Intermediate forms may represent primitive or semi-domesticated weedy forms that are formed by occasional hybridization, as the area of natural distribution of the wild ancestor presents a high degree of overlapping with the area of distribution of the crop (Lester and Niakan, 1986; Lester *et al*., 1986, 1990). This intermediate forms very likely favour the flux of genes from the wild *S. anguivi* into the cultivated *S. aethiopicum*, contributing to a high genetic background and diversity.

Solanum marcrocarpon accessions have also been very variable for the traits studied. An important diversity may be caused by the fact that in this crop some accessions are used for the leaves, others for the fruits, and others for both plant organs (Lester *et al*., 1990; Schippers, 2000; Maundu *et al*., 2009). Therefore, it is expected that accessions used for the leaves will have smaller fruits than those used for the fruits. Also, although a characteristic typical of *S. macrocarpon* is having fruits flattened or subspherical, an accession with elongated fruits has been found. It remains to be investigated if the elongated fruit of this odd accession is caused by a mutation similar to the *SUN* mutation of tomato, which results in extremely elongated fruits (Monforte *et al*., 2014).

The phenotypic results obtained have important implications for germplasm conservation and breeding (Furbank and Tester, 2011). The high diversity found indicates that a large number of accessions will need to be conserved in germplasm banks or represented in core collections in order to have a good representation of the phenotypic variation found in both species (Odong *et al*., 2013). The characterization data and the multivariate analysis performed may be useful to select a subset of accessions that represent most of the morphological diversity of both complexes. At the selection and breeding level, considerable phenotypic differences among and within groups may be used for selection of the best accessions or to select parents for obtaining F1 hybrids heterotic for yield or with intermediate or new characteristics (Lester and Thitai, 1989; Seck, 2000; Adeniji and Aloyce, 2012).

In conclusion, we have found that the combined utilization of conventional and Tomato Analyzer phenomics descriptors is a powerful tool for studying the diversity and relationships of scarlet and gboma eggplants complexes. In particular, Tomato Analyzer allows the detailed description of fruit characteristics and the differentiation of cultivar groups in which few plant morphological differences are found. The detailed characterization information on the germplasm collections will be useful for the enhancement of both crops, including the conservation of genetic resources, selection and breeding.

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3.2.2 Reducing capacity, chlorogenic acid content and biological activity in a collection of scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplants

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Abstract

Scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplants are important vegetables in sub-Saharan Africa. Few studies have been made in these crops on the diversity of phenolics content and their biological activity. We have studied the reducing activity, the chlorogenic acid and other phenolic acids contents in a collection of 56 accessions of scarlet eggplant including the four cultivated groups (Aculeatum, Gilo, Kumba, Shum) and the weedy intermediate *S. aethiopicum*-*S. anguivi* types, and in eight accessions of gboma eggplant including the cultivated *S. macrocarpon* and its wild ancestor *S. dasyphyllum*. A sample of the accessions evaluated in this collection has been tested for inhibition of nitric oxide (NO) using macrophage cell cultures. The results show that there is a great diversity in both crops for reducing activity, chlorogenic acid content and chlorogenic acid peak area (% of total phenolic acids). Heritability (H2) for these traits was intermediate to high in both crops. In all samples, chlorogenic acid was the major phenolic acid and accounted for more than 50% of the chromatogram peak area. Considerable differences were found among and within groups for these traits, but the greatest values for total phenolics and chlorogenic acid content were found in *S. dasyphyllum*. In most groups reducing activity was positively correlated (with values of up to 0.904 in the Aculeatum group) with chlorogenic acid content. Inhibition of NO was greatest in samples having a high chlorogenic acid content. The results show that both crops are a relevant source of chlorogenic acid and other phenolic acids. The high diversity found also indicates that there are good prospects for breeding new scarlet and gboma eggplant cultivars with improved content in phenolics and bioactive properties.

Introduction

Selection of vegetables with improved content in bioactive phenolics is a breeding objective of an increasing number of genetic improvement programmes aimed at developing new varieties with enhanced functional properties (Diamanti *et al*., 2011; Plazas *et al.*, 2013a). Dietary phenolics of vegetables and of other plant products have been shown to have bioactive properties beneficial for human-health resulting from, among others, free-radical scavenging properties, regulation of enzymatic activity or modulation of several cell signaling pathways (Soobrattee *et al*., 2005; Fresco *et al*., 2006; Dai *et al*., 2010; Sato *et al*., 2011; Surh, 2003).

Among phenolic compounds, phenolic acids exert a potent antioxidant activity through the interaction with reactive oxygen and nitrogen species by several mechanisms (Virgili y Marino, 2008). This strong reducing capacity is the key to their biological activity (Rice-Evans *et al*., 2014). Among these phenolic acids, chlorogenic acid (5-*O*-caffeoyl-quinic acid) is abundant in vegetables (Plazas *et al*., 2013a; Manach *et al*., 2008; Alarcón-Flores *et al*., 2014) and has shown to be present multiple beneficial properties, including analgesic, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-microbial, anti-obesity, cardioprotective, hypotensive, and neuroprotective effects (Plazas *et al.*, 2013a; Sato *et al*., 2011; Suzuki *et al*., 2006; Cho *et al*., 2010; Ahn *et al*., 2011; Burgos-Morón *et al*., 2012; Coman *et al*., 2012; Zhao *et al*., 2012; dos Santos *et al*., 2006). Furthermore, chlorogenic acid is highly bioavailable for humans (dos Santos *et al*., 2006). Therefore, selecting vegetable varieties with higher chlorogenic acid content may be of interest (Plazas *et al*., 2013a).

Common eggplant (*Solanum melongena* L.) has been reported as having very high contents in chlorogenic acid, which constitutes the major phenolic compound in the fruit flesh (Plazas *et al*., 2013a; Ahn *et al*., 2011; Stommel y Whitaker, 2003; Whitaker y Stommel, 2003; Prohens *et al*., 2013; Prohens *et al*., 2007), and some breeding programmes for improving total phenolics and chlorogenic acid content have been started (Plazas *et al*., 2013a; Prohens *et al*., 2013; Prohens *et al*., 2007).

Apart from the common eggplant, there are two cultivated eggplants native to Africa, namely the scarlet eggplant (*S. aethiopicum* L.) and the gboma eggplant (*S. macrocarpon* L.) which, despite their importance in sub-Saharan Africa (Schippers, 2000) have largely remained neglected. Both crops are hypervariable at the morphological level, in particular in the case of the scarlet eggplant (Schippers, 2000; Lester, 1986; Lester *et al*., 1986; Bukenya y Carasco, 1994; Lester y Daunay, 2003; Polignano *et al*., 2010; Sunseri *et al*., 2010; Plazas *et al*., 2014). Four cultivar groups of *S. aethiopicum* are recognized: Aculeatum (used as ornamental), Gilo (used for the fruits), Kumba (used for the fruits and leaves), and Shum (used for the leaves) (Lester, 1986; Lester *et al*., 1986; Plazas *et al*., 2014). Also, weedy plants of semi-domesticated forms that are intermediate in characteristics between the cultivated *S. aethiopicum* and its wild ancestor *S. anguivi* L. are commonly harvested (Lester *et al*., 1986; Plazas *et al*., 2014; Lester y Niakan, 1986). For the purposes of this paper these

intermediate forms are referred to as *aethiopicum-anguivi*. The gboma eggplant *S. macrocarpon*, which is cultivated for its fruits and leaves, is generally not differentiated in cultivar groups, although the wild ancestor *S. dasyphyllum* Schum and Thon. is included in the gboma eggplant complex (Bukenya y Carasco, 1994). The wild *S. dasyphyllum* is clearly distinguished from the cultivated *S. macrocarpon* for having greater prickliness and smaller fruits, and is mostly used as medicinal (Schippers, 2000; Bukenya y Carasco, 1994).

Few efforts have been devoted to evaluating the phenolics content of scarlet and gboma eggplants (Stommel y Whitaker, 2003; Prohens *et al*., 2007; Sunseri *et al*., 2010). These studies have shown that, like common eggplant, both species present high levels of total phenolics and of chlorogenic acid. However, the diversity of these crops for their reducing activity, phenolic acids content, or their relationship with biological activity has been barely studied. The largest study on scarlet eggplant diversity related to this subject was performed by Sunseri *et al*. (Sunseri *et al*., 2010), who evaluated 70 accessions of scarlet eggplant for chlorogenic acid content and found a wide range of variation, from 0.20 g/kg to 9.88 g/kg. However, the results were part of a general study of characterization and did not involve studying differences among groups or other related traits, like reducing activity or biological activity of varieties having different chlorogenic acid concentrations (Sunseri *et al*., 2010).

Also, Stommel and Whitaker (2003) evaluated 13 accessions of *S. aethiopicum* for phenolic acids acid in a general study of diversity of phenolics acid composition in common eggplant and found a range of variation for chlorogenic acid from 1.09 to 3.52 g/kg. For the gboma eggplant, we know no studies for the diversity of reducing activity, chlorogenic acid content or biological activity. In consequence, it is desirable to undertake a detailed investigation on the functional properties and compounds and biological activity of both the scarlet and gboma eggplants. Apart from providing information relevant on the properties of both crops, this knowledge will be of interest for selection and breeding of varieties of both crops with improved functional properties. In addition, the common, scarlet and gboma eggplants can be intercrossed giving hybrids of intermediate fertility (Daunay y Lester, 1991; Oyelana y Ugborogho, 2008; Dauynay y Hazra, 2014; Rotino *et al.*, 2014).

Therefore, the three cultivated eggplant species might be used as genetic resources for reciprocal breeding (Rotino *et al.*, 2014; Prohens *et al*., 2012), including introgression of functional quality traits (Mennella *et al*., 2010).

In this work, we characterize the total reducing activity as well as the chlorogenic acid and other phenolic acids content in a collection of scarlet and gboma eggplants from different groups. Moreover, in a selected set of accessions we carried out the study of the biological activity *in vitro* in macrophages. The objective is to provide relevant information on the reducing activity, chlorogenic acid content, and their relationship, and to test the biological activity of the extracts of scarlet and gboma eggplants. This information will be useful for developing eggplants with improved functional properties, i.e., with higher content in chlorogenic acid and enhanced antioxidant and biological activity.

Experimental Section

Plant material

Fifty-six accessions of scarlet eggplant and eight accessions of gboma eggplant were used for the present study (Table 1). Five plants per accession were grown in Valencia (Spain) in the open field during the summer season of 2013 using the standard horticultural practices used for common eggplant. These accessions had been previously characterized morphologically by Plazas *et al*. (2014) and assigned to their respective groups: Aculeatum (5), Aethiopicum-anguivi (6), Gilo (34), Kumba (9), and Shum (2) for the scarlet eggplant, and *S. dasyphyllum* (1) and *S. macrocarpon* (7) for the gboma eggplant.

Table 1. Scarlet eggplant and gboma eggplant groups evaluated, number of accessions and typical characteristics of the fruit of each of the groups (Schippers, 2000; Lester, 1986; Lester *et al*., 1986; Bukenya y Carasco, 1994; Plazas *et al*., 2014).

Group	n	Type	Common use	Fruit weight (g)	Fruit shape	Fruit diameter (cm)	Fruit grooves	Fruit locules	Calyx prickliness
Scarlet eggplant	56								
Aculeatum	5	Cultivated	Ornamental	20 - 40	Flattened	3-8	Many	4-10	Very high
Aethiopicum-anguivi	6	Weedy[a]	Medicinal	3 - 8	Ellipsoid	1-3	None to few	2-3	Absent to low
Gilo	34	Cultivated	Food (fruits)	10 - 70	Subspherical to ellipsoid	3-8	None to few	2-6	Absent to low
Kumba	9	Cultivated	Food (fruits & leaves)	50 - 350	Flattened	5-12	Very many	10-20	None
Shum	2	Cultivated	Food (leaves)	2 - 6	Round	2-3	None to few	2-4	None
Gboma eggplant	8								
S. dasyphyllum	1	Wild	Medicinal	15 - 30	Subspherical	3-5	None	2-5	Very high
S. macrocarpon	7	Cultivated	Food (fruits & leaves)	50 - 150	Subspherical	5-9	None	4-6	Absent to low

[a]Found as a non-cultivated plant in disturbed environments.

One sample of fruit, consisting of either a minimum of 250 g or five fruits (for the small fruited accessions), was obtained for each plant. Commercially ripe fruits (i.e., physiologically immature at the breaker stage) were used (Plazas *et al*., 2014). Fruits were brought to the laboratory, washed and a slice of 1-2 cm wide longitudinal section from stem to blossom-end was cut from the middle of the fruit. The excised tissue was frozen in liquid N_2 and lyophilized. The lyophilized tissue of the fruit from an individual plant was powdered and pooled as a single sample. Powdered tissue of each sample was used for the analyses.

Chemical analysis

Total reducing capacity

Total reducing capacity was determined according to the Folin-Ciocalteu procedure (Singleton y Rossi, 1965; Prior *et al*., 2005). For each sample, 0.125 g of the lyophilized tissue was extracted with 15 ml of acetone:water:glacial acetic acid (70:29.5:0.5, v/v) for 24 h under continuous stirring at room temperature. The extracted sample was then centrifuged at 3500 rpm for 5 min in an Eppendorf 5804 R centrifuge (Eppendorf, Hamburg, Germany) and 1.5 mL of the supernatant were pipetted, poured on Eppendorf tubes and stored at -20ºC until analyzed. Thawed samples were centrifuged at 10000 rpm for 5 min, and 65 µL of the supernatant was mixed with 0.5 mL diluted (10%, v/v) Folin-Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany) and allowed to stand at room temperature for 5 min. Subsequently, 0.5 mL of sodium carbonate (60 g/L) was added to the mixture. After 90 min at room temperature, absorbance was measured at 750 nm in an iMark microplate spectrophotometer (Bio-Rad, Herts, United Kingdom). Chlorogenic acid (Sigma-Aldrich Chemie) was used as a standard, and total reducing capacity was expressed as chlorogenic acid equivalents in g/kg of dry weight (Tables 3 and 4).

Chlorogenic acid and other free phenolic acids

Chlorogenic acid and other phenolic acids (hydroxycinnamic acid conjugates) were extracted according to Helmja *et al*. (2008). Lyophilized samples (0.1 g) were homogenized in 1.8 mL of methanol:water (80:20, v/v) plus 0.1% (w/v) of 2,3-*ter*-butyl-4-hydroxyanisole (BHT). The total extract was

vortexed vigorously, sonicated for 1 h at room temperature, and then centrifuged at 2000 rpm for 3 min in an Eppendorf 5804 R centrifuge. The supernatant was filtered through 0.2 µm polytetrafluoroethylene (PTFE) membrane filters. Standard solutions of chlorogenic acid were prepared using the same protocol.

Determination of the contents in chlorogenic acid and other hydroxycinnamic acid derivatives was performed by high-performance liquid chromatography (HPLC) according to the protocol of Luthria and Mukhopadhyay (2006). Extracts were analyzed on a HPLC 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) operated by the OpenLAB CDS ChemStation Edition software package (Agilent Technologies). Aliquots of 10 µL were injected with the 1220 Infinity LC System automatic sampler into a ZORBAX Eclipse Plus C18 (3.5 µm; 4.6 x 12.5 mm; Agilent Technologies) column protected by a ZORBAX Eclipse Plus C18 guard column (5 µm; 4.6 x 12.5 mm; Agilent Technologies). The method used was a modification of that described by Prohens *et al*. (2013). The binary gradient consisted of 0.1% formic acid (solvent A) and methanol (solvent B). The mobile phase gradient was as follows: 0 min, 95A:5B at 0.5 mL/min; 0–5 min linear increase to 10% B at 0.5 mL/min; 5–10 min, linear increase to 20% B at 0.5 mL/min; 10–18 min, linear increases to 83% B and 0.5 mL/min; 18–23 min, linear increase to 100% B at 0.5 mL/min; 23–27 min, 100% B at 1.0 mL/min; 27–30 min, decrease to 5% B at 1.0 mL/min; 30–40 min, 95A:5B at 0.5 mL/min. Quantification was based on absorbance at 325 nm. The concentration of chlorogenic acid in the extracted samples was calculated using the developed calibration curves. The calibration curve was calculated using unweighted linear regression analysis and fit to linearity was evaluated with the r^2 value (r^2>0.99). The chlorogenic acid peak area and the total peak area of other phenolic acids (hydroxycinnamic acid conjugates) were determined and used to calculate the percentage of total peak area corresponding to chlorogenic acid (Tables 3 and 4).

Biological assays

Cell cultures

The murine macrophage cell line RAW 264.7 (ECACC, Salisbury, UK) was used for all *in vitro* experiments. The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum,

penicillin (100 U/mL) and streptomycin sulfate (100 mg/mL) in a humidified 5% CO_2 atmosphere.

Preparation of extracts for biological assays

A set of eight accessions from the different groups (one for each group and two for the large Gilo group) was chosen based in their differences in chlorogenic acid content (Table 2). Lyophilized fruit samples (1 g) were homogenized in 30 mL of methanol and extracted in an ultrasonic bath (Elmasonic S30, Elma, Singen, Germany) for 1 h. Extracted samples were centrifuged at 2000 rpm for 3 min in an Eppendorf 5804 R centrifuge and the supernatant was collected and filtered with PTFE filters. Samples were dried in a vacuum centrifuge (SpeedVac®, Thermo Scientific, Waltham, MA USA) and redissolved in ultrapure water (MilliQ Millipore, Molsheim, Francia). Finally they were filtered through 0.2 µm sterile PTFE filters and 1:10, 1:50 and 1:100 dilutions in sterile phosphate buffered saline were prepared.

Cell viability assay

The effect of each extract on cell viability was evaluated with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. In brief, murine RAW 264.7 macrophages were exposed to different dilutions of each extract in a 96-well microplate for 24 h, after which 20 µL per well of a 5 mg/mL solution of MTT (Sigma-Aldrich Chemie) was added and cells were incubated for 40 min at 37°C until blue deposits of formazan were visible. Metabolically active cells are able to transform MTT into formazan; therefore, the greater the cell viability the larger will be the blue formazan deposits. This colored metabolite was dissolved in acid isopropanol (0.04 N HCl) and incubated for 1 h at room temperature. Absorbance was measured at 570 nm subtracting the absorbance at 630 nm with a Bio-Rad iMarkTM Microplate reader. The results were expressed in absolute absorbance readings; a decrease in absorbance indicated a reduction in cell viability.

Nitrite determination

Nitric oxide (NO) levels were assessed by nitrite quantification as described by Grisham *et al*. (1996). Briefly, murine RAW 264.7 macrophages were cultured with different dilutions of each extract in a 96-well microplate for 1 h, after which cells were stimulated with lipopolysaccharide (LPS) (Sigma-Aldrich Chemie). After 24 h incubation, 100 µL of culture medium was mixed

with Griess reagent (Sigma-Aldrich Chemie). The latter reacts with NO present in the medium to give a red colour. Absorbance was read at 540 nm with a Bio-Rad iMark microplate spectrophotometer. Therefore, the absorbance values give an indication of the amount of NO present in the medium.

Table 2. Chlorogenic acid (CGA) content and ranking for CGA in the methanolic extracts of the eight accessions for which the biological activity (cytotoxicity and inhibition of NO production in RAW 264.7 macrophages) was evaluated.

Group	Code	CGA content (g/kg)	CGA rank
Scarlet eggplant			
Aculeatum	ACUL	1.02	6
Aethiopicum-anguivi	AE-AN	1.40	5
Gilo	GILO1	0.21	8
	GILO2	3.69	2
Kumba	KUMB	0.23	7
Shum	SHUM	2.21	3
Gboma eggplant			
S. dasyphyllum	DASY	4.87	1
S. macrocarpon	MACR	1.98	4

Data analysis

The mean, minimum and maximum values for the total reducing capacity, chlorogenic acid content and chlorogenic acid peak area of scarlet eggplant and for gboma eggplant accession means were calculated. Analysis of variance (ANOVA) tests were performed separately for accessions of scarlet eggplant and for accessions of gboma eggplant in order to calculate the sums of squares corresponding to accession and to residual, average standard error of accession means, coefficient of phenotypic variation (CV_P; %), coefficient of genotypic variation (CV_G; %), and broad-sense heritability (H^2) (Table 1) (Wricke y Weber, 1986). Broad-sense heritability was calculated using the formula $100 \cdot H_G^2/H_P^2$, where H_G^2 and H_P^2 are, respectively, the genotypic and phenotypic variance calculated from ANOVA mean squares (Wricke y Weber, 1986). Mean values for each accession were used to perform additional ANOVA analyses to detect differences among group means (Table 4). Significant differences among accessions and among group means were detected using the Duncan

multiple range test (Little y Hills, 1978). Pearson linear coefficients of correlation (r) and coefficients of determination (r^2; %) between total reducing activity and chlorogenic acid content were calculated using accession means for all accessions and their significance studied with an F-test (Table 5) (Little y Hills, 1978). The total reducing capacity accounted for by chlorogenic acid (%) was calculated and an ANOVA test was performed to detect differences among the different scarlet eggplant and gboma eggplant groups. The production of nitric oxide in the biological assays is expressed as the mean ± standard error values. Statistical significance was determined with an ANOVA followed by Dunnett's *t*-test for multiple comparisons.

Results and Discussion

Variation among accessions

The mean values for the total reducing capacity of scarlet eggplant and gboma eggplant collections were of 7.45 g/kg and 11.16 g/kg of chlorogenic acid equivalents (i.e., the concentration of pure chlorogenic acid concentration that would be required for accounting for this reducing activity), respectively (Table 3). For chlorogenic acid content the average values were, respectively, of 1.51 g/kg and 1.66 g/kg. These values for total reducing capacity and chlorogenic acid content reveal that, as with the common eggplant (Plazas *et al.*, 2013a; Stommel y Whitaker, 2003; Prohens *et al*., 2013; Cao *et al*., 1996), both the scarlet and gboma eggplants have high levels of total reducing activity and chlorogenic acid content, although there is not a perfect correlation between these two traits.

Our values for chlorogenic acid are similar to those obtained by Stommel and Whitaker (2003). However, Sunseri *et al*. (Sunseri *et al*., 2010) found an average value for chlorogenic acid in scarlet eggplant of around two-fold higher than our values. Differences in extraction procedures and environmental effects, which are important for phenolics content in eggplant as revealed in a recent study (San José *et al*., 2014), as well the stage of fruit harvesting might account for these differences (San José *et al*., 2014; Hanson *et al*., 2006; Luthria y Mukhopadhyay, 2006; Mennella *et al*., 2012; García-Salas *et al*., 2014). Wide ranges of variation were found for both reducing activity and chlorogenic acid content in the two collections, with differences of up to 4.4-fold and 3.2-fold in scarlet and gboma eggplants, respectively, for total

reducing capacity and of up to 21.3-fold and 10.1-fold in scarlet eggplant and gboma eggplants, respectively, for chlorogenic acid content (Table 3). This is in agreement with the results obtained by Sunseri *et al*. (2010), who found a range of variation of 49.6-fold for chlorogenic acid content in a collection of 70 accessions of scarlet eggplant. The percentage of the sums of squares of accession for both traits and in both crops was always above 80%, with differences among accessions being highly significant (P<0.001) (Table 3). A large range of variation for both reducing activity measured with the Folin-Ciocalteu reagent and for chlorogenic acid content has also been found in collections of common eggplant (Stommel y Whitaker, 2003; Prohens *et al*., 2013; Mennella *et al*., 2010; Hanson *et al*., 2006; Raigón *et al*., 2008), suggesting that, in general, reducing activity and chlorogenic acid content levels are very variable in eggplants, and therefore amenable to selection (Wricke y Weber, 1986).

Average values of the chlorogenic acid peak area percentage in the HPLC chromatograms were of 78.62% for scarlet eggplant and of 60.87% in gboma eggplant (Table 3). In all accessions the chlorogenic acid peak area accounted for more than 50% of the total peak area in the chromatogram, with maximum values of 93.3% in scarlet eggplant (in one accession of the Gilo group) and 71.5% in gboma eggplant. This is in agreement with the results of Stommel *et al*. (2003), who found that between 63.4 and 96.0% of the total phenolic acids content of 13 accesions *S. aethiopicum* and 73.0% of the total phenolics content of a single accession of *S. macrocarpon* as evaluated by HPLC corresponded to chlorogenic acid. Values obtained are also similar to those of *S. melongena* (Stommel y Whitaker, 2003; Prohens *et al*., 2007; Mennella *et al*., 2012). Differences among accessions in both collections were highly significant (P<0.001), although the percentage of sums of squares of accession was lower than for total reducing activity and chlorogenic acid content (Table 3). Also, the coefficients of phenotypic and genotypic variation for the chlorogenic acid peak area were much lower than those of total reducing activity and chlorogenic acid (Table 3).

Broad-sense heritability (H^2) values between 0.3 and 0.7 are considered as moderate; while those above 0.7 are regarded as high.

Table 3. Percentage of the total sum of squares for the effects of accession and residual, global mean, **minimum** and maximum accession means, average standard error for accession means (SE), coefficient of phenotypic variation (CV_P), coefficient of genotypic variation (CV_G), and heritability (H^2) for total reducing capacity (expressed as equivalents of chlorogenic acid, CGA), chlorogenic acid content, and percentage of peak area (for high-performance liquid chromatography at 325 nm) corresponding to chlorogenic acid for the fruit traits evaluated in a collection of 56 accessions of scarlet eggplant and 8 accessions of gboma eggplant.

	Sum of squares (%)								
Trait	**Accesion[a]**	**Residual**	**Mean**	**Minimum**	**Maximum**	**SE**	**CV_P (%)**	**CV_G (%)**	**H^2**
Scarlet eggplant (n=56)									
Total reducing capacity (equivalents of CGA; g·kg^{-1})	87.55***	12.45	7.45	3.83	16.92	0.62	28.61	21.79	0.58
Chlorogenic acid (g·kg^{-1})	82.76***	17.24	1.51	0.21	4.47	0.19	39.92	27.69	0.48
Chlorogenic acid peak area (%)	41.56***	58.44	78.62	50.30	95.30	2.82	10.06	6.07	0.36
Gboma eggplant (n=8)									
Total reducing capacity (equivalents of CGA; g·kg^{-1})	84.34***	15.66	11.16	7.15	22.69	1.09	32.37	23.82	0.54
Chlorogenic acid (g·kg^{-1})	94.89***	5.11	1.66	0.48	4.87	0.15	46.26	41.58	0.81
Chlorogenic acid peak area (%)	73.65***	26.35	60.87	50.40	71.50	2.03	9.41	5.73	0.37

[a] *** indicates significant at P<0.001.

In both the scarlet and gboma eggplant collections, H^2 values were moderate for the three traits evaluated, except for chlorogenic acid in the gboma eggplant, in which they were high (0.81) (Table 3). Prohens *et al*. (2013) obtained H^2 values of 0.50 for reducing activity measured with the Folin-Ciocalteu reagent in a collection of common eggplant, which are similar to the values obtained by us in scarlet and gboma eggplants. The H^2 values obtained for scarlet and gboma eggplant for the three traits indicates that selection will be efficient and therefore there are good prospects for a significant genetic advance in selection and breeding programmes (Wricke y Weber, 1986). This is an indication that selection for total reducing activity or chlorogenic acid content may be more efficient than selection for modifying the phenolics acid profile. However, given the positive correlation between total phenolics and chlorogenic acid content (see section "Relationship between total reducing capacity and chlorogenic acid content") selection for one of these traits will also result in the indirect selection for the other (Wricke y Weber, 1986).

Differences between groups

Of the five scarlet and two gboma eggplants groups, the highest total reducing capacity was found in the wild gboma eggplant *S. dasyphyllum* which, with a value of 22.69 g/kg of chlorogenic acid equivalents, had significantly ($P<0.05$) higher levels than the rest of groups studied (Table 4). The scarlet eggplant Kumba group ranked second, with values (12.86 g/kg) significantly higher than those of the rest of scarlet eggplant groups. When considering chlorogenic acid content, again *S. dasyphyllum* presented the highest value (4.87 g/kg), being significantly higher than that of the rest of groups (Table 4). *Solanum dasyphyllum* was followed by the scarlet eggplant group Shum (3.03 g/kg), which had values significantly higher than those of the scarlet eggplant groups Aculeatum, Gilo, and Kumba and of the gboma eggplant *S. macrocarpon* (Table 4). In particular, *S. dasyphyllum* presents values much higher than the rest of groups for both traits, indicating that this species is a source of variation of considerable interest for improving the content of the cultivated *S. macrocarpon*. However, before using *S. dasyphyllum* in breeding programmes it would be advisable to study the presence in this wild species and subsequent generations of potentially toxic compounds, like glycoalkaloids.

Table 4. Means and range (in italics) for total reducing capacity (expressed as equivalents of chlorogenic acid), chlorogenic acid content (CGA), and percentage of peak area (for high-performance liquid chromatography at 325 nm) corresponding to chlorogenic acid.

		Total reducing capacity (equivalents of CGA; g·kg^{-1})[a]		Chlorogenic acid (g·kg^{-1})[a]		Chlorogenic acid peak area (%)[a]	
Group	**N**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Scarlet eggplant	56						
Aculeatum	5	7.39 c	*6.02 - 8.64*	1.28 c	*0.70 - 2.16*	79.4 a	*76.5 - 82.3*
Aethiopicum-anguivi	6	8.01 c	*6.23 - 11.55*	2.25 bc	*1.17 - 4.47*	78.7 a	*67.6 - 87.0*
Gilo	34	6.02 c	*3.83 - 16.45*	1.46 c	*0.21 - 3.69*	79.6 a	*50.3 - 93.3*
Kumba	9	12.86 b	*9.41 - 16.92*	0.99 c	*0.23 - 1.55*	72.5 ab	*58.8 - 82.9*
Shum	2	5.87 c	*3.86 - 7.87*	3.03 b	*2.21 - 3.83*	87.6 a	*86.0 - 89.2*
Gboma eggplant	8						
S. dasyphyllum	1	22.69 a	---	4.87 a	---	50.4 c	---
S. macrocarpon	7	9.51 bc	*7.15 - 16.03*	1.20 c	*0.48 - 1.98*	62.4 bc	*54.3 - 71.5*

[a] Varietal group means within columns separated by different letters are significantly different according to the Duncan multiple range test at P≤0.05.

Also, the availability of a broad range of variation may be useful for developing populations using parents with contrasting values for the study of the inheritance, mapping QTL and validating candidate genes for reducing capacity and chlorogenic acid content (Pérez-de-Castro *et al*., 2012).

Unexpectedly, the Kumba group, which displayed the highest average value among scarlet eggplant groups for the total reducing capacity, had low values for chlorogenic acid content (Table 4). Conversely, the Shum group, which had low average values for total reducing capacity presented high chlorogenic acid contents. This results in differences among groups in the percentage of total reducing capacity accounted for by chlorogenic acid (Figure 1). In this respect, while in the Kumba group, chlorogenic acid accounts for less than 10% of the total reducing capacity, in the case of the group Shum it accounts for more than 50%. The rest of groups present values between 13.0% (*S. macrocarpon*) and 26.6% (*aethiopicum-anguivi*) (Figure 1). In common eggplant it has been found that chlorogenic acid measured by HPLC generally accounts for between 15% and 75% of the reducing activity of common eggplant measured with the Folin-Ciocalteu reagent (Mennella *et al*., 2012; Plazas *et al*., 2013b; Luthria, 2012). Similarly to our results, in common eggplant considerable differences among accessions and between groups of accessions in the percentage of reducing activity accounted for by chlorogenic acid have been found (Mennella *et al*., 2012; Plazas *et al*., 2013b).

The percentage of peak area corresponding to chlorogenic acid in the HPLC chromatogram also presented significant differences among groups (Table 4). In particular, the gboma eggplant groups had values significantly lower than those of the scarlet eggplant groups (with the exception of a non-significant difference between *S. macrocarpon* and the Kumba group). While in all groups chlorogenic acid was the predominant phenolic acid, the HPLC profiles presented considerable differences, in particular between the scarlet eggplant groups and the gboma eggplant groups (Figure 2). This confirms that differences among eggplant relatives for phenolic compounds profile are important and may have a potential use in interspecific chemotaxonomy (Wu *et al*., 2013).

The within group range of variation for total reducing capacity, chlorogenic acid content and chlorogenic acid peak area was large, in

particular for the Gilo group, which was the group with a larger number of accessions studied (Table 4).

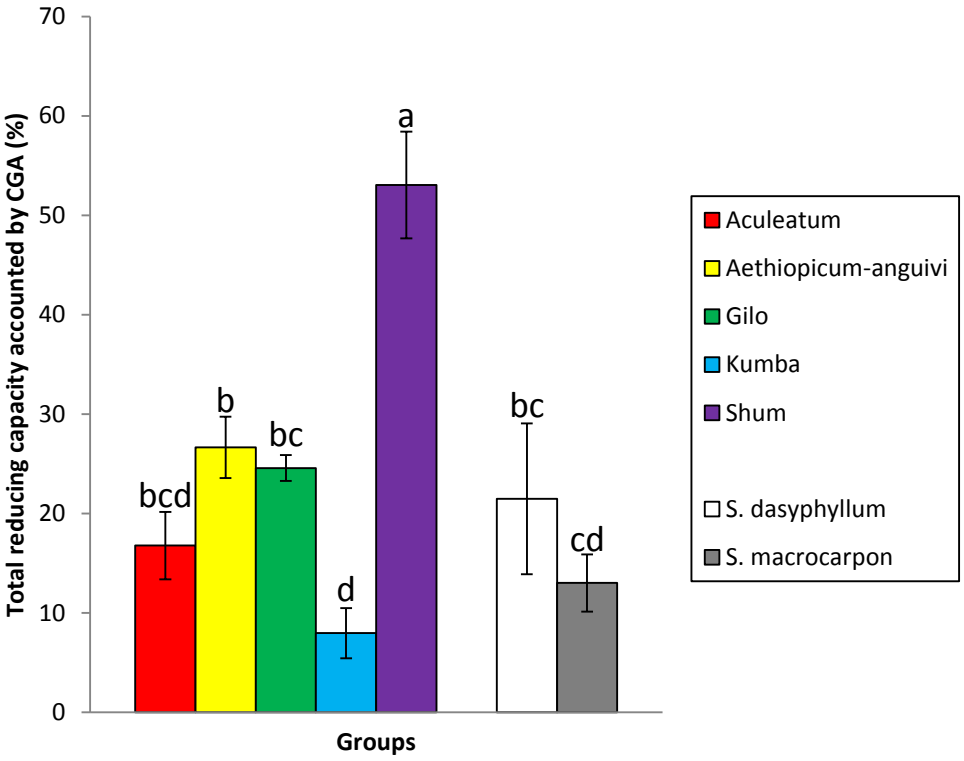

Figure 1. Percentage of total reducing capacity explained by CGA in the different scarlet eggplant (Aculeatum, *aethiopicum-anguivi*, Gilo, Kumba and Shum) and gboma eggplant (*S. dasyphyllum* and *S. macrocarpon*) groups. Bars represent ±standard error of the mean for each of the groups obtained from an ANOVA. Different letters indicate significantly different means at P≤0.05 according to the Duncan's multiple range test.

The Kumba group, despite being represented by 9 accessions, did not contain any accessions with high chlorogenic acid levels, with eight of the nine accessions in this group having values below the average value for the whole scarlet eggplant collection (1.51 g/kg). Availability of a wide range of variation within each of the groups has important implications for breeding, as it shows that it may be possible to select within each of the groups accessions with improved levels of the desired trait/s (Wricke y Weber, 1986).

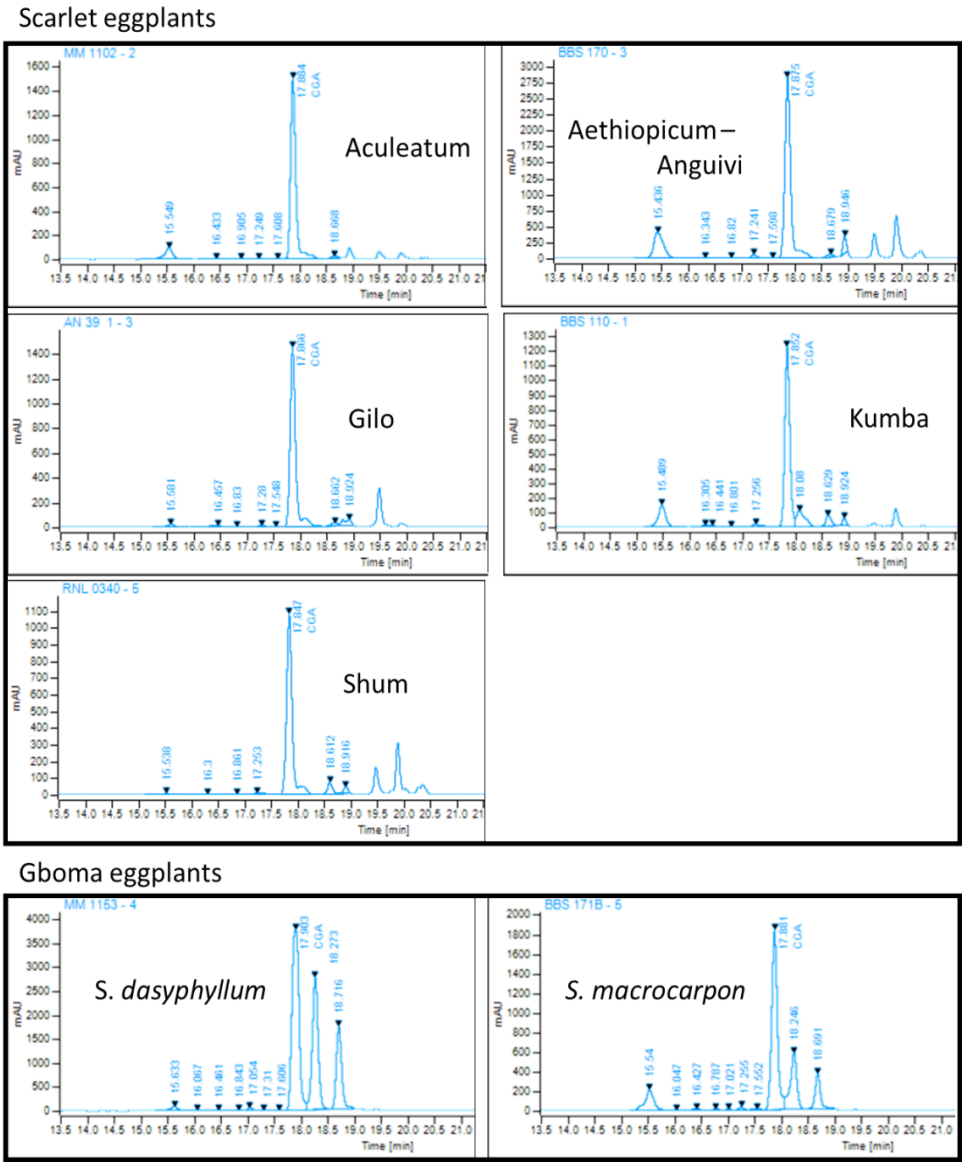

Figure 2. Representative C18-high performance liquid chromatography chromatograms of phenolic compounds (detected at 325 nm) in methanolic extracts of accessions of scarlet eggplant and gboma eggplant groups. The chlorogenic acid (CGA) peak is indicated. Note that different groups may have different peak scales.

Relationship between total reducing capacity and chlorogenic acid content

When considering the whole collection, the linear correlation coefficient between accession means for the total reducing capacity and chlorogenic acid content in all the accessions was significant, but presented a relatively low value (r=0.370) (Table 5). Consequently, the coefficient of determination (r^2) was also low, revealing that only 13.7% of the variation in total reducing activity was explained by the variation in chlorogenic acid. This is contrast with previous results in common eggplant, in which higher values for this correlation have been found (Plazas *et al*., 2013b). However, a closer examination of the data revealed that this low value might be caused by an admixture of different groups in the analysis, each of which has a different relationship pattern (Figure 3). This may result in low correlation values when all accessions are considered together (Little y Hills, 1978).

In this respect, the Kumba group presented a total reducing capacity-chlorogenic acid content relationship different from that of the other groups (Figure 1), which resulted in a non-significant correlation coefficient between total reducing capacity and chlorogenic acid content when the whole collection of scarlet eggplant accessions is considered (Table 5). However, when the correlation analysis was performed separately for each group we found that the Aculeatum, *aethiopicum-anguivi* and Gilo groups presented high within group correlation values, above 0.9 for the two former and of 0.675 for the latter (Table 5). These values are in agreement with previous results in common eggplant in which in a collection of 18 accessions the correlation between reducing activity measured with the Folin-Ciocalteu reagent and CGA was moderate at 0.633 (Plazas *et al*., 2013b).

Table 5. Coefficients of correlation (r) and determination (r^2; %), F-ratio and significance (probability of F) for the linear model for the relationship between total reducing activity and chlorogenic acid for the 64 accessions of scarlet eggplant and gboma eggplant studied.

Group	n	Coef. correlation	Coef. determination (%)	F-ratio	Prob. F
All accessions	64	0.370	13.7	9.83	0.0026
Scarlet eggplant	56	0.197	3.9	2.18	0.1453
Aculeatum	5	0.904	81.6	13.34	0.0354
Aethiopicum-anguivi	6	0.901	81.1	17.17	0.0143
Gilo	34	0.675	45.5	26.72	<0.0001
Kumba	9	-0.179	3.2	0.23	0.6451
Shum[a]	2	---	---	---	---
Gboma eggplant	8	0.893	79.7	23.59	0.0028
S. dasyphyllum[a]	1	---	---	---	---
S. macrocarpon	7	0.499	24.9	1.65	0.2548

[a]For these groups no degrees of freedom were available to evaluate the significance of the linear correlation.

The high values obtained for the total reducing activity and chlorogenic acid in the Aculeatum, Aethiopicum-anguivi and Gilo groups have implications for breeding as they indicate that in these groups selection for one of these two traits will result in indirect selection for the other (Pérez-de-Castro *et al*., 2012). However, for the Kumba group the correlation value obtained was non-significant, revealing that in this group variation in chlorogenic acid content did not contribute to explaining the variation in total reducing capacity. In consequence, compounds other than chlorogenic acid must play a major role in the antioxidant capacity of the Kumba group. When considering the *S. macrocarpon* accessions were studied separately, the correlation value was of 0.499, which was somewhat lower than that of the regular groups of scarlet eggplant (Table 5).

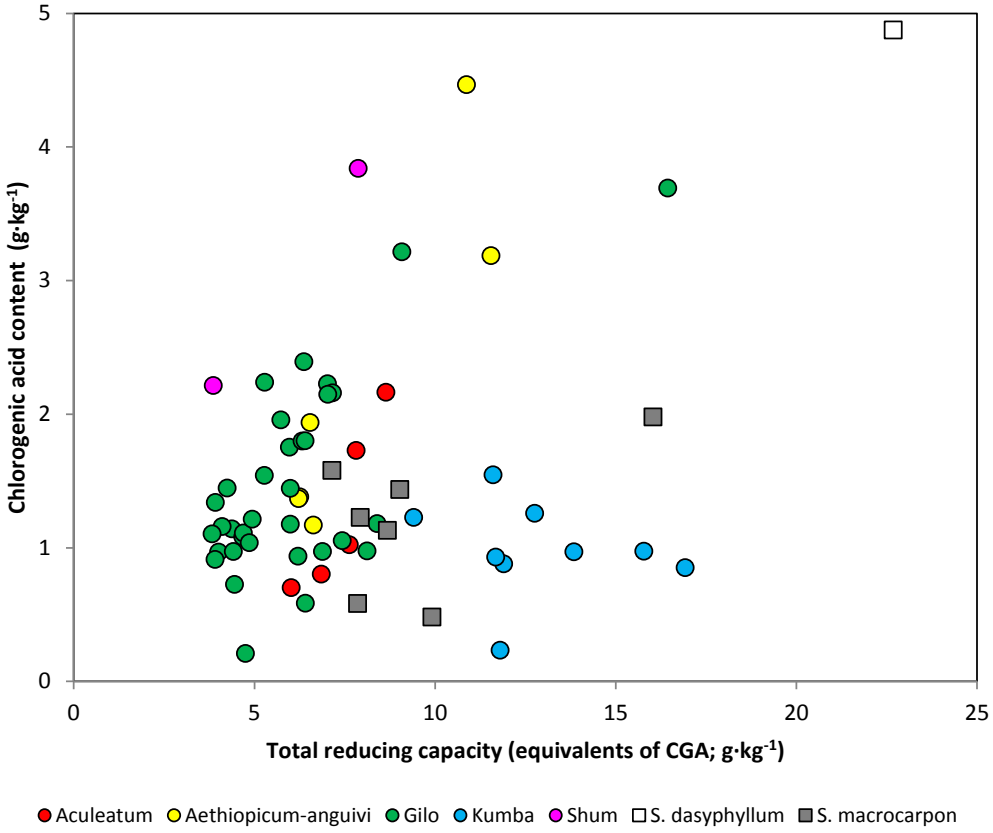

Figure 3. Relationship between the total reducing capacity (expressed as equivalents of CGA; x-axis) and chlorogenic acid (CGA) content (y-axis) in the individual accessions of the different scarlet eggplant and gboma eggplant groups.

Apart from chlorogenic acid, other phenolic acids present in the fruit different from chlorogenic acid and that were detected in the HPLC chromatogram must also have had a contribution to the reducing activity (Stommel y Whitaker, 2003; García-Salas *et al*., 2014). Phenolic acids have different reducing activities (Soobrattee *et al*., 2005), and some compounds present at low concentrations might have a relevant role in accounting for the total reducing capacity. For example, hydroxybenzoic acid has a Trolox equivalent antioxidant capacity (TEAC) more than 30 times lower than that of rosmarinic acid (Soobrattee *et al*., 2005). Also, other non-phenolic antioxidants, proteins and inorganic ions present in the eggplant fruit flesh

may react with the Folin-Ciocalteu reagent (Singleton *et al*., 1999; Everette *et al*., 2010; Sánchez-Rangel *et al*., 2013), which may contribute to the total reducing activity. However, further studies should be made to identify precisely the different compounds that play a major role in accounting for the total reducing activity apart from chlorogenic acid.

Biological activity

Both chronic inflammatory diseases and cardiovascular diseases are associated with an altered nitric oxide (NO) production, a free radical involved in many physiological processes in the human body (Wang y Mazz, 2002). Dietary polyphenols have shown to exhibit beneficial biological activities such as free-radical scavenging, regulation of enzymatic activity, and modulation of several cell signaling pathways which explain their proven antioxidant, anti-inflammatory, anticarcinogenic and preventive effects on coronary diseases (Sato *et al*., 2011; Wang y Mazz, 2002).

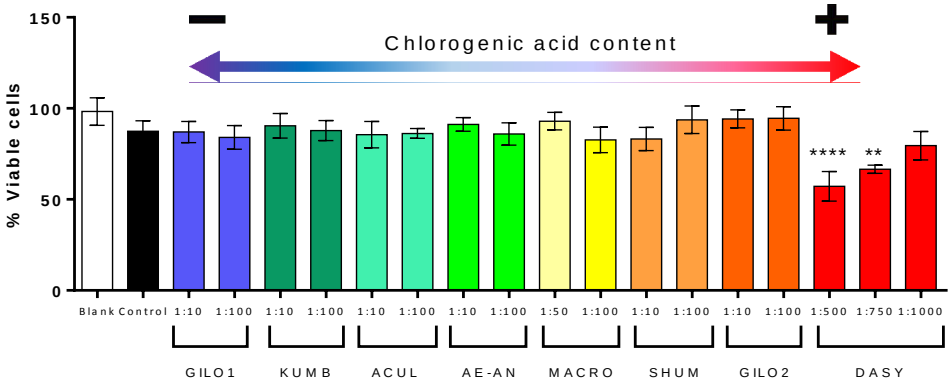

Figure 4. Percentage of viable cells of RAW 264.7 macrophages incubated in different dilutions of methanolic extracts of scarlet and gboma eggplant accessions (see Table 5 for codes description and chlorogenic acid content of individual accessions). Accessions have been ordered according to the chlorogenic acid content of the pure extracts, with lowest values to the left and highest values to the right. Bars represent ±standard error of the mean. Columns tagged with asterisks indicate that the mean values are significantly different from the control (**, P<0.01; ****, P<0.0001) according to Dunnett's multiple comparison test.

In order to test the effect of the eggplant extracts in nitric oxide production, we first evaluated the toxicity of the different extracts of several accessions to determine the non-toxic dilutions. As shown in Figure 4, ten-fold dilutions or higher showed no toxicity, except for the extracts of the gboma eggplant accessions, which had to be used at 1:50 dilution or higher in the case of *S. macrocarpon*, and of 1:1000 in the case of *S. dasyphyllum* (Figure 4). This may suggest that in the gboma eggplants, compounds other than phenolic acids may account for this toxicity. In this respect, Sánchez-Mata *et al*. (2010) found that gboma eggplants had higher glycoalkaloids content than scarlet eggplants. However, further studies should be conducted to identify the underlying cause or compounds reducing cell viability in the gboma eggplants.

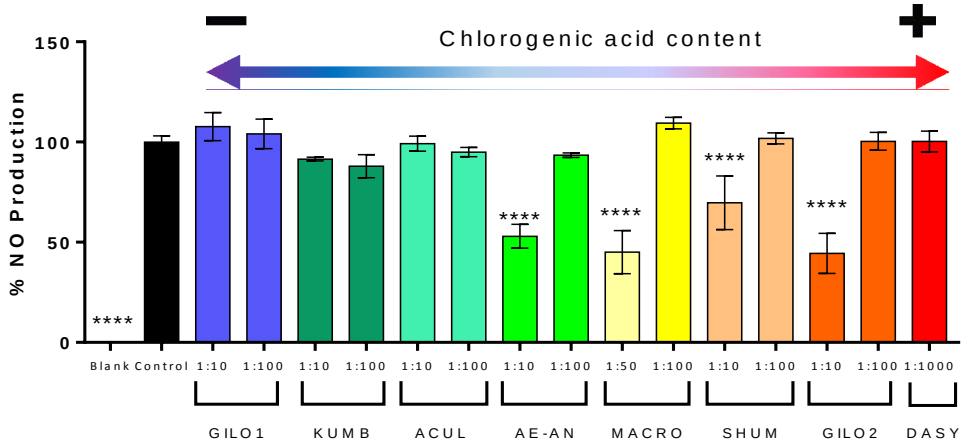

Figure 5. Percentage of NO production of RAW 264.7 macrophages incubated in different non-cytotoxic dilutions of methanolic extracts of scarlet and gboma eggplant accessions (see Table 5 for codes description and chlorogenic acid content of individual accessions). Accessions have been ordered according to the chlorogenic acid content of the pure extracts, with lowest values to the left and highest values to the right. Bars represent ± standard error of the mean. Columns tagged with asterisks indicate that the mean values are significantly different from the control (****, P<0.0001) according to Dunnett's multiple comparison test.

Hwang *et al*. (2014) recently demonstrated *in vitro* in RAW 264.7 macrophages that chlorogenic acid significantly inhibits NO production by inhibiting the inducible nitric oxide synthase without any cytotoxicity. As shown in Figure 5, our results demonstrate that those accessions with a higher

content in chlorogenic acid are able to significantly reduce about 50% the LPS-induced NO production, and this NO inhibition occurs in a dose-dependent manner. This is true in all cases except in the case of *S. dasyphyllum*. The *S. dasyphyllum* accession shows the highest total reducing activity and the highest content in chlorogenic acid (Table 4), but a very high dilution (1:1000) had to be tested given its cytotoxicity (Figure 5) and this may be the cause of the lack of inhibition of NO production.

These materials could be selected for their direct use or for being incorporated in breeding programmes aimed at developing eggplants with healthier properties.

Conclusions

We have found that scarlet eggplant and gboma eggplants present a high diversity for reducing activity and for chlorogenic acid content, which is the main phenolic acid in these eggplants. Heritability values have been moderate to high for these traits, indicating that selection will be efficient. Considerable differences have been found among and within groups of scarlet and gboma eggplants for total reducing capacity, chlorogenic acid content, and chlorogenic acid peak area, with *S. dasyphyllum* having the highest values for reducing capacity and chlorogenic acid content. In most of the groups, chlorogenic acid has been found to be correlated with reducing activity, indicating that it plays a main role in the bioactive properties of scarlet and gboma eggplants. The biological assays showed that gboma eggplants (in particular *S. dasyphyllum*) extracts had a higher cytotoxicity than those of scarlet eggplants, and that, in general, the higher the chlorogenic acid content the higher the inhibition of NO production of LPS stimulated macrophage cells. The results obtained suggest that both crops have important bioactive properties and that selection and breeding in these crops can result in scarlet and gboma eggplants with enhanced reducing activity and chlorogenic acid content as well as improved biological activity.

Acknowledgments

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3.3 Hibridación interespecífica para la mejora del contenido en compuestos bioactivos de la berenjena

3.3.1 Characterization of interspecific hybrids and first backcross generations from crosses between two cultivated eggplants (*Solanum melongena* and *S. aethiopicum* Kumba group) and implications for eggplant breeding

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Abstract

Common (*Solanum melongena* L.) and scarlet (*S. aethiopicum* L.) eggplants are cultivated for their fruits and form part of the same genepool. We have studied plant and fruit characteristics, pollen viability and seed set, phenolics content, and fruit flesh browning in accessions of *S. melongena* and *S. aethiopicum* Kumba group, as well as interspecific hybrids between these species and first backcross generations to each parental species. Respective genotypes were also characterized with 7 polymorphic SSR markers. The results demonstrate that many differences exist for plant and fruit morphology among *S. melongena*, *S. aethiopicum* and the interspecific hybrids. The latter are very vigorous and generally intermediate between the two parents, except for fruit size which is smaller (and parthenocarpic due to a high pollen sterility) than those of any of the parents. Backcross progenies also exhibited morphological variation with moderate heritability values for the attributes evaluated. Variation for fruit size was present in the backcross generations but fruits were small resulting in little variation for fruit shape. Backcross plants with moderate fertility produced seeded fruits. Primary hybrids had fruit phenolics content similar to that of *S. aethiopicum,* the parent with lowest phenolics concentration, and were heterotic for fruit flesh browning. Backcross progenies were quite variable for both traits. SSR markers did not reveal segregation distortion in the backcross generations for these interspecific hybrids. The results demonstrate that generations derived from sexual interspecific hybridization can be a powerful tool for *S. melongena* and *S. aethiopicum* Kumba group breeding.

Introduction

Common eggplant (*Solanum melongena* L.) is one of the most important vegetable crops in the world and, in consequence, has been the subject of considerable efforts in breeding for yield and quality (Daunay, 2008). However, two other cultivated eggplant species, namely the gboma (*S. macrocarpon* L.) and the scarlet (*S. aethiopicum* L.) eggplant have received little attention for genetic improvement. *S. macrocarpon* and *S. aethiopicum* are cultivated mostly in Africa for their fruits and in some cases for their leaves (Schippers, 2000).

Both *S. macrocarpon* and *S. aethiopicum* have been considered as resources of interest for the genetic improvement of *S. melongena*, as the former present some traits of interest, including tolerance to *F. oxysporum* f. sp. melongenae and resistance to *R. solanacearum* in both species (Cappelli *et al*., 1995; Collonnier *et al*., 2001a; Daunay *et al*., 1991; Hébert, 1985; Rizza *et al*., 2002), as well as resistance to spider mites in *S. macrocarpon* (Bletsos *et al*., 2004), and resistance to root-knot nematodes in *S. aethiopicum* (Hébert, 1985). Given that both gboma and scarlet eggplants are cultivated species, they do not present undesirable traits commonly present in wild relatives of eggplant. In comparison with wild eggplant relatives, both gboma and scarlet eggplants produce larger fruits, although they are usually smaller than those of the large-fruited varieties of common eggplant, present few or no prickles, and have low concentrations of saponins and glycoalcaloids (Daunay, 2008; Polignano *et al*., 2010; Sánchez-Mata *et al*., 2010). No attempts have been made to utilize *S. melongena* germplasm for breeding improved cultivars of the gboma and scarlet eggplants. Lester (1986) suggested that these three species together serve mutually as secondary genepools. For example, *S. melongena* could be a source of variation for fruit size and shape in the gboma and scarlet eggplants and might also be useful to increase fruit phenolic antioxidant content of *S. aethiopicum*, which usually presents concentrations much lower than those of *S. melongena* (Stommel and Whitaker, 2003; Prohens *et al*., 2007).

Hybrids between *S. melongena* and *S. macrocarpon* are difficult to obtain and present a high degree of sterility (Bletsos *et al*., 2004). Although a first backcross generation to *S. melongena* has been reported (Schaff *et al*., 1982), no records document introgression of *S. macrocarpon* genes into *S. melongena*. Interspecific sexual and somatic hybrids between *S. melongena* and *S. aethiopicum* are easier to produce and although they present a high degree of sterility, backcross generations to *S. melongena* with introgressions of *S. aethiopicum* have been obtained on occasion (Rizza *et al*., 2002; Toppino *et al*., 2008; Khan and Isshiki, 2010; Mennella *et al*., 2010). Therefore, generations derived from crosses between *S. aethiopicum* and *S. melongena* seem the most promising for the breeding of both crops.

S. aethiopicum is a complex species comprised of four cultivar groups (Gilo, Shum, Kumba, and Aculeatum) (Lester *et al*., 1986). The Gilo and Shum groups are grown for their subspherical to ellipsoid fruits. Leaves of the Gilo group are pubescent while those of the Shum group are glabrous and the fruits of the former are larger than those of the latter; the Kumba group has flattened large fruits with many locules and is grown for both its fruits and leaves; the Aculeatum group has many prickles and yields spherical or subspherical fruits and is used as an ornamental (Lester *et al*., 1986). *S. melongena* also consists of four groups, which are labeled E-H (Lester and Hasan, 1991). Groups E, F, and G correspond to wild and weedy forms (E and F) and primitive cultivars with small fruits (G) from India and Central Asia (E) and South East Asia (F and G), while the group H consists of large-fruited (10-20 cm long and 7-12 cm in diameter) landraces and modern cultivars known and cultivated worldwide (Lester and Hasan, 1991; Daunay, 2008; Weese and Bohs, 2010). Since group H is the economically relevant eggplant group, references to *S. melongena* in the scientific literature (and also in this paper) normally correspond with group H unless otherwise specified.

The *S. aethiopicum* Aculeatum group cytoplasm has been used to develop male-sterile lines of eggplant via sexual hybridization between *S. aethiopicum* (female) and *S. melongena* (male) followed by successive backcrosses to *S. melongena* using the latter as the male parent (Khan and Isshiki, 2010). *S. aethiopicum* Gilo and Aculeatum groups have been used to obtain somatic hybrids resistant to Ralstonia solanacearum (Daunay *et al*., 1993; Collonnier *et al*., 2001b) and to introgress Fusarium wilt resistance into *S. melongena* (Rizza *et al*., 2002; Toppino *et al*., 2008). Reports are not available describing use of the Kumba group for common eggplant breeding. Similarly, use of *S. melongena* for genetic improvement of *S. aethiopicum* has not been reported. In this respect, development of materials derived from interspecific hybridization between *S. melongena* and *S. aethiopicum* Kumba group would be of interest for the breeding of both species and could represent a first step in producing introgression lines (ILs) of *S. melongena* and *S. aethiopicum* Kumba group with discrete chromosomal regions of *S. aethiopicum* Kumba group and *S. melongena*, respectively, for gene mapping studies and eggplant breeding.

Availiability of characterization data for traits of agronomic interest in parental, hybrid and segregating generations is essential for breeding programs. In this respect, morphological descriptors for the characterization of eggplant and related species, like *S. aethiopicum*, are available as a result of the European Eggplant Genetic Resources Network (EGGNET) (van der Weerden *et al*., 2007). These descriptors have been used and validated in a number of characterizations of genetic resources and breeding materials (Prohens *et al*., 2005; Muñoz-Falcón *et al*., 2009; Polignano *et al*., 2010). Recently, a new image processing software tool, Tomato Analyzer, has been developed that allows the detailed characterization of traits related to fruit size and shape (Brewer *et al*., 2006, 2008; Gonzalo and van der Knaap, 2008; Gonzalo *et al*., 2009). Tomato Analyzer has not been applied to the characterization of eggplant fruits. Given that the fruit are the most important plant part for which both *S. melongena* and *S. aethiopicum* are cultivated, we hypothesize that Tomato Analyzer may represent a useful tool for the characterization of fruit attributes in introgressions derived from *S. melongena* x *S. aethiopicum* crosses.

Crosses involving different species of *Solanum* often present segregation distortion resulting from abnormal chromosome pairing and recombination (Kreike and Stiekema, 1997; Chetelat *et al*., 2000; Doganlar *et al*., 2002). This may cause difficulty in obtaining introgressions of specific fragments of one species in the genetic background of the other and in developing a comprehensive set of ILs representing the entire donor species genome in the genetic background of the recurrent species. Co-dominant molecular markers, like SSRs, which are currently available for eggplant molecular characterization (Stàgel *et al*., 2008; Nunome *et al*., 2010; Vilanova *et al*., 2011) may be very useful for studying if abnormal segregation exists in the materials derived from interspecific hybridization between *S. melongena* and *S. aethiopicum*.

Here we evaluate the morphological characteristics of the plant and fruit, fruit phenolics content and fruit flesh browning in *S. melongena* and *S. aethiopicum* Kumba group accessions, interspecific hybrids and backcrosses to both species. Screening with selected SSR markers was performed to assess segregation distortion in interspecific crosses between these species. Our

objective is to obtain information relevant for the genetic improvement of both crops.

Materials and methods

Plant material

Materials used consisted of two accessions of *S. melongena* (M1 and M2), two accessions of *S. aethiopicum* and the intraspecific hybrid among them (A1, A2 and A1×A2), the four possible *S. melongena* × *S. aethiopicum* hybrids, two first backcrosses of interspecific hybrids to the *S. melongena* parent, and two first backcrosses of the interspecific hybrids to the *S. aethiopicum* parent. Details on the origin and main characteristics of these materials are indicated in Table 1. Hybridization was done using the usual procedure used for eggplant (Sidhu *et al*., 2005). In short, flowers of the female parent were manually emasculated before anthesis and bagged and pollen of the male parent was deposited on the stigmas of the emasculated flowers at the time of flower opening followed by flower bagging. *S. melongena* was the female parent of the interspecific hybrids and the latter were used as female parents for obtaining the backcrosses. Synchronization of flowering was achieved thanks to the continuous flowering of both species. Many seeds with a high rate of germination were available for the interspecific hybrids. However, for the backcrosses, and in particular of the backcrosses to *S. melongena*, most of the fruits were parthenocarpic and few seeds were available. In consequence, we could only obtain seeds for four backcrosses out of the 16 possible backcross generations. For the *S. melongena* backcrosses (M1×A1)×M1 and (M2×A2)×M2, 3 and 5 seeds were available, respectively, of which 1 and 4, germinated (Table 1). For the *S. aethiopicum* backcrosses (M1×A2)×A2 and (M2×A2)×A2, 35 seeds of each backcross were used, of which 19 and 27, respectively, germinated (Table 1).

Table 4. Scarlet eggplant and gboma eggplant groups evaluated, number of accessions and typical characteristics of the fruit of each of the groups (Schippers, 2000; Lester, 1986; Lester *et al*., 1986; Bukenya y Carasco, 1994; Plazas *et al*., 2014).

Plant material	Code	n	Fruit type	Origin
Non-segregating generations				
S. melongena				
PI263727	M1	10	Semi-long, purple	Puerto Rico; local name: 'Rosita'
PI470273	M2	10	Semi-long, purple	Kalimatan, Indonesia
S. aethiopicum				
PI413783	A1	10	Very flattened, green; Kumba group	Burkina Faso
PI413784	A2	10	Very flattened, green; Kumba group	Burkina Faso
PI413783×PI413784	A1×A2	10	Very flattened, green; Kumba group	Intraspecific hybrid
S. melongena× *S. aethiopicum*				
PI263727×PI413783	M1×A1	10	Flattened, green	Interspecific hybrid
PI263727×PI413784	M1×A2	10	Flattened, green	Interspecific hybrid
PI470273×PI413783	M2×A1	10	Flattened, green	Interspecific hybrid
PI470273×PI413784	M2×A2	10	Flattened, green	Interspecific hybrid
Segregating generations				
Backcrosses to *S. melongena*				
(PI263727×PI413783)× PI263727	(M1×A1)×M1	1	Variable	First backcross
(PI470273×PI413784)×PI470273	(M2×A2)×M2	4	Variable	First backcross
Backcrosses to *S. aethiopicum*				
(PI263727×PI413784)×PI413784	(M1×A2)×A2	19	Variable	First backcross
(PI470273×PI413784)×PI413784	(M2×A2)×A2	27	Variable	First backcross

Growing conditions

Plantlets of all materials were transplanted on 14 May, 2009, to an open field plot (sandy loamy soil) in the campus of the Universidad Politècnica de València, Valencia, Spain (GPS coordinates of the field plot: lat. 39º 28' 55'' N, long. 0º 20' 11'' W) in a completely randomized design. A completely randomized design was used instead of a block design because the plot is quite uniform and previous experiments using block designs showed no block effect (Prohens *et al*., 2007; Muñoz-Falcón *et al*., 2008). Plants were spaced 1 m between rows and 0.8 m apart within the row and drip irrigated. Fertilization was applied with drip irrigation throughout the growing cycle and consisted of 80 g/plant of a 10N-2.2P-24.9K plus micronutrients commercial fertilizer (Hakaphos Naranja; Compo Agricultura, Barcelona, Spain). Standard horticultural practices for eggplant production in the Mediterranean coastal area of Spain were followed (Baixauli, 2001). No manual pollination was performed in the characterization experiment, although we frequently observed the presence of bees and bumblebees visiting flowers, and so some degree of cross pollination took place (Sambandam, 1964), which could facilitate fruit set in male sterile plants.

Plant and fruits characterization

Plant traits were measured in individual plants (n=10 for respective parental species and hybrids; see Table 1 for backcross generations) using 16 primary descriptors developed by the Eggplant Genetic Resources Network (EGGNET) (Prohens *et al*., 2005; van der Weerden and Barendse, 2007). These descriptors included vegetative (4), leaf (6), and flower and inflorescence (6) traits (Table 2). For plants that produced fruits, five representative fruits (or all fruits if less than five were available) per plant were photographed to display the characteristics and diversity of the fruits produced in respective species and generations (Figure 1). Fruits were cut longitudinally and scanned into digital images and subjected to morphometric analysis with Tomato Analyzer version 2.2.0.0 Software (Brewer *et al*., 2006). A total of 21 traits, corresponding to basic measurements (6), fruit shape index (1), blockiness (3), homogeneity (3), proximal fruit end shape (1), distal fruit end shape (3), asymmetry (3), and latitudinal section (1) were measured (Table 2). Full details on the description of each of the traits can be found elsewhere (Brewer *et al*., 2008).

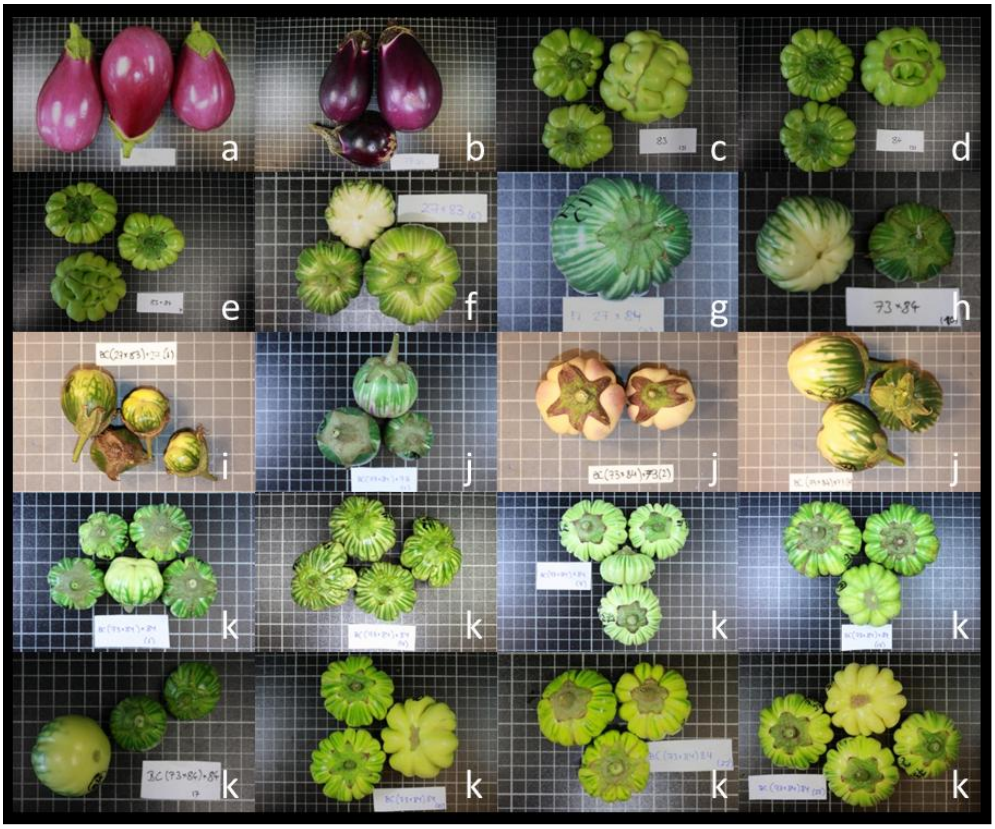

Figure 1. Fruit samples of *S. melongena* M1 (a) and M2 (b) and *S. aethiopicum* A1 (c) and A2 (d), the intraspecific *S. aethiopicum* hybrid A1×A2 (e), the interspecific hybrids M1×A1 (f), M1×A2 (g), M2×A2 (h), and of individual plants of the backcrosses to *S. melongena* (M1×A1)×M1 (i; 1 plant) and (M2×A2)×M2 (j; 3 plants) and to *S. aethiopicum* (M2×A2)×A2 (k; 8 plants).

Pollen viability and seed set

Pollen viability from three plants for each non-segregating generation and from representative plants for each of the backcrosses (in total 2 plants for the *S. melongena* backcrosses, and 10 plants for the *S. aethiopicum* backcrosses) was estimated by staining pollen grains with an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution containing 0.9% of MTT and 54% of sucrose (Khatun and Flowers, 1995). Pollen grains were observed after 1 h and grains stained deep blue were considered viable.

At least 400 pollen grains obtained from several flowers were observed per plant. Presence of seeds was evaluated in the fruits used for the morphometric analyses.

Phenolics content and browning

Fruit phenolics content was measured for each individual plant in a bulked sample derived from the fruits used for the morphometric analyses. For extraction of phenolics, 5 mL of fresh juice obtained with a domestic juicer were mixed with a 10 mL solution of acetone (70% v/v) and glacial acetic acid (0.5% v/v) and left for 24 h at room temperature. Phenolics content was determined according to the Folin–Ciocalteu procedure (Singleton and Rossi, 1965). Chlorogenic acid (Sigma-Aldrich Chemie, Steinheim, Germany) was used as standard. The phenolic acid content was expressed as chlorogenic acid equivalents in mg•kg-1 of fresh fruit flesh.

For browning measurement, fruit flesh color was measured with a Minolta CR-300 chroma-meter (Minolta Co. Ltd., Osaka, Japan) in the fruits used for the morphometric analyses. CIELAB (L*, a*, b*) color measurements were made immediately after the fruits were cut (0 min) and 10 min later. The whiteness of the fruit flesh was measured as the Euclidean distance of the color coordinates to the pure white color coordinates (L*=100; a*=0; b*=0) using the formula $DW((100-L^*)^2+a^{*2}+b^{*2}))^{0.5}$, where DW is the distance to the pure white color. The difference between DW at 10 min (DW10) and at 0 min after the fruit was cut (DW0), i.e., the increase in the distance to pure white, was used as a measure of degree of browning (DB) suffered by the fruit (DB=DW10-DW0) (Prohens *et al*. 2007). DB data of individual fruits were used to obtain an average DB value per plant.

Table 2. Plant and fruit shape traits studied and their description. Further details for the measurement of plant traits can be obtained from Prohens et al. (2005) and van der Weerden and Barendse (2007), and for measurement of fruit shape traits from Brewer et al. (2006, 2008) and Gonzalo and van der Knaap (2008).

Trait	Code	Units / Description
Plant traits		
Vegetative		
Plant height	P-Heigth	cm
Hypocotyl anthocyanin intensity	P-AnthH	0=absent; 9=very strong
Shoot apex anthocyanin intensity	P-AnthA	0=absent; 9=very strong
Number of leaves to first node	P-Leaves1	---
Leaf		
Pedicel length	L-Pedicel	cm
Blade length	L-Length	cm
Blade breadth	L-Breadth	cm
Blade apex angle	L-Apex	1=very acute (<15º); 9=very obtuse (>160º)
Blade lobing	L-Lobing	1=very weak; 9=very strong
Prickles	L-Prickles	0=none; 9=very many
Flower and inflorescence		
Number of flowers per inflorescence	F-Number	---
Corolla color	F-Color	1=greenish white; 9=bluish violet
Number of sepals	F-Sepals	---
Number of petals	F-Petals	---
Number of stamens	F-Stamens	---
Corolla diameter	F-Diameter	cm

Fruit size and shape traits		
Basic measurements		
Weight	Weight	Fruit weight (g)
Perimeter	Perimeter	Perimeter length (cm)
Area	Area	Fruit area (cm^2)
Width at mid-height	Width_MH	The width measured at ½ of the fruit's height (cm)
Maximum width	Max_Width	The maximum horizontal distance of the fruit (cm)
Height at mid-width	Height_MW	The height measured at ½ of the fruit's width (cm)
Maximum height	Max_Height	The maximum vertical distance of the fruit
Fruit shape index		
Fruit shape index external	Fruit_Shape	Ratio Max_Heigth/Max_Width
Blockiness		
Proximal fruit blockiness	P_Blockiness	Ratio of the width at the upper blockiness position to Width_MH
Distal fruit blockiness	D_Blockiness	Ratio of the width at the lower blockiness position to Width_MH
Fruit shape triangle	Triangle	Ratio of the width at the upper blockiness position to the lower blockiness position
Homogeneity		
Ellipsoid	Ellipsoid	The ratio of the error resulting from a best-fit ellipse to the area of the fruit; smaller values indicate that the fruit is more ellipsoid
Circular	Circular	The ratio of the error resulting from a best-fit circle to the area of the fruit fruit; smaller values indicate that the fruit is more circular
Rectangular	Rectangular	The ratio of the rectangle bounding the fruit to the rectangle bounded by the fruit
Proximal fruit end shape		
Proximal angle macro	P_Angle	The angle between best-fit lines drawn through the fruit perimeter on either side of the proximal end point
Distal fruit end shape		
Distal angle macro	D_Angle	The angle between best-fit lines drawn through the fruit perimeter on either side of the distal end point
Distal indentation area	D_Indentation	Ratio of the area of the distal indentation to the total area of the fruit, multiplied by 10
Distal end protrusion	D_Protrusion	Ratio of the area of the distal protrusion to the total area of the fruit, multiplied by 10
Asymmetry		
Obovoid	Obovoid	Calculated according to the formula provided in the tomato Analyzer Manual (Brewer *et al*. 2008). The higher the value, the greater is the area of the fruit below mid height.
Ovoid	Ovoid	Calculated according to the formula provided in the tomato Analyzer Manual (Brewer *et al*. 2008). The higher the value, the greater is the area of the fruit above mid height.
Width widest position	Width_W	Ratio of the height at which the Max_Width occurs to the Max_Height
Latitudinal section		
Lobedness degree	Lobedness	The standard deviation of distances from the center of weight to the perimeter, multiplied by 100

Molecular characterization

Twelve genomic SSR markers that we developed (Vilanova *et al*., 2011) were tested for polymorphism among parental species. Seven polymorphic SSRs were further tested in individual parents, hybrids, and backcrosses (Table 3).

Table 3. Primer sequences, expected size, and linkage group (Vilanova *et al*., 2010, 2011) of the seven SSR markers used for molecular characterization of the materials studied.

SSR locus	Primer sequence (5'-3')	Expected size (bp)	Linkage group
CSM7	F- CGACGATCACCTTGATAACG	201	3
	R- CCTAAATGCAGAGTTTCCAAAG		
CSM12	F- CAATGGTATGTCTCCACTCGTC	210	6
	R- AGCTAAACATGAGATGCCGAT		
CSM16	F- ACGTGCCATTTCAAACTTGG	224	1
	R- TCCTTTTCTTGAGCTGAATTTG		
CSM21	F- ATTTGACAACTGCCACATCG	245	1
	R- ACCATGGGAAAGCGTATGAG		
CSM26	F- CCCAGAAAAGGCTCATTGTTAG	230	3
	R- GTCGAGGCAATCCAAATTACTC		
CSM32	F- TCGAAAGTACAGCGGAGAAAG	248	4
	R- GGGGGTTTGATTTTCATTTTC		
CSM54	F- ATGTGCCTCCATTCTGCAAG	227	9
	R- TGGGTGGGATGCTGAGTAAG		

Genomic DNA from each plant of the segregating (backcross) generations and from a mixture of young leaves from six plants for the non-segregating (parents and interspecific hybrids) generations was extracted from young leaves with the DNeasy Plant Mini Kit (Quiagen Inc., Valencia, California, USA) using the protocol recommended by the manufacturer. The DNA quality was assessed after electrophoresis on a 0.8 % agarose gel, and the DNA concentration of each of the samples was determined with a Nanodrop ND-1000 (Nanodrop Technologies, Wilminton, Delaware, USA) spectrophotometer. DNA was diluted to a concentration of 10 ng/µl in order to perform PCRs. SSRs were tested following the M13-tail PCR method of

Schuelke (2000), which involves an M13-tailed forward primer used in combination with a standard M13 primer dye-labeled with FAM, NED, PET or VIC fluorophores at its 5'-end.

The PCR reaction consisted of 1× PCR buffer, 1.5mM $MgCl_2$, 0,2 mM dNTPs, 0.04 units Taq DNA polymerase, 0.05 µM forward primer, 0.25 µM reverse primer, 0.2 µM M13-labeled primer, 10 ng DNA, and distilled H_2O in an 10 µl total reaction volume. Amplifications were carried out in an Eppendorf thermocycler with an initial step at 94 ºC for 3 min, 35 cycles of 94 ºC for 30 s, 58 ºC for 45 s, 72 ºC for 1 min and a final 10 min extension at 72 ºC. PCR products were separated in an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA). The analysis was performed using Genscan and Genotyper (Applied Biosystems) software.

Data analyses

Data for the plant and fruit morphology and of fruit phenolics content and flesh browning were subjected to analysis of variance (ANOVA). Given that segregating generations are expected to be more variable than non-segregating generations if genetic variation exists among parents, two ANOVAs were made for each of the traits studied, one which included the non-segregating (parents and hybrids) generations and another which included the segregating (first backcross) generations. The average (pooled) variance and standard deviation for each of the traits studied was obtained from the corresponding ANOVAs. Therefore, one variance value was obtained for the 90 plants of non-segregating generations and another one for the 51 plants of the segregating generations. Broad-sense heritability (H2) of each trait was calculated as H2=VG/(VG+VE) using the variances obtained for the segregating and non-segregating generations. The variance of the segregating generations represented both genetic (VG) and environmental (VE) variances, whereas the variance of the non-segregating generations, which were genetically homogeneous as determined by SSR markers (see below), estimated only the environmental (VE) variance. For those traits where considerable mean differences were observed among generations and for which a relationship between the mean and standard deviation was observed, in order to avoid scaling effects, the log transformed data were used to estimate H2. Principal

components analyses (PCA) were performed for standardized plant and fruit traits using pairwise Euclidean distances among individuals.

For SSR data, chi-square ($\chi 2$) tests were performed to assess the goodness-of-fit of the segregation of individual markers in the first backcrosses to a 1 homozygous: 1 heterozygous segregation model. Heterogeneity $\chi 2$ tests for the results obtained for individual markers were performed in order to evaluate if pooled data for individual markers could also be tested for goodness-of-fit to a 1:1 distribution (Little and Hills, 1978). Where applicable, Yates correction was used to calculate the $\chi 2$ values. In order to evaluate the regular and independent segregation of SSR markers in first backcross generations, we compared the observed frequency of individual plants displaying markers in heterozygosis with the expected frequencies of a theoretical binomial distribution corresponding to the actual (0.46 homozygous : 0.54 heterozygous) and theoretical (1 homozygous: 1 heterozygous) segregations for these generations.

Results

Plant traits

Few differences were found between the *S. melongena* M1 and M2 parents. Differences ($p<0.05$) were mostly related to leaf size, number of flowers per inflorescence, and number of petals and stamens per flower (Table 4). Similarly, few differences were found between the *S. aethiopicum* A1 and A2 parents and their intraspecific hybrid A1×A2. However, considerable differences were found between the parents we used of *S. melongena* and those of *S. aethiopicum*, as well as among the parents of each species and the interspecific hybrids (Table 4). The materials of used by us *S. melongena* and *S. aethiopicum* differed mostly in anthocyanin pigmentation of the hypocotyl and apex which was present in *S. melongena* parents and absent in *S. aethiopicum* parents, leaf size (L-Pedicel, L-Length and L-Breadth) which was larger in *S. melongena*, leaf apex and leaf lobing which were greater in *S. aethiopicum*, number of flowers per inflorescence which were also greater in *S. aethiopicum*, in particular with respect to *S. melongena* M2, and flower color and flower diameter which were both greater in *S. melongena*. Interspecific hybrids had greater plant height, in particular in hybrids with *S. melongena* M1, stronger

anthocyanin pigmentation, more pointed leaf apex, and higher number of flowers per inflorescence than any of the parents; also, interspecific hybrids, in particular the M1×A1 hybrid, presented prickles in the leaves, while the parents did not. For the remaining traits, interspecific hybrids were intermediate between both parents, although for leaf size traits and flower color they were more similar to *S. melongena* parents and for leaf lobing to *S. aethiopicum* parents.

Average values for most traits of the first backcross generations were intermediate between those of the respective parents, *S. melongena* or *S. aethiopicum*, and the interspecific hybrids (Table 4). However, the backcrosses to *S. melongena* had smaller values ($p<0.05$) than either *S. melongena* or the interspecific hybrids for leaf size traits and the number of flower sepals, petals, and stamens. For both *S. melongena* and *S. aethiopicum* backcrosses, plant height is more similar to the *S. melongena* and *S. aethiopicum* recurrent parent than to the interspecific hybrids; in the case of the *S. melongena* backcrosses, flower diameter is more similar to the interspecific hybrids, while for *S. aethiopicum* backcrosses, the number of flowers per inflorescence is more similar to *S. aethiopicum*. Contrary to what occurs in the interspecific hybrids, leaf prickles are absent in *S. melongena* backcross individuals and are present in just a few individuals of one of the backcrosses to *S. aethiopicum*. Backcross generations were more variable than non-segregating generations, and in all cases, with the exception of leaf prickles the pooled standard deviations were greater in the former than in the latter. As a result, estimates of broad sense heritability (H2) reached values of up to 0.66 for the number of flowers per inflorescence. H2 values of 0.5 or above were also obtained for other traits like plant height, anthocyanins in the hypocotyl and apex and leaf blade breadth.

Table 4. Mean values for the plant traits evaluated for each of the parental generations, interspecific hybrids and first backcrosses (BC). Average (pooled) standard deviations (SD) for non-segregating and segregating generations, as well as broad-sense heritability values (H^2) are also presented.

	Non-segregating generations										Segregating generations					
	S. melongena		*S. aethiopicum*			*S. melongena* × *S. aethiopicum*					BC *S. melongena*		BC *S. aethiopicum*			
Trait[a]	M1	M2	A1	A2	A1×A2	M1×A1	M1×A2	M2×A1	M2×A2	SD	(M1×A1)×M1	(M2×A2)×M2	(M1×A2)×A2	(M2×A2)×A2	SD	H^2
N	10	10	10	10	10	10	10	10	10		1	4	19	27		
P-Height	118.9	119.9	110.1	116.1	109.6	175.8	181.4	131.9	144.7	12.43	125.0	128.3	111.0	124.3	24.71	0.50
P-AnthH	4.4	5.8	0.0	0.0	0.0	8.0	7.2	6.2	6.6	1.17	5.0	7.0	2.7	1.2	2.80	0.58
P-AnthA	4.4	6.4	0.0	0.0	0.0	8.0	7.4	6.4	6.6	1.18	5.0	7.0	2.6	1.3	2.84	0.59
P-Leaves1	10.8	11.3	9.6	8.7	8.8	9.9	10.4	12.0	9.9	1.74	10.0	11.5	8.3	10.4	2.39	0.27
L-Pedicel	9.0	6.7	4.6	4.2	6.4	6.5	7.5	6.5	7.1	0.79	4.6	4.9	4.0	5.4	1.28	0.38
L-Length	24.8	27.1	19.3	21.7	21.5	22.7	25.0	20.5	22.3	1.43	18.0	21.3	20.4	21.5	2.76	0.48
L-Breadth	15.1	16.1	12.8	12.0	13.7	14.6	15.5	13.0	14.5	0.96	15.5	13.2	12.7	14.0	1.91	0.50
L-Apex	5.1	4.6	5.6	5.8	5.8	4.7	3.6	3.2	3.6	0.60	5.6	2.9	4.6	4.5	1.01	0.41
L-Lobing	4.6	5.0	6.4	5.6	6.8	6.2	6.4	5.8	6.4	0.88	5.0	6.5	7.0	6.3	0.96	0.08
L-Prickles	0.0	0.0	0.0	0.0	0.0	2.4	0.4	0.1	0.3	0.49	0.0	0.0	0.1	0.0	0.14	0.00
F-Number	2.8	1.4	3.3	2.9	3.3	7.1	6.6	6.6	6.3	0.66	4.0	5.8	3.6	3.3	1.91	0.66
F-Color	5.6	5.6	3.4	3.0	3.0	5.0	5.0	4.8	4.8	0.69	7.0	5.0	3.7	3.5	0.90	0.24
F-Sepals	6.8	6.2	6.4	6.6	6.5	6.2	6.3	5.6	5.6	0.34	5.6	5.7	6.1	6.0	0.58	0.43
F-Petals	7.1	5.8	6.5	6.5	6.5	6.1	6.2	5.5	5.6	0.33	5.4	5.4	6.1	5.9	0.51	0.35
F-Stamens	7.1	5.9	5.6	6.7	7.2	6.1	6.1	5.6	5.6	0.45	5.6	5.6	6.4	6.2	0.77	0.43
F-Diameter	5.3	4.9	2.9	2.6	2.7	3.8	3.7	3.5	3.5	0.25	3.9	3.5	3.2	3.1	0.42	0.41

[a]Descriptions of each trait are listed in Table 2.

The first and second components of the plant morphology PCA account for 32.1% and 18.7% of the total variation, respectively. The third component accounted for 10.2% of the total variation, and its inclusion in the analyses did not improve the interpretations. The first component was positively correlated with pigmentation of the plant and flower, leaf and flower size, plant vigour (P-Height and P-Leaves1), number of flowers per inflorescence and leaf prickles, and negatively with leaf lobing and leaf blade apex angle and with the number of flower parts (Figure 2). Traits having a greater positive correlation with the second component included number of flowers per inflorescence, leaf lobing, and plant height, while those having a greater negative correlation included the number of flower parts, leaf and flower size, and leaf blade apex. Related traits, like those referring to pigmentation, leaf size, or number of flower parts plot together in the PCA (Figure 2).

Projecting individual accessions on the plant traits PCA plot clearly separates *S. melongena* (positive values of the first component and negative for the second), *S. aethiopicum* (negative for the first component), and the interspecific hybrids (positive for the first component and positive or small negative values for the second component) (Figure 3). The *S. melongena* backcrosses cluster with the interspecific hybrids, while the backcrosses to *S. aethiopicum* mostly plot in the area intermediate between the interspecific hybrids and *S. aethiopicum* or overlap with *S. aethiopicum*. The PCA graph also shows that backcross generations are dispersed over a greater area of the graph than each of the non-segregating generations, indicating a higher variation in the former than in the latter (Figure 3).

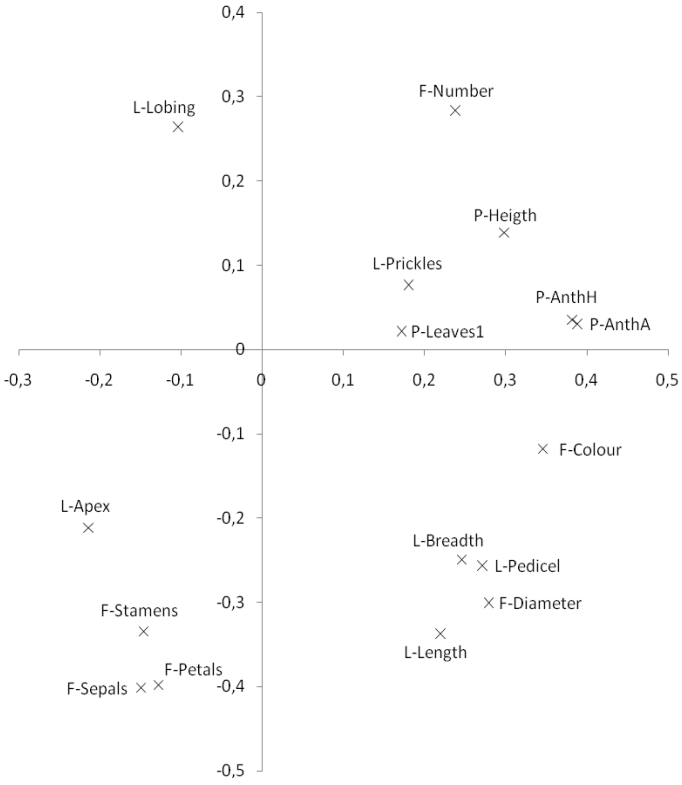

Figure 2. Principal components analysis of plant traits (see Table 2) for *S. melongena* and *S. aethiopicum* parents, hybrids and backcrosses. First (X-axis) and second (Y-axis) components of the PCA account for 32.1% and 18.7% of the total variation, respectively.

Pollen viability and fruit set

All *S. melongena* and *S. aethiopicum* parental plants and intraspecific hybrids were highly male and female fertile as evidenced by pollen stainability (>85%) and fruits with many seeds. The interspecific hybrids had low pollen stainability (0-2%) and only 11 (seven of M1×A1, two of each of M1×A2 and M2×A2 and none of M2×A1) of the 40 interspecific hybrid plants (27.5%) produced several fruits, all of which were seedless. Backcrosses were quite variable for pollen stainability, with values ranging from 1% to 62% in the 12 plants measured. Twenty-seven of 51 first backcross plants (52.9%) produced fruits. Although many of these were seedless, 9 plants of the backcrosses to *S. aethiopicum* (2 of the [M1×A2]×A2 backcross and 7 of the [M2×A2]×A2

backcross) and 3 of the backcrosses to *S. melongena* (1 of the [M1×A1]×M1 backcross and 2 of the [M2×A2]×M2 backcross) produced some seeded fruits. The remainder of the backcross plants that yielded fruit did not produce seed.

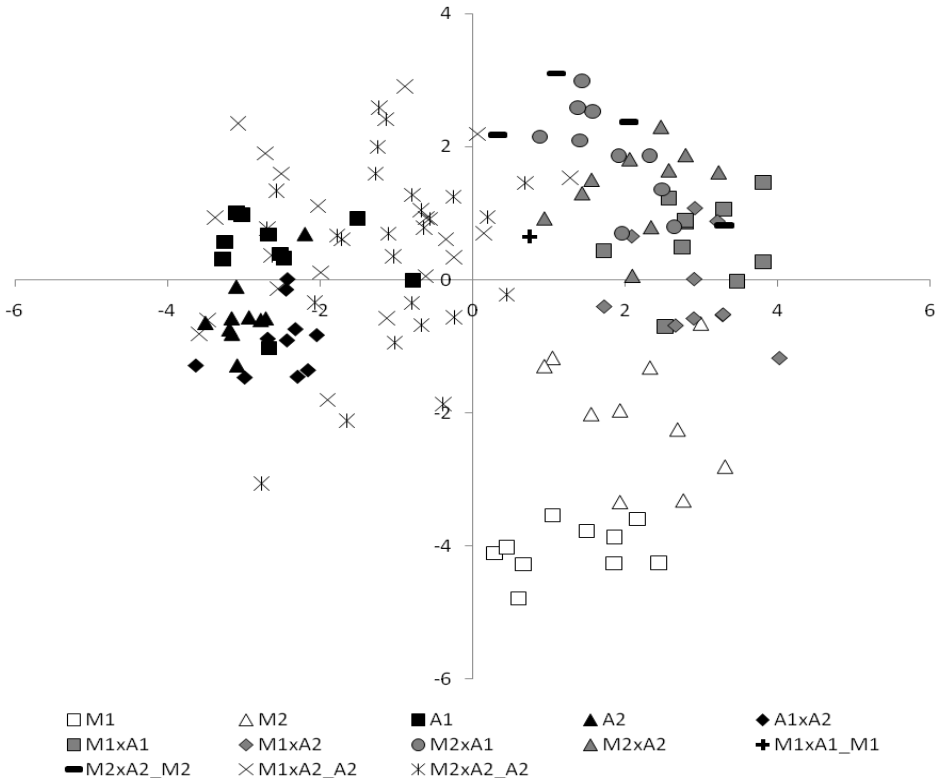

Figure 3. Similarities based on 16 plant traits (see Table 2) among the individual plants of the *S. melongena* (M1 and M2) and *S. aethiopicum* (A1 and A2) parent lines, intraspecific *S. aethiopicum* hybrid (A1×A2), interspecific *S. melongena* x *S. aethiopicum* hybrids (M1×A1, M1×A2, M2×A1, and M2×A2), and backcrosses to *S. melongena* ((M1×A1)×M1 and (M2×A2)×M2) and *S. aethiopicum* ((M1×A2)×A2 and (M2×A2)×A2) represented on the first (X-axis) and second (Y-axis) components of the PCA (32.1% and 18.7% of the total variation, respectively).

Fruit size and shape traits

Fruit size and shape traits could only be evaluated for those plants that produced fruit. Representative fruit for the different generations studied are displayed in Figure 1. Similar to plant traits, few differences ($p<0.05$) were found between the two *S. melongena* parents, among the two *S. aethiopicum*

parents and the intraspecific hybrid between them, and among the interspecific hybrids (Table 5). Differences between the *S. melongena* parents were mostly related to a somewhat larger fruit size (e.g., Area, Max_Width, and Max_Height) and a greater distal end protrusion of M2 in comparison to M1. For *S. aethiopicum*, the most important differences were the higher proximal fruit blockiness of A2 with respect to A1 and A1×A2 and the lower Circular values (i.e, more circular shape) of the hybrid A1×A2 with respect to its parents. The most relevant differences among the interspefic hybrids were caused by a larger fruit size (e.g., Area, Max_Width, and Max_Heigth) of M1×A1, smaller distal angle of M1×A2, and higher distal indentation of M2×A2.

Many differences ($p<0.05$) were found between the *S. melongena* and *S. aethiopicum* parents, as well as between the parents and the interspecific hybrids. In comparison to the *S. aethiopicum* parents, fruits of the *S. melongena* parents were much larger (larger Perimeter, Area, and Max_Height), more elongated (Fruit_Shape), with a lower proximal fruit blockiness and higher distal blockiness, less triangular, less ellipsoid (i.e., larger values of Ellipsoid), less circular (i.e., larger values of Circular), lower proximal angle, smaller distal indentation and higher distal protrusion, more obovoid (higher Obovoid and lower Ovoid) and greater in width at the widest position. Fruits of interspecific hybrids were smaller than those of either parent, with lower values for Perimeter, Area, Width_MH, Max_Width, Height_MW, and Max_Height. Interspecific hybrids also had a smaller lobedeness degree than any of the parents. For other fruit traits, interspecific hybrids were mostly intermediate between parents, although they had Ellipsoid and Circular values more similar to those of *S. melongena*, and like *S. aethiopicum*, no distal end protrusion (D_Protrusion).

Table 5. Mean values for fruit traits of the parental generations, interspecific hybrids and first backcrosses (BC). Average (pooled) standard deviations (SD) for non-segregating and segregating generations, as well as broad-sense heritability values (H^2) are also presented.

	Non-segregating generations									Segregating generations					
	S. melongena		*S. aethiopicum*			*S. melongena* × *S. aethiopicum*				BC *S. melongena*		BC *S. aethiopicum*			
Trait[a]	M1	M2	A1	A2	A1×A2	M1×A1	M1×A2	M2×A2	SD	(M1×A1)×M1	(M2×A2)×M2	(M1×A2)×A2	(M2×A2)×A2	SD	H^2
N	10	10	10	10	10	7	2	2		1	3	8	15		
Weight	283.0	345.1	118.5	107.7	92.4	64.1	40.9	23.1	64.06	7.1	36.3	68.5	56.6	24.74	0.21[b]
Perimeter	41.8	45.1	27.7	27.0	25.8	22.1	19.2	16.0	3.94	10.5	18.5	22.6	20.9	3.16	0.20[b]
Area	107.4	120.4	39.3	39.9	33.4	32.2	23.8	17.0	14.64	7.7	23.3	32.2	28.3	7.69	0.19[b]
Width_MH	8.9	9.6	9.5	9.7	9.0	7.2	6.3	5.3	1.04	3.1	5.4	7.7	7.1	1.15	0.30[b]
Max_Width	9.3	10.0	9.6	9.8	9.2	7.3	6.4	5.3	1.06	3.2	5.5	7.7	7.2	1.16	0.30[b]
Height_MW	14.4	15.1	4.1	4.1	3.6	4.9	4.1	3.6	1.32	3.0	5.0	4.6	4.3	0.53	0.00[b]
Max_Height	14.7	15.5	5.5	5.1	4.8	5.4	4.4	3.9	1.42	3.1	5.3	5.1	4.8	0.61	0.00[b]
Fruit_Shape	1.60	1.56	0.57	0.52	0.53	0.74	0.69	0.73	0.154	0.96	0.98	0.66	0.68	0.080	0.04[b]
P_Blockiness	0.57	0.58	0.65	0.80	0.70	0.63	0.71	0.67	0.099	0.54	0.67	0.69	0.70	0.042	0.00
D_Blockiness	0.72	0.72	0.52	0.64	0.54	0.71	0.69	0.75	0.063	0.69	0.65	0.69	0.68	0.039	0.00
Triangle	0.79	0.82	1.35	1.32	1.38	0.91	1.04	0.90	0.289	0.81	1.04	0.99	1.03	0.066	0.00
Ellipsoid	0.92	0.88	0.47	0.58	0.46	0.90	0.89	0.92	0.081	0.95	0.94	0.85	0.87	0.079	0.00
Circular	0.87	0.89	0.38	0.33	0.24	0.86	0.82	0.86	0.071	0.97	0.96	0.72	0.77	0.115	0.39
Rectangular	0.48	0.48	0.51	0.58	0.52	0.54	0.54	0.56	0.030	0.49	0.53	0.54	0.55	0.026	0.00
P_Angle	123.5	138.3	210.5	221.8	207.3	164.1	157.9	159.4	31.18	144.6	167.2	174.8	177.7	23.78	0.00
D_Angle	128.4	131.2	144.9	150.0	149.1	123.2	80.1	122.8	33.38	201.1	151.0	138.8	149.4	25.52	0.00
D_Indentation ($x10^{-3}$)	1.0	0.2	10.8	14.4	19.6	2.2	7.5	18.8	10.73	9.3	6.7	6.5	17.5	19.91	0.46
D_Protrusion ($x10^{-3}$)	19.0	65.5	0.0	0.0	0.0	0.0	0.0	0.0	47.70	0.0	0.0	0.0	0.0	0.00	0.00
Obovoid ($x10^{-3}$)	245.4	227.5	27.2	12.6	21.6	123.1	109.4	123.5	39.64	167.7	94.9	73.7	62.8	41.16	0.10[b]
Ovoid ($x10^{-3}$)	1.3	2.6	152.5	169.2	173.3	33.1	0.0	16.5	47.6	28.5	51.6	54.8	51.6	39.5	0.00[b]
Width_W	0.63	0.61	0.44	0.44	0.43	0.52	0.51	0.53	0.031	0.54	0.49	0.48	0.49	0.024	0.00
Lodebness	11.5	10.7	10.4	10.3	10.7	5.8	6.3	5.5	2.81	2.7	3.4	7.1	6.7	1.44	0.00

[a]Descriptions of each trait are listed in Table 2.

[b]In order to avoid scaling effects caused by the fact that for these traits means of each generation are proportional to standard deviations, H^2 values were calculated using log transformed data.

Fruit size traits (perimeter, Area, Width_MH, Max_Width, and Heigth_MW) for backcross generations ranged from similar, to intermediate to smaller than the interspecific hybrid or recurrent parent (Table 5). For other fruit traits, similar to plant traits, average values for fruit of backcross generations were intermediate between those of their respective parents, *S. melongena* or *S. aethiopicum* and the interspecific hybrids (Table 5). However, fruits of the backcrosses to *S. melongena* were smaller than those of the backcrosses to *S. aethiopicum*. Fruit of backcrosses to *S. melongena* were less circular (i.e., larger Circular values) and had a lower lobedness than fruits of *S. melongena* or the interspecific hybrids, had a higher distal angle, and were less obovoid (i.e., smaller Obovoid and larger Ovoid values) than either parent. In the case of the backcrosses to *S. aethiopicum*, Ellipsoid and Circular values were more similar to those of the interspecific hybrids in comparison to *S. aethiopicum*. Variation within backcross generations for most traits was lower than that for non-segregating generations, likely a result of small fruit size. Transformation of data to avoid scaling effects resulted in more reliable estimates of H2 for fruit size traits and for traits where wide differences existed among generation means. Trait heritabilities were moderate to low, ranging from 0 for 13 out of 21 traits, to 0.46 for distal indentation area. Additional traits with moderate heritability included Circular (0.39), fruit width (0.30 for Width_MH and Max_Width), fruit perimeter (0.20) and fruit area (0.19).

The first and second components of the PCA for fruit size and shape account for 50.0% and 24.0% of the total variation, respectively. For the first component, traits with the highest positive correlation values were mostly related to fruit size (Perimeter, Area, Height_MW, Max_Heigth, Width_W), as well as fruit shape index and Circular (i.e., less circular fruits) and obovoid shape (Obovoid). First component traits with the largest negative correlation values were related to ovoid, triangular and rectangular shape, as well as to proximal blockiness (Figure 4). The traits with the highest positive correlation with the second component of the PCA included fruit size traits (Perimeter, Area, Width_MH, Max_Width) as well as the degree of lobedness and ovoid shape. Traits with the largest negative correlation with this second component included Circular (i.e., less circular fruits) and distal blockiness (Figure 4). Plotting the accessions on the PCA graph shows *S. melongena* with positive

values for the first component and intermediate/high values for the second component, *S. aethiopicum* with negative values for the first component and intermediate/high values for the second, and the interspecific hybrids with intermediate values for the first component and low values for the second (Figure 5). Similar to plant data, the *S. melongena* backcrosses plot coincident with the interspecific hybrids, while the backcrosses to *S. aethiopicum* generally plot coincident witht the interspecific hybrids or intermediate between the interspecific hybrids and *S. aethiopicum*. Contrary to the plant traits, the backcrosses do not show a greater dispersion than several non-segregating generations (Figure 5).

Figure 4. Principal components analysis of fruit traits evaluated (see Table 2) for *S. melongena* and *S. aethiopicum* parents, hybrids and backcrosses. First (X-axis) and secondY-axis) components of the PCA account for 47.6% and 23.3% of the total variation, respectively.

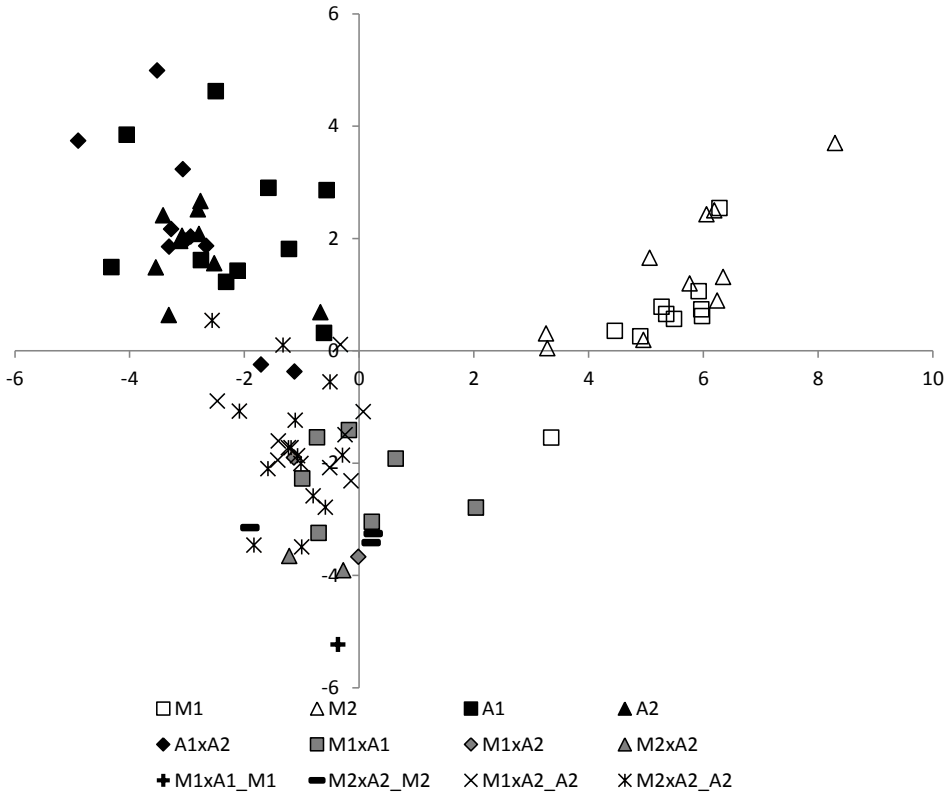

Figure 5**.** Similarities based on 22 fruit traits (see Table 2) among the individual plants of the *S. melongena* (M1 and M2) and *S. aethiopicum* (A1 and A2) parent lines, intraspecific *S. aethiopicum* hybrid (A1×A2), interspecific *S. melongena* x *S. aethiopicum* hybrids (M1×A1, M1×A2, and M2×A2), and backcrosses to *S. melongena* ((M1×A1)×M1 and (M2×A2)×M2) and *S. aethiopicum* ((M1×A2)×A2 and (M2×A2)×A2) represented on the first (X-axis) and second (Y-axis) components of the PCA (47.6% and 23.3% of the total variation, respectively).

Phenolics and browning

Phenolics content of the fruits of the *S. melongena* parents was much higher, with average values above 500 mg•kg-1, than that of the fruits of the *S. aethiopicum* parents, with average values below 200 mg•kg-1 (Table 6). However, important variations were found among plants of the non-segregating generations, in particular for the *S. melongena* parents (Figure 6). Phenolics content of interspecific hybrids was also low, consistent with that of the *S. aethiopicum* parents. For the backcrosses to *S. melongena* and *S.*

aethiopicum, phenolics content was skewed towards that of the recurrent parents. Heritability of the phenolics content in the backcross generations was low (H2=0.20).

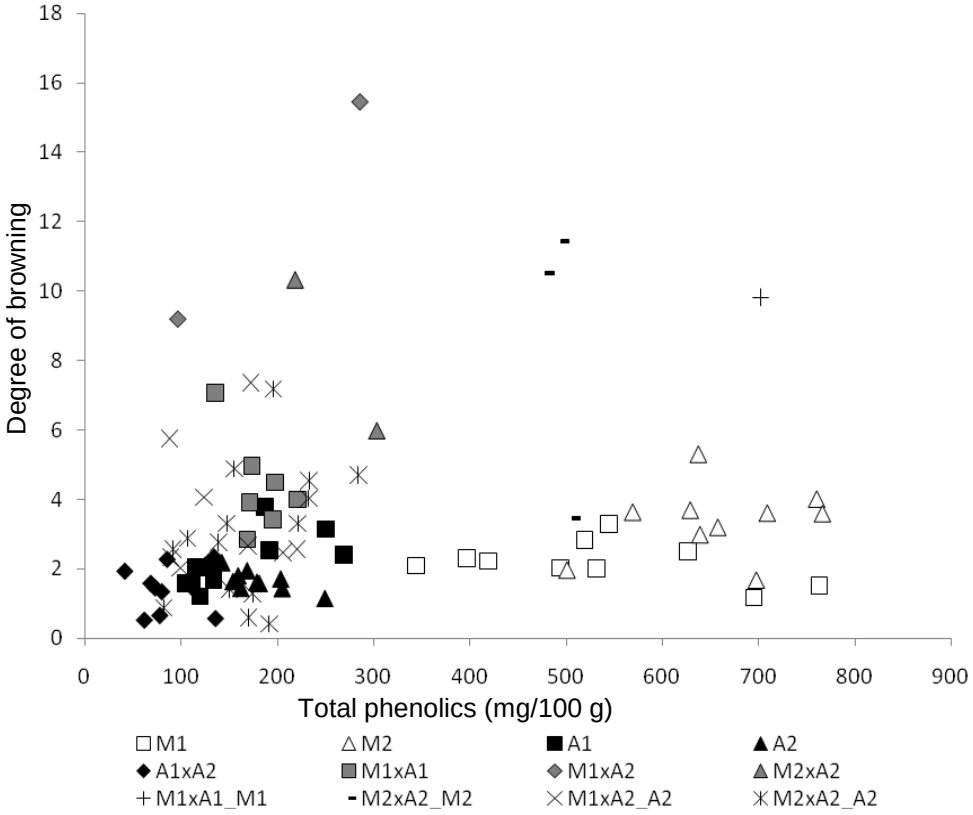

Figure 6. Relationship between total phenolics content and the degree of browning of fruit flesh among individual plants of the *S. melongena* (M1 and M2) and *S. aethiopicum* (A1 and A2) parent lines, intraspecific *S. aethiopicum* hybrid (A1×A2), interspecific hybrids *S. melongena* x *S. aethiopicum* hybrids (M1×A1, M1×A2, M2×A1, and M2×A2), and backcrosses to *S. melongena* ((M1×A1)×M1 and (M2×A2)×M2) and *S. aethiopicum* ((M1×A2)×A2 and (M2×A2)×A2).

The degree of browning for cut fruit of *S. melongena* and *S. aethiopicum* was low, especially for *S. aethiopicum* (Table 6). However, hybrids had very high browning values. For backcrosses, flesh browning was much higher than that of the recurrent parent, especially for *S. melongena*. For the backcross generations, a moderate positive relationship ($r=0.68$: $P<0.001$) occurred between phenolics content and browning (Figure 6).

Table 6. Mean values for total phenolics content and browning (degree of browning; DB) evaluated for parental lines, interspecific hybrids and first backcrosses (BC). Average (pooled) standard deviations (SD) for non-segregating and segregating generations, as well as broad-sense heritability values (H^2) are also presented.

	Non-segregating generations									Segregating generations					
	S. melongena		*S. aethiopicum*		*S. melongena* × *S. aethiopicum*					BC *S. melongena*		BC *S. aethiopicum*			
Trait	M1	M2	A1	A2	A1×A2	M1×A1	M1×A2	M2×A2	SD	(M1×A1) ×M1	(M2×A2) ×M2	(M1×A2) ×A2	(M2×A2) ×A2	SD	H^2
N	10	10	10	10	10	7	2	2		1	3	8	15		
Phenolics (mg·kg^{-1})	533.2	656.6	161.7	180.1	87.1	180.4	191.2	261.2	74.38	701.9	493.1	146.2	171.6	52.97	0.20[a]
Browning (DB)	2.20	3.36	2.21	1.64	1.40	4.39	12.32	8.16	1.095	9.82	8.47	3.66	2.98	2.233	0.51

[a]H^2 values were calculated using log transformed data.

Molecular characterization

The seven SSR markers tested produced amplification products in both *S. melongena* parents, while for *S. aethiopicum*, one of the markers (CSM12) was null in both parents. SSR fingerprints of hybrids exhibited alleles of both parents, with the exception of CSM12 in which only the *S. melongena* allele was present. For the first backcrosses to *S. melongena* and *S. aethiopicum*, the segregation values for each of the SSR markers was compatible with a 1:1 ratio for homozygous:heterozygous alleles of the recurrent parent (Table 7). χ^2 heterogeneity tests for individual markers in respective generations supported marker homogeneity and pooling of marker data. Similar to individual markers, pooled data also support a 1:1 ratio for homozygous: heterozygous SSR marker distribution and denoted lack of segregation distortion in the backcross generations (Table 7).

Table 7. Number of individuals in respective first backcross (BC) generations to *S. melongena* and *S. aethiopicum* displaying SSR markers homozygous (Hom.) and heterozygous (Het.) for the recurrent parent allele for individual and pooled marker data. χ^2 test for goodness-of-fit to a 1:1 distribution and for heterogeneity of pooled data are shown.

SSR Marker	BC *S. melongena* (n=5)				BC *S. aethiopicum* (n=46)			
	Hom.	Het.	χ^2	Prob. χ^2	Hom.	Het.	χ^2	Prob. χ^2
CSM7	2	3	0.000	1.000	19	27	1.065	0.302
CSM12	---	---	---	---	24	22	0.022	0.883
CSM16	4	1	0.800	0.371	17	29	2.630	0.105
CSM21	3	2	0.000	1.000	20	26	0.543	0.461
CSM26	4	1	0.800	0.371	22	24	0.022	0.883
CSM32	2	3	0.000	1.000	28	18	1.761	0.185
CSM54	4	1	0.800	0.371	18	28	1.761	0.185
Heterogeneity χ^2 test								
Total			2.400	0.879			7.804	0.350
Pooled	19	11	1.633	0.201	148	174	1.941	0.164
Heterogeneity			0.767	0.979			5.863	0.434

Combined SSR marker data for *S. aethiopicum* backcrosses are compatible with observed and expected 1:1 homozygous:heterozygous backcross segregation ratios for SSR markers in individual plants, denoting independent segregation of the SSR markers tested (observed 0.46

homozygous:0.54 heterozygous, P=0.62; expected 1 homozygous : 1 heterozygous, P=0.42) (Figure 7).

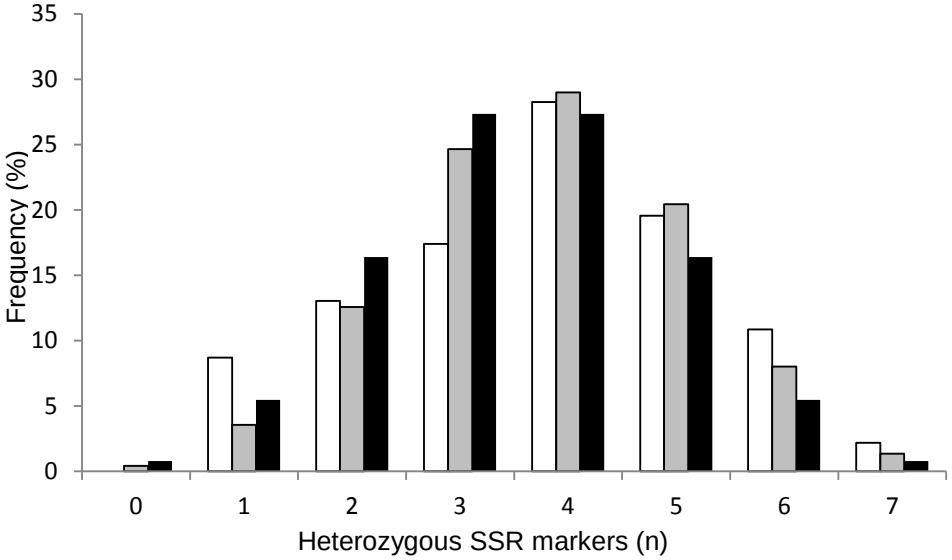

Figure 7. Observed frequencies in all *S. aethiopicum* backcrosses of individuals heterozygous for SSR markers (white bars), and frequencies corresponding to a binomial distribution for observed 0.46 homozygous: 0.54 heterozygous segregation (grey bars), and for expected 1 homozygous:1 heterozygous segregation (black bars).

Discussion

Crop relatives constitute genetic resources of interest for improving and increasing the genetic variation of domesticates. In this respect, wild relatives have been frequently used in the genetic improvement of major crops, mostly as sources of resistance to biotic and abiotic stresses (Zamir, 2001). However, the use of related domesticates for improving cultivated species is less frequent, as it is not common to find related crops which, like the cultivated Brassicas (Snowdon, 2007), form part of the same primary or secondary genepools. One of these cases corresponds to the three African eggplant species, which include the common, gboma, and scarlet eggplant (Daunay, 2008). Given that the greatest compatibility among these three species is between the common and scarlet eggplant (Collonnier *et al*., 2001a; Daunay, 2008; Oyelana and Ugborogho, 2008), we evaluated the interest of interspecific hybrids and their first backcrosses with *S. melongena* and *S.*

aethiopicum Kumba group to explore the feasibility of breeding between these two crops. Contrary to the *S. aethiopicum* Gilo and Aculeatum groups, to our knowledge the Kumba group has not been exploited for common eggplant breeding. Eggplant breeders have commonly utilized the less important domestic crop, in this case *S. aethiopicum*, for the improvement of the predominant domesticated commodity, *S. melongena* and neglected reciprocal improvement of *S. aethiopicum*.

S. melongena and *S. aethiopicum* have been frequently intercrossed and high seed set is usually obtained (Behera and Singh, 2002; Oyelana and Ugborogho, 2008; Khan and Isshiki, 2010). Seeds of interspecific hybrids are typically viable and produce highly vigorous plants (Behera and Singh, 2002; Oyelana and Ugborogho, 2008; Gisbert *et al*., 2011). However, sexual hybrids often have a high degree of sterility (Daunay *et al*., 1993; Isshiki and Taura, 2003). Development of progenies with increased fertility from hybrids has been obtained by sexual crosses using the interspecific hybrids as female parents (Khan and Isshiki, 2010) or by means of somatic hybridization followed by anther culture of the hybrids or of progrenies obtained after crossing the tetraploid somatic hybrids with tetraploid *S. melongena* (Daunay *et al*., 1993; Rizza *et al*., 2002; Toppino *et al*., 2008; Mennella *et al*., 2010). In our case, by using the recurrent parents as males first backcross seeds, which we have used here for the characterization of the plants and fruits, could be obtained. Although the number of seeds available to us for the *S. melongena* backcrosses was very low, seed counts for the backcrosses to *S. aethiopicum* were comparatively large. Contrary to the crossing barriers encountered with utilization of *S. aethiopicum* for improvement of *S. melongena*, our results support utilization of *S. melongena* for *S. aethiopicum* Kumba group improvement. In agreement with Khan and Isshiki (2010), we have found here that fertility in the first backcross generations improved and we have found plants with moderate fertility and seed set under open field conditions. Under these same conditions, the interspecific hybrids did not seed seeded fruits.

EGGNET plant descriptors (Prohens *et al*., 2005; Muñoz-Falcón *et al*., 2009; Polignano *et al*., 2010) proved to be of great utility for obtaining detailed morphological characterization of the parents of *S. melongena* and *S. aethiopicum*, as well as of their interspecific hybrids and segregating backcross

generations, confirming the utility of these descriptors in exotic cultivated forms of the species. Tomato Analyzer, which has been used successfully for fruit characterization of tomato and other crops (Brewer *et al*., 2006, 2008; Gonzalo and van der Knaap, 2008; Gonzalo *et al*., 2009), had not previously been evaluated for characterization of eggplant fruit. In our case, it has allowed for detailed characterization of fruit size and shape which is of great relevance for the improvement of both species, and demonstrates the utility of this tool for eggplant breeding. In any case, Tomato Analyzer characterization does not replace the recording of simple traits of great agronomic interest, like fruit weight; instead, it allows obtaining information additional and complementary to them.

Interspecific hybrids obtained from *S. melongena* x *S. aethiopicum* were transgressive for several traits with values above or below those obtained for any of the parents. For example, interspecific hybrids were very vigorous, in particular the hybrids involving one of the *S. melongena* parents (M1). In this respect, Gisbert *et al.* (2011) found that interspecific hybrids between *S. melongena* and *S. aethiopicum* are of interest as eggplant rootstocks due to the high degree of vigor, earliness and yield that they confer to the scion. Collonnier *et al*. (2001b) found that somatic hybrids between *S. melongena* and *S. aethiopicum* produced plants that were more vigorous than any of the parents. The differences we found in the current study for vigor among interspecific hybrids suggests that exploiting variation in combining ability for hybrid vigor will produce superior rootstocks.

Transgressive segregation was also evident for prickles. Interspecific hybrids between *S. melongena* x *S. aethiopicum* had some prickles, while the parents were unarmed. Similarly, Schaff *et al*. (1982) found that interspecific hybrids between *S. melongena* and *S. macrocarpon* had more prickles than any of the parents. Lester (1986) reported that crosses between prickle-free plants of *S. macrocarpon* and *S. aethiopicum* Kumba group produced F1 plants with prickles and suggested that different loss-mutations had occurred in these two species. A similar situation in our study may have occurred for the number of inflorescences, where hybrids produced inflorescences with more flowers than the parents. In this respect, the wild ancestors of *S. melongena* and of *S. aethiopicum* (*S. incanum* and S. anguivi, respectively) have more flowers per

inflorescence than most of the cultivated varieties (Lester *et al*., 1986; Lester and Hasan, 1991). Collonnier *et al*. (2001b) reported that somatic hybrids between *S. melongena* and *S. aethiopicum* Gilo and Aculeatum groups had more flowers per plant than any of the parents.

Fruit of interspecific hybrid plants was smaller than those of any of the parents. We attribute this to the concurrence of two factors. First, fruits of interspecific hybrids were seedless and in Solanaceae, parthenocarpic fruits are smaller than seeded fruits (Cuartero *et al*., 1987; Prohens and Nuez, 2001; Kikuchi *et al*., 2008). Second, smaller hybrid fruit size suggests that there are different genes affecting fruit size in both species so that the alleles for large fruit size are recessive (Doganlar *et al*., 2002). Lester (1986) reported that the "gigantic" features of *S. aethiopicum* Kumba group are recessive to the smaller features of the wild ancestor S. anguivi. Our results are similar to those found in other groups of *S. aethiopicum*. For example, Daunay *et al*. (2003) and Oyelana and Ogunwenmo (2009) found that the fruit size of interspecific sexual hybrids between these two species was smaller than that of any of the parents and that very few fruits were produced per plant. However, somatic hybrids between *S. melongena* and *S. aethiopicum* Gilo and Aculeatum groups had fruit weights intermediate between both species and in many cases the somatic hybrids set fruits with viable seeds (Collonnier *et al*., 2001; Rizza *et al*., 2002; Daunay *et al*., 2003). Conversely, dihaploids from these somatic hybrids had low fertility and parthenocarpic fruits which generally were smaller than any of the parents (Rizza *et al*., 2002), indicating that fertility restoration and seed set likely contribute to increased fruit size.

Little information is available in the literature regarding interspecific hybrid backcross generations to *S. melongena* and *S. aethiopicum*. First backcrosses to *S. aethiopicum* had plant characteristics generally intermediate between the hybrids and the parents, as is reflected in the data of the individual traits as well as in the PCA analyses. This contrasts with backcrosses to *S. melongena*, where plant characteristics were skewed to the hybrids. Interestingly, the backcross generations display a loss in plant vigor observed in interspecific hybrids, the number of flowers is considerably reduced, and the presence of prickles is present in just a few individuals, demonstrating rapid recovery of the characteristics typical of the recurrent parent. For plant traits,

the backcross generations were more variable than the non-segregating generations, demonstrating that selection within the first backcross generation can be efficient. For fruit traits, we found that the average fruit size of the first backcross generations was, similar to the case of interspecific hybrids, smaller than that of any of the parents. However, we found that most fruits size traits in the backcross generations were less variable than the non-segregating generations, which is reflected in low heritability values and in the limited area of the PCA plots covered by individual plants. When transformed data were used to correct for scaling effects we found a moderate heritability for these traits indicating that selection for fruit size can also be efficient in these generations. Contrary to fruit size traits, fruit shape traits were generally no more variable than the non segregating generations, and for many traits the estimates of heritability were low (<0.3) or 0. We attribute this phenomenon not to lack of genetic variation for fruit shape genes, but to the fact that small fruited varieties of both crops (*S. melongena* and *S. aethiopicum*) tend to be more uniform in fruit shape than large fruited varieties. Similar observations were observed in tomato (Brewer *et al*. 2007). Furthermore, we found that in the interspecific hybrids and backcrosses to *S. aethiopicum*, fruits were very similar in shape to the recurrent parent, suggesting dominance for *S. aethiopicum* fruit shape.

Measurements of fruit phenolics content in the parental lines confirms the occurrence of low total fruit phenolics in *S. aethiopicum* relative to *S. melongena* (Stommel and Whitaker, 2003; Prohens *et al*., 2007; Mennella *et al*., 2010) and denote that *S. melongena* could be utilized for improving the phenolics content of *S. aethiopicum*. Low fruit phenolics in interespecific hybrids suggest dominance of the *S. aethiopicum* alleles. However, backcroses to *S. aethiopicum* had low phenolics content and backcrosses to *S. melongena* had high values similar to the recurrent parent, suggesting that few genes are implicated in the differences observed for phenolics content. Mennella *et al*. (2010) found that introgression lines of *S. aethiopicum* Aculeatum and Gilo groups in a *S. melongena* genetic background had phenolics content similar to that of the *S. melongena* recurrent parents, also suggesting that few genes are implicated in the differences observed. The high variation found for phenolics content by us and also by Luthria *et al*. (2010) in non-segregating generations of eggplant suggests that clonal replicates of individual plants and or marker

assisted selection should be used in breeding programmes aimed at introgressing the high phenolics content of *S. melongena* into the *S. aethiopicum* genetic background.

In the case of flesh browning, interspecific hybrids generally had higher values than any of the parents, suggesting complementation for polyphenol oxidase enzymes from *S. melongena* and *S. aethiopicum* may have taken place. In the backcrosses, we found that some plants had browning values similar to those of the recurrent parents, while others had values similar to those of the hybrids, which leads us to speculate that some of the backcross plants with high browning might have two forms of the enzyme while others only have the recurrent parent form and resultant lower browning. Although we did not find substantial difference between *S. melongena* and *S. aethiopicum* accessions for flesh browning, Mennella *et al*. (2010) reported much higher PPO activity in *S. melongena* than in *S. aethiopicum*, indicating that differences exist in the activity of the PPO that is expressed in the fruit in both species.

Despite the fact that interspecific hybrids were highly sterile and a number of backcross plants were also sterile, we did not find segregation distortion for the SSR markers studied. This indicates that polyploidy or other chromosome abnormalities that affect the number of chromosomes did not occur. Although a high level of segregation distortion has been found in interspecific crosses of *S. melongena* with S. linneanum (16%) and *S. incanum* (23%) (Doganlar *et al*., 2002; Vilanova *et al*., 2010), our results indicate that segregation distortion in sexual crosses of *S. melongena* with *S. aethiopicum* is low and similar to those of sexual intraspecific eggplant crosses (6.5%) (Barchi *et al*., 2010). However, our data have to be taken with caution, as it would be advisable to us a larger number of markers spread throughout all the genome to confirm that no large values of segregation distortion are found. In any case, our preliminary results suggest that synthesis of a comprehensive set of *S. melongena*/*S aethiopicum* introgression lines may be feasible. It is important to note that Barchi *et al*. (2010) found segregation distortion in a dihaploid eggplant population obtained after crossing an *S. melongena* cultivar with an *S. melongena*/*S. aethiopicum* introgression line. On the other hand, the F2 generation of the same cross showed much less segregation distortion, suggesting that dihaploid populations, like the ones used by Mennella *et al*.

(2010) to obtain introgression lines of *S. aethiopicum* in the *S. melongena* genome may not be appropriate for capturing the full range of genetic variation present in the parental lines.

In conclusion, we have found that interspecific sexual hybridization between *S. melongena* and *S. aethiopicum* Kumba group may be a useful tool for the genetic improvement of both crops. The use of EGGNET descriptors, Tomato Analyzer software, as well as the measurement of phenolics content and browning are powerful tools to help breeders in selecting useful recombinants in backcross generations. Heritability values obtained suggest that considerable improvements can be obtained for many divergent traits of interest between *S. melongena* and *S. aethiopicum* Kumba group. Since segregation distortion was not observed, introgression of specific traits or the development of sets of introgression lines will be facilitated. The information obtained will be of great utility to eggplant breeders, especially for those using interspecific hybridization to transfer traits of interest among species. Finally, the fact that it is possible to obtain large backcross populations to *S. aethiopicum* suggests that it will be easier to use *S. melongena* to improve *S. aethiopicum* than viceversa. In this way, apart from the high phenolics content of *S. melongena* many other traits present in *S. melongena*, like parthenocarpy, purple fruit colour, or low content in saponins (Daunay, 2008) could be introgressed into the genetic background of *S. aethiopicum*, through the backcrossing of the hybrids with *S. aethiopicum*. Our results provide a valuable example for use of an extensively bred crop for genetic improvement of a neglected crop.

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3.3.2 Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant (*Solanum melongena*) and its wild ancestor (*S. incanum*)

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Abstract

Solanum incanum, the wild ancestor of eggplant (*S. melongena*), has been considered as a source of variation for high content of phenolic acid conjugates in breeding programs aimed at improving the functional quality of eggplant. We have evaluated the morphological and phenolic acids content in an interspecific family including *S. incanum* (P1), *S. melongena* (P2), their interspecific hybrid (F1), progeny from the selfing of the F1 (F2), and the backcross of the F1 to P2 (BC1P2). Many morphological differences were found between parents, while the F1 was intermediate for most traits. However, F1 plants were taller and pricklier, and presented higher fruit flesh browning than any of the parents. F2 and BC1P2 were morphologically highly variable and the results obtained suggest that a rapid recovery of the characteristic combination of *S. melongena* traits can be achieved in a few backcross generations. Segregation for prickliness was found to be compatible with simple genetic control, prickliness being dominant over non-prickliness. A total of 16 phenolic acid conjugates were studied, of which chlorogenic acid (5-*O*-(*E*)-caffeoylquinic acid) was the most common compound in all samples, averaging 77.8% of all hydroxycinnamic acid derivatives. Contents of total phenolic acid conjugates were much higher in *S. incanum* than in *S. melongena* fruit flesh, and no major differences were found in the profile of phenolic acids among parents. The interspecific hybrid (F1) was intermediate between the two parents in phenolic acids content. Non-segregating generations presented considerable variation in phenolic acids content, but the range of variation was wider in segregating F2 and BC1P2 generations. Additive genetic effects were the most important in explaining the results obtained for the phenolic acids content. A number of BC1P2 plants presented a good combination of phenolic acids content and fruit weight or flesh browning. Overall, the results demonstrate that improvement of functional quality in *S. melongena* can be obtained using *S. incanum* as a donor of alleles for high phenolic acids content.

Introduction

Eggplant (Solanum melongena L.) is one of the vegetables ranked highest in total antioxidant capacity, which is attributed to its high content of phenolics (Cao *et al*., 1996; Hanson *et al*., 2006; Okmen *et al*., 2009; Akanitapichat *et al*., 2010; Lo Scalzo *et al*., 2010). The main phenolics in eggplant fruit are hydroxycinnamic acid (HCA) conjugates, predominantly

chlorogenic acid (5-O-(E)-caffeoylquinic acid; 5-CQA), which constitutes up to 95% of the total phenolic acids present in the fruit flesh (Stommel and Whitaker, 2003; Whitaker and Stommel, 2003; Singh *et al*., 2009). In addition, purple pigmented eggplant accessions contain anthocyanins derived from delphinidin, in the fruit epidermis (Azuma *et al*., 2008). These anthocyanins also have potent antioxidant activity (Azuma *et al*., 2008), but their concentration in eggplant fruit is relatively small compared with HCA conjugates present in the flesh.

Chlorogenic acid has many beneficial properties for human health, including free radical scavenging, anti-carcinogenic, cardioprotective, anti-obesity, anti-inflammatory, analgesic, and antipyretic activities, and it also regulates the level of glucose in blood (Sawa *et al*., 1998; Triantis *et al*., 2005; dos Santos *et al*., 2006; Lee and Zhu, 2006; Kwon *et al*., 2008; Cho *et al*., 2010; Dai *et al*., 2010; Coman *et al*., 2012). Potential health benefits, including anti-HIV activity, have also been identified for other minor HCA conjugates in fruits of eggplant and wild relatives (McDougall *et al*., 1998; Ma *et al*., 2011). Furthermore, chlorogenic acid and other HCA conjugates present in eggplant are highly stable (Friedman & Jürgens, 2000; Lo Scalzo *et al*., 2010). In fact, it has been reported that the levels of bioavailable phenolic acids in eggplant subjected to different cooking treatments are even higher than those present in the raw product (Lo Scalzo *et al*., 2010).

Given the increasing interest in vegetables with a higher content of functional compounds, the development of eggplant cultivars with increased amounts of hydroxycinnamic acids in general, and of chlorogenic acid in particular, in the fruit flesh is warranted (Stommel and Whitaker, 2003; Prohens *et al*., 2007). However, oxidation of phenolics causes browning of the fruit flesh after cutting and exposure to air, which is an undesired trait (Queiroz *et al*., 2008). Therefore, selection against fruit flesh browning in breeding programmes has resulted in the indirect selection for low phenolics content. As a consequence, modern varieties of eggplant often have a lower concentration of phenolic acids in comparison with traditional local varieties (Prohens *et al*., 2007).

In order to be efficient, breeding programmes aimed at increasing the phenolics content of eggplant fruit require germplasm resources with sufficiently high levels of variation for these compounds. Although several fold differences in the content of phenolic acid conjugates within the cultivated species have been reported (Stommel and Whitaker, 2003; Hanson *et al*., 2006; Prohens *et al*., 2007; Okmen *et al*., 2009; Akanitapichat *et al*., 2010), the highest content has been found in wild relatives of eggplant, including *S. incanum* L. (Stommel and Whitaker, 2003; Ma *et al*., 2010, 2011). *S. incanum* is the wild ancestor of eggplant (Lester and Hasan, 1991; Meyer *et al*., 2012) and hybrids with the cultivated eggplant *S. melongena* are easily obtained. Those hybrids are completely fertile and present regular meiosis and segregation of markers (Lester & Hasan, 1991; Vilanova *et al*., 2010; Daunay, 2012). Therefore, *S. incanum* represents a viable source of variation for phenolics content that can be effectively introduced into breeding programmes aimed at increasing eggplant fruit phenolics content. In addition, *S. incanum* presents other traits of interest that may be useful in eggplant breeding. These attributes include resistance to Fusarium oxysporum and tolerance to abiotic stresses such as drought (Yamakawa and Mochizuki, 1979; Lester and Hasan, 1991).

One of the disadvantages of including *S. incanum* as source of variation for phenolics content in an eggplant breeding programme is that many unfavourable traits from the agronomic and commercial point of view are present in this wild species, including the presence of prickles, small fruit, and lack of anthocyanins in the skin (Lester and Hasan, 1991). These traits have to be selected against and removed in the backcross breeding programme, which may be difficult depending on the number of genes and genetic control involved in each trait (Fita *et al*., 2010). Furthermore, the genes of interest in the wild species that are responsible for the traits of interest might be linked to unfavourable genes (linkage drag). Therefore, as in other breeding programmes in eggplant involving interspecific hybridization (Prohens *et al*., 2012), breeding for high phenolics content in eggplant must take into account not only phenolics content in segregating generations between *S. melongena* and *S. incanum* but also morphological and agronomic traits of interest.

Here we evaluate the inheritance of morphological attributes that contribute to fruit quality, as well as the content of phenolic acid conjugates, in segregating F2 and backcross generations resulting from an interspecific cross between *S. incanum* and *S. melongena*. The objective was to obtain information of relevance for improvement of the functional quality of eggplant as well as to assess the feasibility of developing materials of commercial interest from interspecific crossings between *S. melongena* and its wild relative *S. incanum*.

Material and Methods

Plant material

One interspecific family consisting of one parental accession of *S. incanum* (MM577; P1), one parental accession of *S. melongena* (ANS26; P2), the interspecific hybrid between them (ANS26×MM577; F1), the selfed hybrid (F1×F1; F2) and the first generation hybrid backcrossed to the *S. melongena* parent (ANS26×F1; BC1P2). The *S. incanum* parent originated in Israel, and its fruit are small and green; the *S. melongena* parent is a landrace from Spain with large purple fruit. Six plants were evaluated for each of the P1, P2, and F1 non-segregating generations. For the F2 and BC1P2 generations, 41 and 64 plants were evaluated, respectively. Both P1 and P2 are genetically uniform and highly homozygous as revealed with SSR markers (Vilanova *et al*., 2010).

Growing conditions

Plantlets of all materials were transplanted on 6 May, 2009, to an open field plot (sandy loamy soil) on the campus of the Universidad Politécnica de Valencia, Valencia, Spain (GPS coordinates of the field plot: lat. 39º 28’ 55’’ N, long. 0º 20’ 11’’ W) in a completely randomized design. Plants were spaced 1.2 m between rows and 1.0 m apart within the row and drip irrigated. Fertilization was applied with drip irrigation throughout the growing cycle and consisted of 80 g/plant of a 10N-2.2P-24.9K plus micronutrients commercial fertilizer (Hakaphos Naranja; Compo Agricultura, Barcelona, Spain). Standard horticultural practices for eggplant production in the Mediterranean coastal area of Spain were followed.

Morphological characterization

Morphological traits were measured in individual plants using 16 primary descriptors developed by the Eggplant Genetic Resources Network (EGGNET) (Table 1; Prohens *et al*. 2005; van der Weerden and Barendse 2007). For leaf, flower and fruit traits, five measurements were taken for each individual plant. The fruit skin primary (i.e., predominant) colour was measured in the CIELAB 1976 colour coordinates L* (0 = black; 100 = white), a* (positive values = red; negative values = green), and b* (positive values = yellow; negative values = blue), using a Minolta CR-300 chroma-meter (Minolta Co. Ltd., Osaka, Japan). Fruit flesh browning was measured as DW10 –DW0, where DW10 and DW0 are, respectively, the distances to pure white colour (L* = 100; a* = 0; b* = 0) of the fruit flesh color measured at 0 min and at 10 min after the fruit were transversally cut with a sharp knife. Values of DW were calculated as DW = $((100\text{-}L^*)^2+a^{*2}+b^{*2}))^{0.5}$ (Prohens *et al*., 2007). Fruit skin primary colour and flesh browning were measured in five fruit per plant. The measurement of the fruit flesh colour was made at the mid-point between the center of the fruit and the pericarp.

Phenolic acid conjugate analysis

Fruit (three to five) of individual plants were harvested and brought to the laboratory, where they were washed, peeled, and a 2-cm wide longitudinal section from stem to blossom end was cut from the middle of the fruit. The excised tissue was frozen in liquid N2 and lyophilized. The lyophilized tissue of the fruit from an individual plant was powdered and pooled as a single sample. The powdered samples were shipped from the Universitat Politècnica de València (Spain) to the USDA Beltsville Agricultural Research Center (USA) using a courier service. After being received, samples were stored at –80 ºC until analyzed.

Sub-samples of the lyophilized powdered fruit tissue (0.2 g) were extracted by vigorous stirring for 15 min at room temperature in 10 mL of methanol-water, 4:1, in a 15-mL plastic centrifuge tube that was sealed after flushing with N2. The tube was then centrifuged at 4000 g for 5 min, the first extract was decanted, and the process was repeated on the same tissue sample. The first and second extracts were combined and 4 mL were passed through a Whatman PTFE syringe filter (0.2 µm pore size). One mL aliquots of

each filtered extract were transferred to amber HPLC vials and the solvent evaporated under a stream of N_2 at 40 °C. The residue was dissolved in 1.0 mL of water-methanol, 4:1, plus 0.02% phosphoric acid. Each vial was flushed with N2 before it was sealed with a Teflon-lined septum cap. Samples were stored at –80 °C until analyzed by HPLC.

Table 1. Morphological traits evaluated and their description. Further details for the measurement of plant traits can be obtained from Prohens *et al*. (2005) and van der Weerden and Barendse (2007).

Trait	Code	Units / Description
Plant height	P-Heigth	cm
Angle between main branches	S-Angle	Sexagesimal degrees
Shoot apex anthocyanins intensity	A-Anthocyanins	0=absent; 9=very strong
Stem anthocyanins intensity	S-Anthocyanins	0=absent; 9=very strong
Stem prickles	S-Prickles	number of prickles between two internodes
Leaf pedicel length	L-Pedicel	cm
Leaf blade length	L-Length	cm
Leaf blade breadth	L-Breadth	cm
Leaf blade length/breadth ratio	L-Length/Breadth	
Leaf blade lobing	L-Lobing	1=very weak; 9=very strong
Leaf anthocyanins intensity	L-Anthocyanins	0=absent; 9=very strong
Leaf prickles	L-Prickles	0=none; 9=very many
Leaf longest prickle length	L-PrickleLength	mm
Flowers per inflorescence	Fl-Number	
Corolla diameter	Fl-Diameter	mm
Fruit weight	Fr-Weight	g
Fruit skin L* primary colour	Fr-L*	CIELAB L* colour coordinate
Fruit skin a* primary colour	Fr-a*	CIELAB a* colour coordinate
Fruit skin b* primary colour	Fr-b*	CIELAB b* colour coordinate
Fruit browning index	Fr-Browning	Difference of distance to pure white between 10 and 0 min after fruit cut

Phenolic acid conjugates in the fruit tissue extracts were separated and quantified by RP-HPLC in 50 µL injections onto a Luna C18(2) column (5 µm particle size, 250 mm long, 4.6 mm i.d.) from Phenomenex (Torrance, CA) using an HP 1100 Series instrument with a quaternary pump, autosampler, and

photodiode array detector (Agilent Technologies). Data were analyzed with Agilent ChemStation software (Revision B.03.01). The method used was a modification of that described by Whitaker and Stommel (2003). The binary gradient consisted of 0.02% H3PO4 in water (A) and methanol (B) as follows: 0 min, 90A:10B at 1.0 mL/min; 0–15 min, linear increase to 25% B at 1.0 mL/min; 15–25 min, linear increase to 50% B at 1.0 mL/min; 25–28 min, linear increases to 80% B and 1.2 mL/min; 28–30 min, linear increase to 100% B at 1.2 mL /min; 30–32 min, 100% B at 1.2 mL/min; 32–35 min, decrease to 10% B at 1.2 mL/min; 35–38 min, 10% B with linear decrease to 1.0 mL/min. Relative quantification was based on absorbance at 325 nm (caffeoyl and feruloyl conjugates) and 280 nm (dihydrocaffeoyl conjugates).

Sixteen compounds were quantified, including esters and/or amides of caffeic, dihydrocaffeic, and ferulic acid. With the exception of three putative feruloyl esters of quinic acid, all the compounds have been identified by a combination of LC-MS and NMR analyses in prior studies (Whitaker and Stommel, 2003; Ma *et al*., 2011). The 16 phenolic acid conjugates were numbered according to the order of HPLC elution but were otherwise distributed in four groups on the basis of their chemical structures (Table 2) as follows: Group 1 – four isomers of caffeoylquinic acid (3-O-E, 4-O-E, 5-O-E, and 5-O-Z) plus 3,5-di-O-E-caffeoyquinic acid; Group 2 – six hydroxycinnamoyl amides of polyamines, including N-caffeoylputrescine, N-caffeoylspermidine, N,N′-bis(dihydrocaffeoyl)spermidine, N-caffeoyl-N′-dihydrocaffeoyl-spermidine, N-dihydrocaffeoyl-N′-caffeoylspermidine, and N,N′-bis(caffeoyl)spermidine; Group 3 – three isomers of feruloylquinic acid (3-O-E, 4-O-E, and 5-O-E); and Group 4 – two isomers of malonylcaffeoylquinic acid (3-O-malonyl-5-O-E-caffeoyl and 5-O-malonyl-4-O-E-caffeoyl).

Table 2. Phenolic acid grouping based on identification or tentative identification of the 16 hydroxycinnamic acid conjugates in eggplant fruit extracts that were quantified by HPLC-UV.

Phenolic peak	Elution time (min)	UV A_{max} (280-330 nm)	Abbreviation	Conjugate identification
Group 1 – Mono- and di-caffeoylquinic acid esters				
3	15.1	326, 296 (sh)	3-CQA	3-*O*-(*E*)-caffeoylquinic acid
8	21.2	326, 296 (sh)	5-CQA	5-*O*-(*E*)-caffeoylquinic acid
10	22.4	326, 296 (sh)	4-CQA	4-*O*-(*E*)-caffeoylquinic acid
12	24.3	319	5Z-CQA	5-*O*-(*Z*)-caffeoylquinic acid
16	28.1	328, 296 (sh)	3,5-diCQA	3,5-di-*O*-(*E*)-caffeoylquinic acid
Group 2 – Hydroxicinnamic acid – polyamine amides (HCAA)				
1	8.2	317, 292	Caff-Put	*N*-(*E*)-caffeoylputrescine
2	12.9	318, 292	Caff-Spd	*N*-(*E*)-caffeoylspermidine
4	16.5	280	Bis-dhCaff-Spd	*N*,*N*´-bis(dihydrocaffeoyl)spermidine
5	18.6	319, 288	Caff/dhCaff-Spd-1	*N*-caffeoyl-*N*´-dihydrocaffeoylspermidine
6	19.7	319, 288	Caff/dhCaff-Spd-2	*N*-dihydrocaffeoyl-*N*´-caffeoylspermidine
7	20.9	319, 293	Bis-Caff-Spd	*N*,*N*´-bis(caffeoyl)spermidine
Group 3 – Mono-feruloylquinic acid esters				
9	21.8	328, 299 (sh)	3-FQA	3-*O*-(*E*)-feruloylquinic acid
13	25.4	328, 299 (sh)	5-FQA	5-*O*-(*E*)-feruloylquinic acid
15	27.1	328, 298 (sh)	4-FQA	4-*O*-(*E*)-feruloylquinic acid
Group 4 – Malonylcaffeoylquinic acid esters				
11	23.9	327, 297 (sh)	3-Mal-5-CQA	3-*O*-malonyl-5-*O*-(*E*)-caffeoylquinic acid
14	25.6	327, 297 (sh)	5-Mal-4-CQA	5-*O*-malonyl-4-*O*-(*E*)-caffeoylquinic acid

Data analyses

For morphological data, the mean and range of each character within individual generations were obtained. Data from individual plants were subjected to analysis of variance (ANOVA), so that two ANOVAs were conducted for each of the traits studied, one which included the non-segregating (parents and hybrids) generations and another which included the segregating (F2 and BC1P2) generations. The average (pooled) variance and standard deviation for each of the traits studied was obtained from the corresponding ANOVAs. Principal components analyses (PCA) were performed for standardized morphological traits using pairwise Euclidean distances among individuals.

For phenolic acids, the percentage of individual phenolic acid conjugates in each plant, and the mean, maximum and minimum values were computed and utilized to quantify phenolic acids content for each of the four groups of phenolic acid conjugates and total phenolic acids content. Mean values and ranges for the P1, P2, F1, F2 and BC1P2 generations are reported. Heritability of phenolic acids content was determined using an additive-dominance model in which midparent and genetic effects (m = midparent, [a] = additive, [d] = dominance) were estimated using Cavalli's weighted method. Parameterization coefficients for gene effects were as follows: m = 1 for all generations; [a] = 1 for P1, -1 for P2, 0 for F1 and F2, and -1/2 for BC1P2; [d] = 0 for P1 and P2, 1 for F1 and 1/2 for F2 and BC1P2. Model goodness of fit was tested using a weighted $\chi 2$ (joint-scaling-test) (Mather and Jinks, 1977). Correlation coefficients among the different groups of phenolic acids and with total phenolic acids content were calculated.

Results

Morphological characterization

Important differences in plant and fruit morphology were found between the *S. incanum* (P1) and *S. melongena* (P2) parents. Lack of overlap in the reported range for 12 out of the 20 traits evaluated illustrates the high level of phenotypic divergence between these species (Table 3). In this respect, the *S. incanum* parent presented no anthocyanin pigmentation in the shoot apex (A-Anthocyanins), stem (S-Anthocyanins), or leaf (L-Anthocyanins),

whereas the *S. melongena* parent presented anthocyanins in all of these organs. P1 was prickly in both the stem (S-Prickles) and leaf (L-Prickles), while P2 was non-prickly (Table 3). In addition, P1 presented shorter leaf pedicel (L-Pedicel), smaller corolla diameter (Fl-Diameter), and particularly, significantly smaller fruit weight (Fr-Weight). The fruit of P1 were green, while those of P2 dark purple (Figure 1), which resulted in higher values for L* (i.e., less black) and b* (i.e., more yellow), and lower values for a* (i.e., more green) in P1 compared with P2. Although fruit browning (Fr-Browning) scores for P1 and P2 overlapped at the very extremes of their respective low and high score distributions, the mean Fr-Browning score was more than three times higher in P1 than in P2 (Table 3).

Heterosis for morphological attributes was evident in F1 hybrid progeny from this interspecific cross. In comparison to parental lines, F1 progeny exhibited greater plant height (P-Height), leaf length (L-Length), leaf lobing (L-Lobing), and prickle length (L-PrickelLength) (Table 3; Figure 1). Presence of prickles (L-Prickle) in the F1 was similar to P1. The interspecific hybrid exhibited anthocyanin pigmentation in the vegetative plant parts studied, with values similar to those observed for P2. Likewise, fruit anthocyanin pigmentation (Fr-L*, Fr-a* and Fr-b*) in the hybrid was similar to that in P2. The flowers of the F1 were about the same size (Fl-Diameter) as those of P2. F1 fruit size (Fr-Weight) was skewed towards the considerably smaller fruit size characteristic of P1. Fruit browning (Fr-Browning) scores for the F1 were greater than those of either parent (Table 3).

The distribution range and accompanying variance for traits evaluated in the segregating generations (F2 and BC1P2) were generally greater than those observed in the non-segregating generations (P1, P2, and F1) (Table 3). In particular, variation was greatest for plant architecture attributes including plant height (Pl-Height) and angle between main branches (S-Angle) and fruit characteristics (Table 3, Figure 1). For the F2, mean values for plant and fruit attributes were generally intermediate between both parents, but in some case skewed towards P1 or P2, e.g. prickle length and flower diameter.

Table 3. Mean value, range, and standard deviation (SD) of the plant traits evaluated for *S. incanum* (P1) and *S. melongena* (P2) parents, the interspecific hybrid (F1), the selfed interspecific hybrid (F2), and F1backcross to *S. melongena* (BC1P2) generations.

	Non-segregating generations							Segregating generations					
	P1		P2		F1			F2			BC1P2		
Trait	Mean	Range	Mean	Range	Mean	Range	SD[a]	Mean	Range	SD	Mean	Range	SD
n	6		6		6			41			64		
P-Heigth	112.3	93-141	139.5	124-156	185.0	175-197	15.1	133.3	84-181	26.5	155.6	86-212	25.0
S-Angle	90.8	65-120	70.8	60-80	82.5	75-90	11.9	82.3	45-125	18.6	77.8	55-115	13.3
A-Anthocyanins	0.0	0-0	6.7	5-9	5.3	3-7	1.2	5.3	0-9	1.5	5.9	3-9	2.5
S-Anthocyanins	0.0	0-0	6.3	5-7	4.7	3-5	0.8	5.4	0-9	2.0	5.8	3-9	1.9
S-Prickles	7.0	5-9	0.0	0-0	9.8	8-14	1.6	6.6	0-16	6.6	4.0	0-14	4.0
L-Pedicel	4.3	3.9-4.5	8.9	6.5-11.0	6.8	6.4-7.3	0.9	6.6	3.5-10.0	1.5	7.1	4.3-10.5	1.3
L-Length	19.5	17.5-20.5	19.3	16.0-21.1	22.0	20.5-23.0	1.5	18.4	13.9-27.0	2.2	19.2	14.5-23.3	2.1
L-Breadth	13.3	13.0-13.5	14.6	12.0-15.6	14.7	13.8-15.7	1.0	12.3	9.7-16.7	1.7	13.1	7.8-17.2	1.5
L-Length/Breadth	1.46	1.30-1.58	1.32	1.25-1.43	1.50	1.46-1.57	0.07	1.51	1.26-1.88	0.15	1.47	1.28-1.86	0.11
L-Lobing	3.7	3-5	3.3	3-5	7	7-7	0.8	6.2	3-7	1.1	6.5	3-7	0.9
L-Anthocyanins	0.0	0-0	6.3	5-7	4.7	3-5	0.8	4.7	0-9	2.1	5.4	3-9	1.8
L-Prickles	4.3	3-5	0.0	0-0	5	5-5	0.6	3.2	0-7	2.2	2.3	0-7	2.5
L-PrickleLength	4.8	4-8	0.0	0-0	9.0	8-11	1.0	5.2	0.0-10.0	3.6	3.6	0.0-11.0	3.8
Fl-Number	5.5	4.0-7.0	4.0	3.0-6.0	6.2	5.0-8.0	1.1	4.8	2.8-7.0	1.1	4.4	2.3-8.0	1.1
Fl-Diameter	32.3	28.0-37.5	48.4	40.0-60.0	47.9	43.8-52.5	5.0	47.8	32.5-60.0	6.4	51.5	41.3-62.5	4.5
Fr-Weight	6.5	3.8-8.9	195.0	158.0-221.5	17.2	12.9-20.3	14.7	40.8	13.5-110.2	24.2	68.1	14.0-205.0	36.8
Fr-L*	52.1	47.8-57.4	23.8	19.8-28.3	29.6	26.6-34.5	3.4	43.2	20.0-85.9	17.5	43.3	19.98-87.5	19.0
Fr-a*	-18.6	-23.3- -15.9	5.0	3.0-6.6	6.3	0.4-9.7	2.5	4.6	-14.27-23.54	9.9	4.5	-16.31-22.06	9.3
Fr-b*	20.7	14.2-26.3	2.1	-1.2-6.2	0.4	-1.8-1.7	2.8	7.0	-13.1-22.0	9.1	6.4	-3.5-31.6	8.8
Fr-Browning	10.6	4.5-15.9	3.1	2.4-5.3	16.8	9.6-26.7	4.1	16.0	5.1-30.2	6.5	15.3	2.8-36.2	7.2

[a]Pooled values for non-segregating generations obtained from ANOVA analyses

Figure 1. Fruit samples of *S. incanum* (P1; a), *S. melongena* (P2; b), the interspecific hybrid (F1; c), the selfed F1 (F2; d), and the F1 backcrossed to *S. melongena* (BC1P2; e). Fruits are not depicted at the same scale; the size of the grid cells is 1 cm x 1 cm.

However, for leaf lobing (L-Lobing) and fruit browning (Fr-Browning), the mean values exceeded those observed for both P1 and P2 but were similar to those of the F1. Fruit weight in the F2 ranged between 13.5 g and 110.2 g, with an average value (40.8 g) much greater than that of the F1 (17.2 g) (Table 3; Figure 1). Approximately 25% of the F2 plants lacked prickles (10 without prickles, 41 with prickles), which is a very good fit ($\chi 2$=0.008; P=0.928) for a 3:1 segregation coinciding with the action of a single major gene. In the case of the BC1P2, mean values for plant and fruit attributes were also generally intermediate between P2 and F1 or skewed toward the F1 or recurrent parent. For example, fruit weight in the BC1P2 ranged between 14.0 g and 205.0 g,

with an average value of 68.1 g intermediate to parent lines. Conversely, traits such as leaf lobing (L-Lobing) and fruit browning (Fr-Browning) were skewed towards those of the F1. Unlike P2 and F1, fruit of some BC1P2 plants lacked anthocyanins, which resulted in average Fr-L* values much greater than those of any of the parents (Table 3; Figure 1). Fifty percent (32 out of the 64) of the BC1P2 plants lacked prickles, which is a perfect fit for a 1:1 segregation ($\chi 2$=0.000; P=1.000) and simple inheritance.

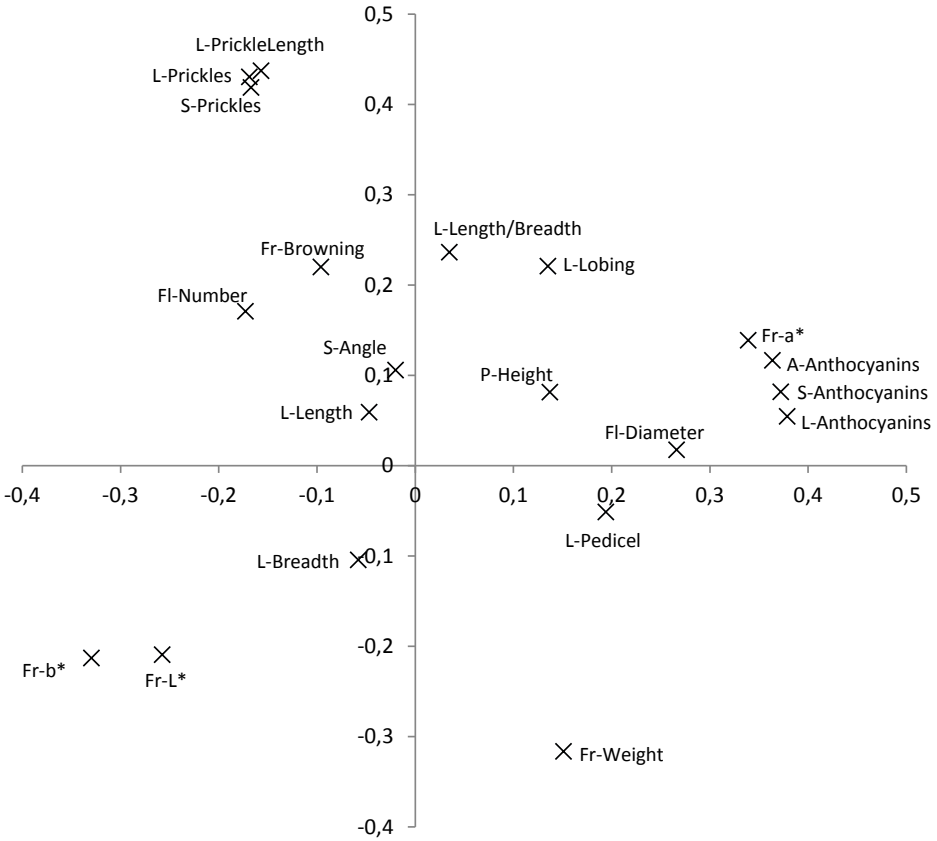

Figure 2. Principal components analysis of plant traits (see Tables 1 and 3) for *S. incanum* (P1) and *S. melongena* (P2) parents, interspecific hybrid (F1), selfing of the interspecific hybrid (F2) and backcross to *S. melongena* (BC1P2) generations. First and second components of the PCA account for 24.8% and 16.7% of the total variation, respectively.

The first and second components of the PCA performed with the morphological data accounted for 24.8% and 16.7% of the total variation,

respectively. The inclusion of the third or subsequent components in the PCA did not improve the interpretations. We considered as relevant those traits having correlation values with the first or second principal component greater than 0.2 (Figure 2). The first component was positively correlated with presence of anthocyanins in fruit (high values for Fr-a*) and vegetative plant parts (S-Anthocyanins, A-Anthocyanins, L-Anthocyanins) and with flower size (Fl-Diameter) (Figure 2). The first and second components were negatively correlated with fruit green colour (Fr-b*) and more luminous fruits (Fr-L*). The second component was positively correlated with the presence of prickles (S-Prickles and L-Prickles) and prickle size (L-PrickleLength), as well as with the leaf length/breadth ratio (L-Length/Breadth) and lobing (L-Lobing), and fruit browning (Fr-Browning). The second component was also negatively correlated with fruit weight (Fr-Weight).

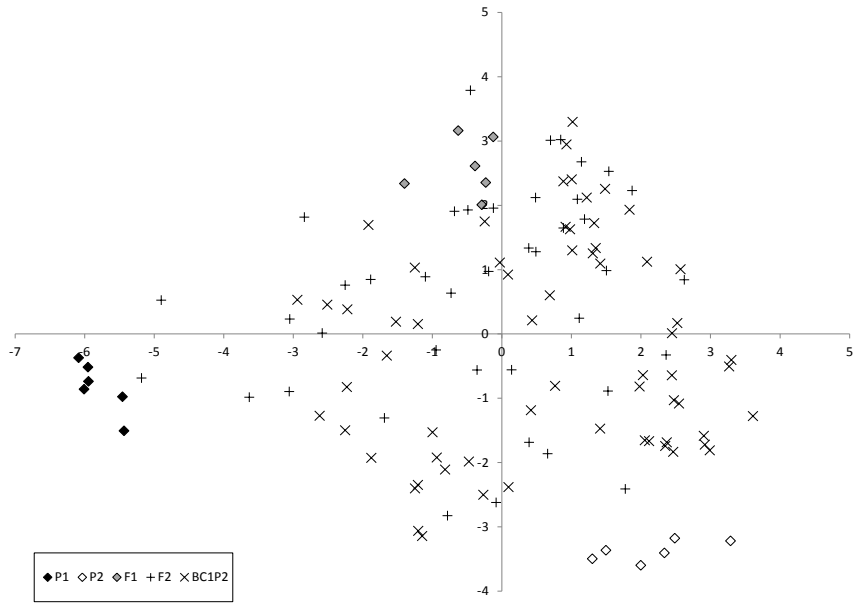

Figure 3. Similarities based on 20 morphological traits (see Tables 1 and 3) among the individual plants of the *S. incanum* (P1) and *S. melongena* (P2) parents, interspecific *S. incanum*x *S. melongena* hybrid (F1), selfing of the interspecific hybrid (F2), and backcross to *S. melongena* (BC1P2) represented on the two first components of the PCA (24.8% and 16.7% of the total variation, respectively).

The projection of the individual plants on the morphological traits PCA plot clearly separates P1 (low values for the first component and intermediate values for the second component), P2 (high values for the first component and low values for the second component), and F1 (intermediate values for the first component and high values for the second component) (Figure 3). The cluster of F1 individuals is approximately equidistant from P1 and P2 clusters. The F2 and BC1P2 individuals generally plot in the triangular area delimited by P1, P2 and F1. F2 individuals were uniformly distributed across the area bounded by P1, P2, and the F1. BC1P2 individuals were plotted most distant to P1 (Figure 3).

Phenolic acid conjugate content

The most common phenolic compound among all the hydroxycinnamic acid conjugates identified in parental, hybrid, and F2 and BC1P2 progeny was 5-CQA, with an average value of 77.8%, and a range from 58.1% to 88.7% (Table 4). For fruit from the parental lines, 5-CQA composed a higher mean percentage of total phenolics in *S. melongena* (75.2%) compared with *S. incanum* (65.4%). All other phenolic acid conjugates were generally present in low concentrations with values below 10%. The highest average values for the minor compounds were recorded for Caff-Put (group 2; 5.5%), 3-Mal-5-CQA (group 4; 4.0%), and bis-Caff-Spd (group 2; 4.0%). However, the levels of some of these individual minor phenolic acid conjugates ranged up to 21.3%, as was the case for 3-Mal-5-CQA in one sample of *S. incanum*. Sixteen hydroxycinnamic acid esters and amides were identified across P1, P2, F1, F2 and BC1P2 progeny. Hydroxycinnamic acid conjugate profiles varied among individual plants. Presence of individual compounds ranged from 10 to 16 in the individual samples analyzed. Seven out of 16 of the hydroxycinnamic acid conjugates (5-CQA, 4-CQA, Caff-Put, bis-Caff-Spd, 5-FQA, 3-Mal-5-CQA, and 5-Mal-4-CQA) were present in all the samples analyzed (Table 4). As shown in Table 4, some individuals present values of 0.0% for the contents of some of the minor hydoxycinnamic acid conjugates. However, for six of the nine compounds for which values of 0.0% are shown (5Z-CQA, 3,5-diCQA, Caff-Spd, bis-dhCaff-Spd, Caff/dhCaff-Spd-1, and Caff/dhCaff-Spd-2), we found variation for their presence/absence in all of the non-segregating generations, with one or more individuals presenting values greater than zero. Among the three remaining compounds for which some individuals with 0.0% values are shown,

we found 3-CQA present in all plants of both parents and absent in all plants of the F1, while 3-FQA and 4-FQA were absent in all plants of *S. melongena* (P2) and the F1, and present in some and absent in other plants of the *S. incanum* (P1) parent.

Table 4. Average, maximum and minimum values for the percentage of each of the 16 hydroxycinnamic acid conjugates identified in fruit tissue extracts from 123 plants of the family constituted by *S. incanum* and *S. melongena* parent lines, the interspecific hybrid (F1), the selfed interspecific hybrid (F2), and F1 backcross to *S. melongena* (BC1P2) generations

Phenolic acid conjugate	Average	Min	Max
	Group 1 – Mono- and di-caffeoylquinic acid esters		
3-CQA	0.1	0.0	0.2
5-CQA	77.8	58.1	88.7
4-CQA	1.4	0.5	6.0
5Z-CQA	0.8	0.0	7.5
3,5-diCQA	0.5	0.0	1.9
Total Group 1	80.6	62.0	90.8
	Group 2 – Hydroxicinnamic acid – polyamine amides (HCAA)		
Caff-Put	5.5	0.2	21.3
Caff-Spd	0.4	0.0	1.3
bis-dhCaff-Spd	0.7	0.0	3.8
Caff/dhCaff-Spd-1	1.7	0.0	6.6
Caff/dhCaff-Spd-2	0.6	0.0	1.4
bis-Caff-Spd	4.0	0.5	12.0
Total Group 2	12.8	2.7	27.0
	Group 3 – Mono-feruloylquinic acid esters		
3-FQA	0.1	0.0	1.1
5-FQA	0.6	0.1	2.0
4-FQA	0.1	0.0	0.9
Total Group 3	0.8	0.1	3.0
	Group 4 – Malonylcaffeoylquinic acid esters		
3-Mal-5-CQA	4.0	0.1	21.3
5-Mal-4-CQA	1.9	0.1	10.4
Total Group 4	5.9	0.5	30.5

As a consequence of high 5-CQA concentrations, group 1 mono- and di-caffeoylquinic acid esters comprised the most abundant class of hydroxycinnamic acids conjugates, with an average value of 80.6% (Table 4). With the exception of 5-CQA, the individual phenolic acids in group 1 were present at very low levels, generally below 1.5%. The hydroxycinnamic acid-polyamine amides (HCAA; group 2) ranked second in concentration, with an average value of 12.8%. Group 2 was followed by the malonylcaffeoylquinic acid esters (group 4), with an average value of 5.9%, and finally by the mono-feruloylquinic acid esters (group 3), with a very low value, 0.8% on average (Table 4).

The *S. incanum* parent (P1) exhibited much higher values than the *S. melongena* parent (P2) for all four groups of phenolic compounds studied (Figure 4; Table 5). The average concentrations of groups 1, 2, 3, and 4 in P1 were 2.3, 2.1, 6.8, and 5.6 times greater, respectively, than in P2, and total phenolic levels were 2.7 times higher in P1 compared with P2. In F1 fruit, concentrations of group 1, 2, and 3 compounds as well as total phenolics were intermediate to those in fruits of the two parents. However, abundance of the two malonylcaffeoylquinic acid isomers in group 4 was less in F1 fruit than in fruit of either parent (Table 5). The range of variation within each of the individual plants of non-segregating generations was high and, except for group 3, overlap was found between the two parents. Accordingly, comparison of the total phenolic acid conjugate content in fruit from single plants of each of the parents or the F1 hybrid revealed that for P1, P2, and F1, respectively, individuals with the highest concentration presented values that were 2.2, 3.0, and 1.7 times greater than those for individuals with the lowest concentration (Table 5).

The F2 and BC1P2 total phenolic acid conjugate concentrations were, on average, similar to those of the F1, with slightly higher levels present in the F2 compared with the BC1P2 (Table 5). As expected, the range of variation for total phenolics and for each of the four groups was generally greater in the F2 and, in particular, in the BC1P2 than in the F1 and parental generations.

Table 5. Mean values and range (HPLC peak area units at 325 nm; 280 nm for bis-dhCaff-Spd), and estimated values ± SE of genetic parameters (m = midparent, [a] = additive, [d] = dominance), χ^2 statistic and probability of χ^2 for goodness of fit to the additive-dominance genetic model proposed for the four groups of hydroxycinnamic acid conjugates identified in fruit tissue extracts for *S. incanum* (P1) and *S. melongena* (P2) parents, the interspecific hybrid (F1), the selfed interspecific hybrid (F2), and F1 backcross to *S. melongena* (BC1P2) generations.

	Group 1[a]	Group 2	Group 3	Group 4	Total
Generation					
P1 (n=6)					
Mean	32456	3061	952	10534	47004
Range	22307-38380	1197-4823	278-1870	1853-18750	28508-61533
P2 (n=6)					
Mean	13979	1491	139	1875	17483
Range	7737-23990	1124-2168	94-166	1009-3547	10052-29799
F1 (n=6)					
Mean	22804	2845	214	924	26787
Range	16781-26849	748-4564	27-355	228-1925	18983-31904
F2 (n=41)					
Mean	23320	3846	194	1894	29254
Range	11765-35208	1672-9573	58-529	319-11172	14699-42565
BC1P2 (n=64)					
Mean	20619	3343	198	1173	25333
Range	7003-32155	756-6670	48-716	108-6128	8200-40246
Genetic parameters					
m	23627±4118	2355±613	301±186	5075±2633	32506±6764
[a]	8794±4312	829±615	162±187	3281±2678	13919±7155
[d]	-412±6343	1440±1521	-114±258	-4217±2771	-5208±9349
χ^2	0.100	0.816	1.033	0.373	0.149
Prob.	0.951	0.665	0.606	0.830	0.928

[a]Group 1: mono- and di-caffeoylquinic acid esters; Group 2: hydroxicinnamic acid – polyamine amides (HCAA); Group 3: mono-feruloylquinic acid esters; Group 4: malonylcaffeoylquinic acid esters.

Based on chi-square (χ2) analysis, the variation observed in the five generations studied was adequately explained by a simple additive-dominance (m[a][d]) inheritance model (Table 5). In all cases, additive genetic variance [a] was positive. This indicates that the alleles of P1 contributed positively to high

levels of total phenolics as well as to the contents of each of the four groups of phenolic acid conjugates. However, additive variance was only significant ($P<0.05$) for group 1. The dominance parameter [d] was negative in all cases, except for group 2 and non-significant at $P<0.05$. Addition of digenic interactions to the additive-dominance model to produce four or five parameter models provided only marginal improvements to model R2 values for the respective groups (data not shown).

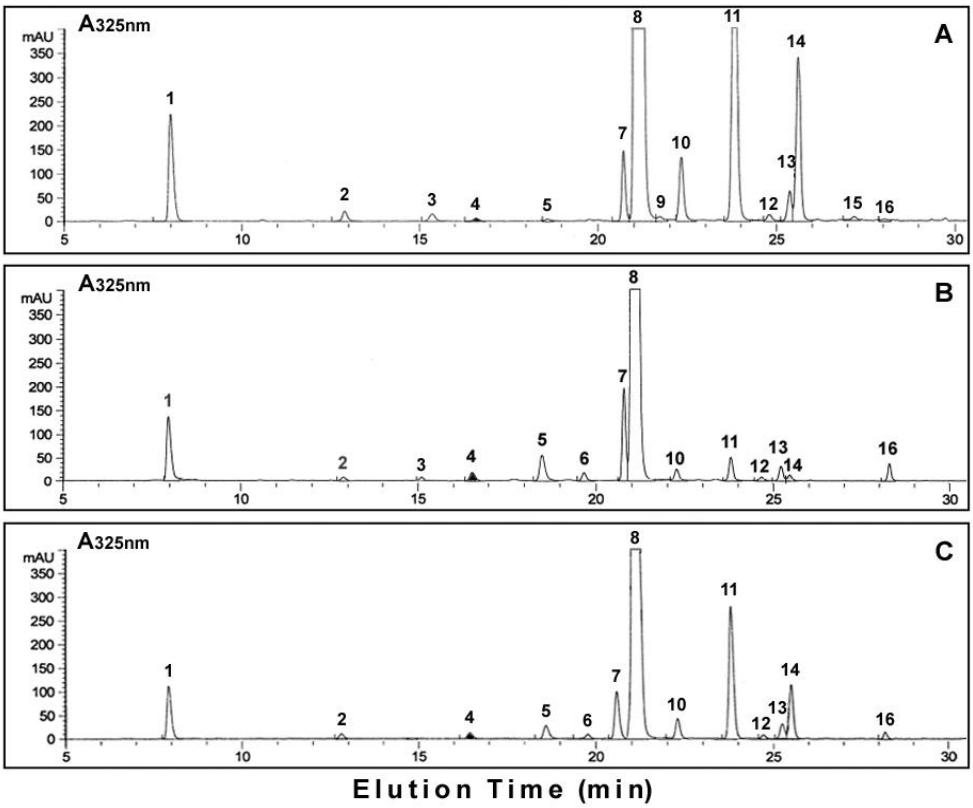

Figure 4. Representative C_{18}-HPLC-UV chromatograms of phenolic compounds in methanolic extracts of fruit tissues from A, *Solanum incanum* parental (P1); B, *S. melongena* parental (P2); and C, *S. incanum* X *S.melongena* hybrid (F1) lines. Numbering of the peaks corresponds to the 16 hydroxycinnamic acid conjugates identified in Table 2. All compounds were detected at 325 nm except peak 4, bis(dihydrocaffeoyl)spermidine, which was detected at 280 nm. Note that the *S. melongena* sample (B) was injected at twice the concentration of the other two samples to better enable comparison of the relative abundance of the 16 phenolics.

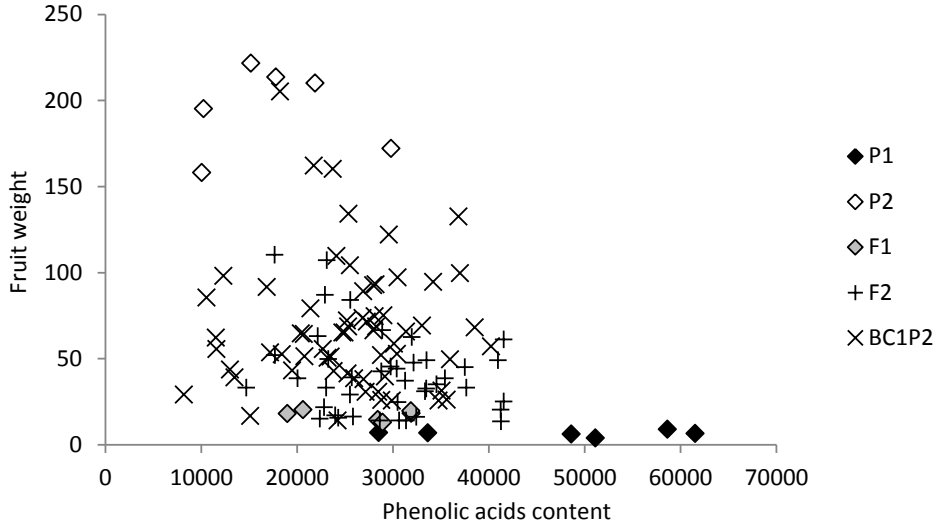

Figure 5. Relationship between total phenolic acid content (summed HPLC-UV peak areas; X-axis) and fruit weight (g; Y-axis) in the individual plants of the *S. incanum* (P1), *S. melongena* (P2) parents, interspecific *S. incanum* x *S. melongena* hybrid (F1), the selfed interspecific hybrid (F2), and backcross to *S. melongena* (BC1P2).

Correlations between the four groups of phenolic acid conjugates were highly significant (P<0.001) (Table 6). Exceptions included non-significant correlations between group 2 and groups 3 and 4. Total phenolics were significantly correlated with each of the four groups, in particular with group 1, in which the correlation coefficient was greater than 0.95 (Table 6). Correlation between total phenolics content and fruit weight was negative (r=-0.3609; P<0.001). However, when considering only the individuals of the segregating generations, the correlation coefficients were non-significant (r=-0.3618 for F2; r=-0.0061 for BC1P2) (Figure 5). BC1P2 individuals with high total phenolic acid conjugate content and moderate fruit weights were identified (Figure 5). The correlation between total phenolics and fruit flesh browning was low and non-significant (P>0.05) when considering all individuals (r=0.1160), or just those of the F2 (r=0.2452) or the BC1P2 (r=0.0567) generations (Figure 6). Among the materials studied, individual plants in the F2 and BC1P2 exhibited intermediate fruit browning combined with high phenolic acid conjugate content.

Table 6. Correlations for the concentrations of the 4 groups of hydroxycinnamic acid conjugates identified in fruit tissue extracts of 123 plants of the family constituted by *S. incanum* (P1) and *S. melongena* (P2) parents, the interspecific hybrid (F1), the selfed interspecific hybrid (F2), and F1 backcross to *S. melongena* generations (BC1P2)

	Group 2	Group 3	Group 4	Total
Group 1	0.3263***	0.5820***	0.5115***	0.9596***
Group 2		0.0219^{ns}	-0.1032^{ns}	0.3723***
Group 3			0.7796***	0.7019***
Group 4				0.6949***

^{ns,} *** indicate non-significant, or significant at P<0.001, respectively.

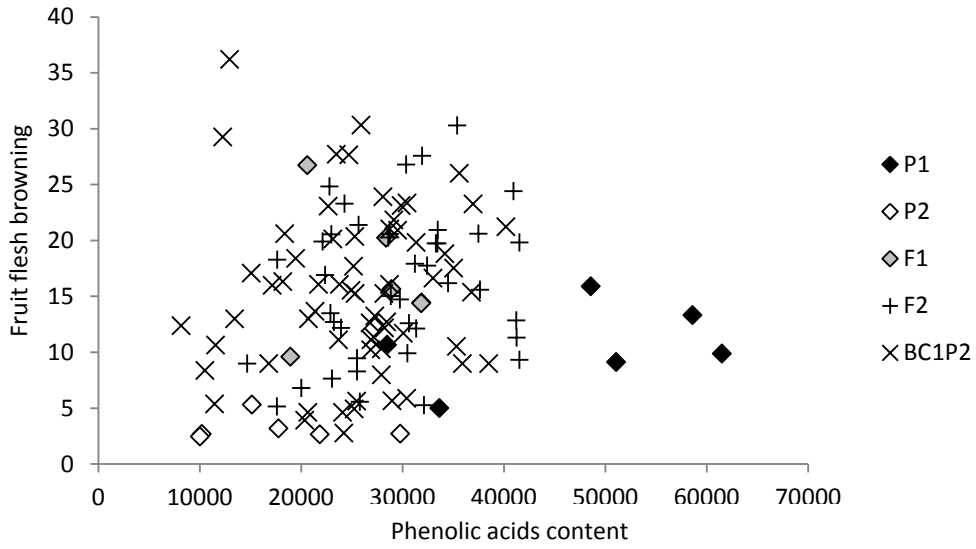

Figure 6. Relationship between total phenolic acid content (summed HPLC-UV peak areas; X-axis) and fruit flesh browning (browning index; Y-axis) in the individual plants of the *S. incanum* (P1) and *S. melongena* (P2) parents, interspecific *S. incanum* x *S. melongena* hybrid (F1), selfing of the interspecific hybrid (F2), and backcross to *S. melongena* (BC1P2).

Discussion

Breeding for nutraceutical quality is an increasingly important objective for vegetable improvement (Jenks and Bebeli, 2011). In the case of eggplant, the most important group of functional compounds present in the fruit is phenolics, which consist of anthocyanins in the skin and hydroxycinnamic acid derivatives, primarily chlorogenic acid, in the flesh (Whitaker and Stommel, 2003, Azuma *et al*., 2008). The concentration of hydroxycinnamic acids present in the fruit is much higher than that of anthocyanins, and consequently the former are the major contributor to fruit antioxidant capacity (Hanson *et al*., 2006; Okmen *et al*., 2009; Singh *et al*., 2009; Lo Scalzo *et al*., 2010). As a consequence, we have initiated a breeding programme aimed at improving fruit phenolics content (Prohens *et al*., 2008).

Although there is marked intraspecific variation for fruit phenolic content in germplasm of the cultivated eggplant *S. melongena* (Stommel and Whitaker, 2003; Hanson *et al*., 2006; Prohens *et al*., 2007; Okmen *et al*., 2009; Akanitapichat *et al*., 2010), phenolic concentrations present in fruit of the wild eggplant ancestor *S. incanum* exceed the highest levels observed in *S. melongena*. Consequently, hybridization with *S. incanum* affords new opportunities to improve nutritive value of cultivated eggplant (Stommel and Whitaker, 2003; Ma *et al*., 2011). Ancestral and present day selection for low fruit browning (Prohens *et al*., 2007), together with the genetic bottleneck suffered during eggplant domestication and evolution (Meyer *et al*., 2012), likely resulted in the elimination of alleles in the cultivated species that contribute to high fruit phenolic content. Given that the genes involved in the biosynthetic pathway for chlorogenic acid have been identified in the Solanaceae (Clé *et al*., 2008), future studies on the diversity and expression of these genes may help to elucidate key genetic factors involved in the differences between wild and cultivated species in chlorogenic acid content.

Because *S. melongena* and *S. incanum* are sexually compatible, (Lester and Hasan, 1991; Daunay, 2012), *S. incanum* is an appropriate source of variation to breed for enhanced phenolic acid conjugate content in the cultivated form of the crop. However, in order to be of practical use for breeding programs, it is important that recombinant individuals are identified

in which high fruit phenolics content is combined with acceptable horticultural and fruit quality (Fita *et al*., 2010).

In this work we describe fundamental differences between *S. melongena* and its wild ancestor *S. incanum* that contribute to horticultural quality and fruit nutritive value. Our results demonstrate that, as in other cultivated Solanaceae (Prohens *et al*., 2003; Paran and van der Knapp, 2007), eggplant domestication has resulted in a profound change in morphology and chemical constitutents (Weese and Bohs, 2010). For example, when compared with the wild ancestor *S. incanum*, the cultivated eggplant accession we have used is typified by much larger fruit, lack of prickles, and anthocyanin pigmentation in the fruit skin and vegetative plant parts. We have found that the interspecific hybrids are intermediate for many traits relative to the parental species. However, interspecific hybrids were very vigorous and heterotic for plant height. Frary *et al*. (2003) found a major QTL with an overdominant effect for plant height in an interspecific hybrid between *S. melongena* and S. linnaeanum Hepper & Jaeger. It has been suggested that the high vigor of the hybrids between *S. melongena* and *S. incanum* may be exploited for developing rootstocks that afford improved earliness and greater fruit yield (Gisbert *et al*., 2011). Interestingly, interspecific hybrids were pricklier than the wild species. A similar phenomenon was described in interspecific hybrids between *S. melongena* and *S. macrocarpon* (Lester, 1986) and between *S. melongena* and *S. aethiopicum* L. (Prohens *et al*., 2012) where the parents were non-prickly, but the interspecific hybrids presented prickles. Similar to the cultivated *S. melongena*, *S. melongena* x *S. incanum* interspecific hybrids were anthocyanin pigmented. Fruit of interspecific hybrids exhibited a higher degree of browning in comparison with both parental species, suggesting that increased polyphenol oxidase gene expression and/or enzyme activity occurs in the interspecific hybrid. Similar observations were noted for interspecific hybrids between *S. melongena* and S. viarum Dunal (Prabhu *et al*., 2009).

As reported in backcross generations from interspecific hybrids between *S. aethiopicum* and *S. melongena* (Prohens *et al*., 2012), segregating F2 and BC1P2 generations from interspecific hybrids between *S. incanum* and *S. melongena* were generally more variable than the non-segregating

generations, affording valuable opportunities for selection in a breeding program. For most of the traits measured, average values for the F2 generation were similar to those obtained for the F1, while for the BC1P2 attributes were often intermediate between hybrids and the *S. melongena* parent. However, some important exceptions were found. Heterosis was evident for plant height. Segregation for prickliness in F2 and BC1P2 generations suggests that one major gene controls the presence of prickles in this interspecific cross. In accord with this, Doganlar *et al*. (2002) found that one major QTL in linkage group 6 explained most of the variation found for prickliness in an interspecific cross between *S. melongena* and S. linnaeanum. Fruit color values in the F2 varied from those in the F1, due partly to lack of anthocyanins (present in P2) and/or chlorophylls (present in P1), and divergent stripe patterns. This same phenomenon occurred in the BC1P2. The data suggest that several genes for fruit color segregate in this cross and that presence of fruit anthocyanin is dominant to lack of anthocyanin (Tigchelaar *et al*., 1968; Doganlar *et al*., 2002).

Despite considerable variation present in F2 and BC1P2 progeny, PCA analysis revealed that backcross progeny were distant to *S. melongena* individuals. This suggests that additional backcross generations from selected plants will be needed in order to recover the combination of traits typical of the cultivated species. Although many traits related to domestication in eggplant are simply inherited or influenced by major QTL, they are situated in different linkage groups (Doganlar *et al*., 2002; Frary *et al*., 2003). As a result, we expected considerable segregation in the F2 and BC1P2 generations. For traits of economic importance such as fruit weight and reduced browning, individual F2 and BC1P2 plants that combined attributes similar to those of the cultivated species were identified.

Our results confirm that *S. incanum* is a useful source of variation for phenolic acid content that may be exploited to improve levels of these functional compounds in eggplant (Stommel and Whitaker, 2003; Ma *et al*., 2011). The most common phenolic acid identified in all samples was chlorogenic acid (5-CQA). This has important implications for the improvement of the functional quality of eggplant, as many human-health benefits have been demonstrated for 5-CQA (Sawa *et al*., 1998; Triantis *et al*., 2005; dos Santos *et al*., 2006; Lee and Zhu, 2006; Kwon *et al*., 2008; Cho *et al*., 2010; Dai

et al., 2010). Other minor phenolics related to 5-CQA were also detected, but their concentrations were low. Hydroxycinnamic acid amides, mostly Caff-Put and bis-Caff-Spd, and malonylcaffeoylquinic acid esters, mainly 3-Mal-5-CQA, comprised the major compounds in the groups present at lesser concentrations. Mono-feruloylquinic acid esters were the least abundant hydroxycinnamic acid conjugates. These results demonstrate that 5-CQA is preferentially accumulated in eggplant and its wild ancestor, suggesting possible functional roles for this compound such as defense against plant pests, pathogens and abiotic stresses (Bradfield and Cohen, 2004; López-Gresa *et al*., 2011). Whereas fruit phenolic acid constituent profiles were fairly similar between *S. melongena* and *S. incanum*, significant differences in concentration of constituent compounds was evident. These results illustrate opportunities to increase total phenolic acid conjugate content and modify concentrations of individual constituents depending on their potential functional value. Nonetheless, breeding for improvement in the concentration of minor compounds may prove to be difficult because the study of their inheritance is complicated, e.g. in some cases the non-segregating generations are not uniform for the presence/absence of these individual compounds.

Wide variation present for total phenolic acid content, as well as for each of the four sub-groups described, may be attributed in part to environment as illustrated by Luthria *et al*. (2010) in a comparison of phenolic compounds in eggplants produced in conventional and organic agriculture systems. In fact, we found that even in non-segregating generations, variation for presence/absence of some minor compounds occurred, indicating that environmental effects have an important influence not only on the concentration of major compounds, but also on the presence/absence of minor phenolic compounds. This has important implications for phenotypic characterization of fruit constituents and selection of valuable germplasm for introduction into breeding programmes. When substantive environmental effects reduce trait heritability or multiple genes influence a trait, the efficiency with which desirable genotypes can be identified is reduced. In this case, increased replication, by using clonal replicates of each individual plant of the segregating generations and/or analyzing several individual fruit of each plant instead of a bulked sample, would result in lower estimates of the environmental effects.

The genetic analysis of phenolic acid conjugate content demonstrated that additive effects account for a large portion of the genetic variance in phenolic constituents and total concentration. Similar results have been reported for coffee (Ky *et al*., 1999). The highest phenolic acid conjugate content will be obtained when alleles from the wild species are present in homozygosis. Development of molecular markers linked to trait-relevant structural and regulatory genes are valuable for improvement of quantitatively inherited traits. Relevant to phenolic acid quinate esters, Comino *et al*. (2009) recently developed gene specific markers in artichoke for a chlorogenic acid hydroxycinnamoyltransferase.

Correlations identified between groups 1 (mono- and di-caffeoylquinic acid esters), 3 (mono-feruloylquinic acid esters), and 4 (malonylcaffeoylquinic acid esters) are in accord with the interconnected biosynthetic pathways for these three groups of compounds and suggest that relatively few markers may be required for co-selection of these phenolic acid conjugates. Synthesis of the feruloylquinic acid isomers in group 3 rather than the corresponding caffeoylquinic acid isomers in group 1 requires the action of a single enzyme, caffeoyl-CoA 3-O-methyltransferase (Do *et al*., 2007). Similarly, synthesis of the two malonylcaffeoylquinic acid isomers in group 4 from 5-CQA and 4-CQA in group 1 is most likely catalyzed by an as yet undiscovered malonyl-CoA caffeoylquinate acyltransferase (Ma *et al*., 2011). Thus, as few as two genes could be involved in linking biosynthesis of compounds in group 1 with those in groups 3 and 4. By contrast, no correlation was indicated between the hydroxycinnamic acid amides (HCAA) of polyamines in group 2 and the quinate esters in groups 3 and 4, and only a low correlation was noted between HCAA in group 2 and the caffeoylquinates in group 1. This is not surprising in light of the requirement for putrescine or spermine as substrates for HCAA biosynthesis, as well as the involvement of a specialized set of acyltransferases that form amide linkages between the hydroxycinnamic acid and polyamine moieties in HCAA (Bassard *et al*., 2010).

Illustrative of the potential to exploit the high phenolic acid conjugate content of *S. incanum* fruit in an eggplant breeding program, we identified plants from the F2 and BC1P2 generations with combinations of high fruit phenolic content, reduced fruit flesh browning, and moderate fruit weight.

Utilizing a collection of cultivated eggplant, Prohens *et al*. (2007) determined that total phenolics accounted for only 16% of the total variation in fruit browning. Although a negative correlation between fruit weight and phenolic acid conjugate content was found in our F2 population, the values obtained are low (-0.3609), which means that only 13% of the variation in phenolic compounds is explained by the variation in fruit weight. Correlations between fruit weight and phenolic content were non-significant for backcross individuals. Hence, we expect that significant progress can be made in breeding market size fruit with high phenolic acid conjugate content. Also, because *S. incanum* presents comparatively high levels of solasonine and solamargine (Fukuhara and Kubo, 1991), levels of glycoalkaloids will have to be monitored in the introgression materials. In this respect, it has been shown that eggplant lines derived from introgression of allied species with high content in glycoalkaloids presented the typical low values of cultivated eggplant (Mennella *et al*., 2010).

In summary, the results obtained indicate that *S. incanum* is a valuable source of variation for improving eggplant phenolic acid conjugate content. Wide variation found for morphological traits, phenolic content, and fruit browning in segregating generations descended from this *S. melongena* x *S. incanum* interspecific cross suggests that the desirable cultivated eggplant characteristics could be recovered expeditiously in a backcrossing breeding program.

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Discusión general

1. Aumento del valor añadido de la berenjena mediante el incremento del contenido en polifenoles

La demanda de hortalizas con mayor contenido en compuestos bioactivos está propiciando el desarrollo de programas de mejora dirigidos a aumentar este tipo de metabolitos. Algunos ejemplos de estas nuevas variedades comerciales mejoradas e innovadoras incluyen la sandía 'Fashion', que tiene un alto contenido en licopeno y citrulina, los tomates 'Lycomate' y el 'Doublerich', que tienen, respectivamente, un alto contenido en licopeno y vitamina C, y la berenjena de Almagro, con alto contenido en ácido clorogénico (Watada *et al.*, 1976; Tarazona-Díaz *et al.*, 2011; Hurtado *et al.*, 2014). El alto contenido en compuestos bioactivos de estas variedades hace que tengan un valor añadido importante, alcanzando mayores precios en el mercado (Diamanti *et al*., 2011).

En el caso de la berenjena, se sabe que es una de las hortalizas con mayor poder antioxidante (Cao *et al*., 1996), conferido en gran parte por los compuestos fenólicos, en particular el ácido clorogénico (Cao *et al*., 1996; Hanson *et al*., 2006; Okmen *et al*., 2009; Akanitapichat *et al*., 2010; Lo Scalzo *et al*., 2010). El creciente interés en el ácido clorogénico se debe a sus múltiples propiedades beneficiosas en la prevención de enfermedades metabólicas y cardiovasculares y trastornos alimenticios (Plazas *et al*., 2013). No obstante, varios estudios muestran que las berenjenas modernas han sufrido una reducción en el contenido de ácido clorogénico debido a la selección indirecta en contra de este carácter al seleccionar plantas con un bajo pardeamiento (Whitaker y Stommel, 2003; Prohens *et al*., 2007; San José *et al*., 2014).

Al igual que en otros cultivos, como por ejemplo el pimiento (Rodríguez-Burruezo *et al.*, 2009b), el éxito de los programas de mejora del contenido en compuestos bioactivos de la berenjena depende de la disponibilidad de fuentes de variación adecuadas (Rodríguez-Burruezo *et al.*, 2009a; Diamanti *et al.*, 2011; Acquaah, 2012). En esta tesis hemos optado por la explotación de la variabilidad tanto intraespecifíca como interespecífica. En el primer caso, hemos utilizado materiales tradicionales de berenjena, ya que éstos presentan una alta variación en el contenido de compuestos bioactivos (Stommel y Whitaker, 2003; Hanson *et al*., 2006; Prohens *et al*., 2007) y, al

igual que en otros cultivos hortícolas (Koch y Goldman, 2005; Mou, 2005; Rodríguez-Burruezo *et al.*, 2005; Burger *et al.*, 2006; Perkins-Veazie *et al*., 2010), muchas de las variedades tradicionales presentan valores mucho más altos que los de las variedades comerciales modernas. En el caso de la utilización de la variabilidad interespecífica, las especies relacionadas pueden tener valores hasta diez veces mayores en compuestos bioactivos que las especies cultivadas (Willits *et al*., 2005; Prohens *et al.*, 2013). De esta forma, hemos seleccionado una especie silvestre del germoplasma primario de la berenjena (*S. incanum*) y dos especies cultivadas (*S. aethiopicum* y *S. macrocarpon*) del germoplasma secundario (Knapp *et al*., 2013). A pesar de que el uso de germoplasma secundario genera mayores dificultades que usar el germoplasma primario, la ventaja de utilizar estas dos especies cultivadas radica en que no presentan los caracteres indeseables propios de las especies silvestres ya que han sido eliminados en la domesticación (Meyer *et al*., 2013), acortando así los programas de mejora que incluyen este tipo de materiales. Por otra parte, hay que tener en cuenta que al ser especies cultivadas, al igual que la berenjena común, se puede aprovechar para mejora recíproca, es decir mejorarlas a las tres utilizando genes de las otras (Plazas *et al*., 2013). Además, los complejos escarlata (Lester *et al.*, 1986; Lester y Niakan, 1986; Plazas *et al.*, 2014; Kouassi *et al.*, 2014) y gboma (Lester y Hasan, 1990 y 1991; Daunay, 2008; Weese y Bohs, 2010) son muy variables por lo que esta variabilidad se puede explotar para la mejora de la berenjena común.

En definitiva, en esta tesis hemos realizado trabajos encaminados a la utilización de la variación para el estudio de la diversidad, y también se han desarrollado metodologías y material vegetal de interés en la mejora genética de la berenjena, lo cual creemos que puede contribuir al desarrollo de variedades con alto valor añadido, que es un objetivo importante dentro del sector hortícola.

2. La diversidad en contenido en polifenoles en berenjena en el contexto de una mejora integral

2.1. *La mejora integral de la berenjena*

El desarrollo de una variedad exitosa y con salida en el mercado actual de hortalizas requiere de una mejora integral, entendida como la obtención de una variedad que satisface a los distintos actores de la cadena que va del productor al consumidor (Cubero, 2003). Esto implica que una variedad exitosa no sólo tendrá que tener un alto contenido en compuestos bioactivos (polifenoles) introgresados de nuestras fuentes de variación, sino que además tendremos que tener en cuenta los caracteres indeseables que podemos arrastrar en el proceso de mejora (disminución del rendimiento, perdida de uniformidad en el fruto, pardeamiento de la carne, aumento de glicoalcaloides, etc.) (Hajjkar y Hodgkin, 2007).

Tradicionalmente uno de los caracteres que más depreciaban la calidad aparente de la berenjena era el pardeamiento de la carne. Este se produce al cortar el fruto y exponerlo al contacto del aire, produciendo una oxidación enzimática de los polifenoles a causa de las polifenol oxidasas (PPOs) que contiene la carne (Fujita y Tono, 1988; Todaro *et al*., 2011). Por ello, seleccionando variedades con bajo pardeamiento, los mejoradores han reducido mediante selección indirecta el nivel de polifenoles de la carne del fruto en las variedades modernas de berenjena (Whitaker y Stommel, 2003; Prohens *et al*., 2007). Sin embargo, nuestros estudios (Plazas *et al*., 2013) muestran que el pardeamiento y el nivel de polifenoles del fruto de la berenjena son caracteres con una correlación baja a moderada, con lo que es factible aumentar la cantidad de polifenoles en el fruto controlando los niveles de pardeamiento del mismo.

Sin embargo, a la vez, tenemos que tener en cuenta otros caracteres necesarios para el éxito de la variedad, como podrían ser: la producción, la forma del fruto, el color, la resistencia a enfermedades, el sabor, el comportamiento durante el procesado, etc. (Daunay y Hazra, 2012). En este sentido, es de interés el estudio de la variabilidad para caracteres morfológicos y agronómicos para poder seleccionar aquellos materiales que presenten una

mejor combinación de caracteres de interés para el éxito de una variedad de berenjena (Cubero, 2003; Daunay y Hazra, 2012). Por ejemplo, en el caso de querer aumentar el contenido en compuestos bioactivos en la berenjena, nos interesa tanto el carácter de elevado contenido en polifenoles, así como todos aquellos caracteres que pueden verse afectados de forma indirecta por ese incremento, principalmente el pardeamiento enzimático de la carne (Prohens *et al*., 2005; Prohens *et al*., 2007; Muñoz-Falcón *et al*., 2008; Hurtado *et al*., 2012; Plazas *et al*., 2013).

2.2. *Diversidad en berenjena común*

Varios estudios muestran que existe una amplia diversidad en berenjena para polifenoles y ácido clorogénico (Stommel y Whitaker, 2003; Hurtado *et al*., 2012; Prohens *et al*., 2013). Además podemos encontrar estudios que indican que el contenido en polifenoles de la berenjena está correlacionado con el pardeamiento (Prohens *et al*., 2007; Massolo *et al*., 2011; Mishra *et al*., 2012, 2013). Efectivamente, el pardeamiento de la berenjena, depende de forma directa del contenido en polifenoles y de la actividad PPO, aunque puede haber otros factores implicados. Eliminar el efecto negativo que produce el pardeamiento en la calidad aparente de la berenjena no es fácil, especialmente teniendo en cuenta que no debemos reducir los niveles de polifenoles. Alguno de estos estudios se han hecho con variedades orientales (Mishra *et al*., 2012, 2013), las cuales son genéticamente diferentes a las occidentales (Vilanova *et al*., 2012; Cericola *et al*., 2013) y dado que no son las que más importancia tienen en Europa, África, Medio Este y América se ha visto justificado la necesidad de realizar un estudio similar en variedades occidentales.

Hasta ahora no había ningún estudio que mostrase cuál es la relación entre polifenoles, actividad PPO y pardeamiento en berenjena. Nosotros hemos encontrado que la alta diversidad que se conoce en la berenjena para polifenoles y ácido clorogénico se confirma en una colección de variedades locales españolas. Además, existe también una alta variación para actividad PPO y pardeamiento, medido de dos formas distintas. También hemos encontrado que estas variedades locales presentan una alta diversidad a nivel

molecular en el estudio que hemos realizado con marcadores SSR (Vilanova *et al*., 2014).

Centrándonos en la necesidad de obtener plantas con un alto contenido en polifenoles y bajo pardeamiento se han buscado fuentes de variación en la amplia variabilidad existente en variedades tradicionales de este cultivo, así como en especies silvestres y cultivadas relacionadas con *S. melongena* (Knapp *et al*., 2013).

En el caso del pardeamiento hemos encontrado que solamente el 18.9% de la variación total de este carácter es debida a las diferencias en polifenoles totales que contiene el fruto. Esta baja correlación confirma lo que ya se observó en otros estudios (Prohens *et al*., 2007; Prohens *et al*., 2013). Estos mismos resultados se han observado también en otros cultivos (Coseteng y Lee, 1987; Radi *et al*., 1997). La falta de correlación entre la actividad PPO y el pardeamiento obtenido en nuestros datos nos lleva a proponer que la actividad PPO del fruto no es el factor limitante para la producción de pardeamiento en los materiales ensayados, es decir que no supone un factor limitante en el pardeamiento. Ello no implica que seleccionar para muy baja actividad PPO no dé lugar a frutos con bajo pardeamiento.

Por otra parte, en esta tesis hemos comprobado la existencia de una alta actividad funcional antioxidante de los frutos de berenjena, la cual está altamente correlacionada con el contenido en ácido clorogénico y la capacidad del captar radicales libres que posee la berenjena (Plazas *et al*., 2013). Se ha encontrado también una correlación muy alta entre el contenido en ácido clorogénico y el pardeamiento del extracto líquido (procedente del zumo del fruto) lo que podría ser muy útil para realizar una medida rápida del contenido en ácido clorogénico presente en el fruto, sin necesidad de efectuar ninguna extracción química.

Se ha podido observar que el factor ambiental es importante a la hora de hacer una selección para caracteres de interés, ya que las accesiones se comportan de forma distinta, acumulando más o menos compuestos dependiendo de cómo y dónde se cultiven (San José *et al*., 2014). Hemos constatado que hay una correlación ambiental positiva entre actividad PPO y el

contenido en ácido clorogénico, observándose que las condiciones que inducen un aumento en el contenido en ácido clorogénico podrían activar también la expresión de los genes de PPO en frutos de berenjena (López-Gresa *et al*., 2011; Mayer, 2006)

En definitiva esta Tesis muestra que es posible seleccionar variedades de berenjena con alto contenido en polifenoles y ácido clorogénico y pardeamiento bajo o moderado. También sugerimos que la búsqueda de variedades con muy baja actividad PPO podría permitir obtener variedades con pardeamiento muy bajo.

2.3. *Diversidad en berenjenas escarlata y gboma*

Las berenjenas escarlata y gboma, a pesar de ser de gran importancia en África (Schippers, 2000; Kouassi *et al*., 2014) han recibido poca atención en lo que respecta a estudios sobre su diversidad y mejora genética. Esto ha permitido que en sus lugares de origen se conservaran un gran número de variedades locales y que junto con las accesiones conservadas en los bancos de germoplasma se disponga de estos recursos genéticos para la mejora de ambos cultivos (Lester *et al*., 1990; Bukenya y Carasco, 1994; Schippers, 2000; Sekara *et al*., 2007; Kouassi *et al*., 2014). Entre estas dos berenjenas se pueden obtener híbridos con una fertilidad intermedia (Oyelana y Ugborogho, 2008). Además del interés por sí mismas, como especies cultivadas, estas dos berenjenas pertenecen al germoplasma secundario de la berenjena común lo que las hace muy interesantes para utilizar la variabilidad encontrada como fuente de variación para la mejora genética de *S. melongena* (Daunay *et al*., 1991; Oyelana y Ugborogho, 2008; Prohens *et al*., 2012; Khan *et al*., 2013).

Para conocer la diversidad existente en estos dos complejos de berenjena, esencial en una mejora integral (Cubero, 2003), nos hemos centrado en definir de forma precisa los caracteres morfológicos, utilizando los descriptores de EGGNET (Prohens *et al*., 2005; van der Weerden y Barendse, 2007; Muñoz-Falcón *et al*., 2008b), y fenotípicos propios de cada uno de los complejos, usando la herramienta informática Tomato Analyzer (Brewer *et al*., 2006, 2007; Gonzalo y van der Knapp, 2008; Rodríguez *et al*., 2010) que da información sobre un elevado número de parámetros de forma del fruto.

También se ha medido la concentración de ácido clorogénico, con técnicas cromatográficas (Naranjo *et al*., 2003); la actividad reductora, como equivalente del contenido en polifenoles totales por el método de Folin-Ciocalteu (Singleton y Rossi, 1965); y su actividad biológica, evaluada como la capacidad de inhibición de óxido nítrico en cultivos celulares de macrófagos (Grisham *et al*., 1996). Aunque la caracterización se ha realizado en condiciones de clima mediterráneo, las cuales son diferentes de la zona de origen (África tropical) de las berenjenas escarlata y gboma, los caracteres evaluados, y en particular los de forma del fruto, presentan en general una alta heredabilidad (Hurtado *et al.*, 2013), por lo que es de esperar que los resultados obtenidos por nosotros sean extrapolables a otras condiciones.

Analizados los datos morfológicos, fenómicos, químicos y biológicos llegamos a la conclusión que estos dos complejos de berenjenas son hipervariables (Plazas *et al*., 2014), y que existe una amplia diversidad entre cada una de las especies estudias, confirmando estudios previos (Lester, 1986; Lester *et al*., 1986; Lester y Daunay, 2003; Raigón *et al*., 2008) y al igual que ocurre en la berenjena común (Cao *et al*., 1996; Stommel y Whitaker, 2003; Plazas *et al*., 2013b; Prohens *et al*., 2013).

La combinación de todos los caracteres morfológicos y fenómicos estudiados en todas las accesiones de *S. aethiopicum* y en *S. anguivi* ha sido suficiente para conseguir distinguir todos los grupos entre sí. Además utilizando el Tomato Analyzer es posible distinguir con los frutos a *S. macrocarpon* de su ancestro *S. dasyphyllum* (Bukenya y Carasco, 1994), lo cual tiene interés para el manejo de recursos genéticos e identificación de materiales.

S. dasyphyllum es la especie con valores más elevados de ácido clorogénico y actividad reductora, aunque también se han encontrado otros polifenoles que podrían estar interviniendo en su elevada actividad reductora. Le siguen el grupo Kumba para capacidad reductora y el grupo Shum en el contenido en ácido clorogénico, de gran interés, al ser cultivadas, para realizar una mejora integral en la berenjena común. Además, los materiales con mayor contenido en polifenoles presentan una mayor actividad biológica, lo cual confirma el papel importante de éstos en las propiedades bioactivas de las

berenjenas, previamente estudiado por Sato y colaboradores en 2011 utilizando ácido clorogénico y ácido caféico.

La existencia de una alta diversidad en *S. aethiopicum* y *S. macrocarpon* conlleva la necesidad de conservar gran cantidad de accesiones en los bancos de germoplasma o en colecciones nucleares para poder tener una buena representación de la variabilidad genética que tienen ambas especies (Odong *et al*., 2013). Los datos de caracterización, el análisis multivariante, la actividad reductora, la actividad biológica y el contenido en ácido clorogénico pueden ser muy útiles para seleccionar grupos de accesiones que representen mucha de la diversidad morfológica de ambos complejos. En cuanto a nivel de selección y mejora genética, las diferencias fenotípicas entre y dentro de los grupos se pueden utilizar para seleccionar las mejores accesiones o los mejores padres para obtener híbridos F1 con efecto heterótico en el rendimiento o con características nuevas o intermedias (Lester y Thitai, 1989; Seck, 2000; Rodríguez-Burruezo *et al*., 2008; Adeniji y Aloyce, 2012). En definitiva, nuestros estudios revelan que las berenjenas escarlata y gboma presentan una amplia diversidad, la cual es de interés para la mejora de la berenjena común y también para la mejora de estos dos cultivos marginados.

3. Hibridación interespecífica para la mejora del contenido en compuestos bioactivos

3.1. Especies relacionadas de interés para la mejora del contenido en compuestos bioactivos

La berenjena común puede cruzarse con un amplio número de especies, aunque con distinto grado de éxito, así como de viabilidad y fertilidad en los híbridos y generaciones siguientes (Daunay y Harza, 2012; Rotino *et al*., 2014). El éxito de la hibridación suele estar relacionado con lo alejada que se encuentre, evolutivamente hablando, la especie con la que se quiere cruzar *S. melongena*.

Dependiendo de la facilidad de cruzamiento, las especies silvestres relacionadas con la berenjena se pueden agrupar en germoplasma primario,

secundario y terciario. El germoplasma primario es el más cercano evolutivamente con la berenjena común, siendo los híbridos totalmente fértiles, mientras que el terciario es el más alejado y los híbridos sólo se pueden obtener utilizando técnicas especiales como rescate de embriones. Por tanto, dependiendo del grupo al que correspondan las accesiones que queramos cruzar con berenjena, tendremos mayor o menor dificultad.

Dentro de las especies más interesantes para la hibridación con la berenjena se encuentra *S. aethiopicum*, porque es cultivada, muy importante en África e hibrida con *S. melongena* mucho mejor que la otra especie cultivada (*S. macrocarpon*) (Oyelana y Ugborogho, 2008). Ambas especies pertenecen al germoplasma secundario de la berenjena común (Daunay y Hazra, 2012). Otra especie interesante es *S. incanum*, la cual es filogenéticamente muy próxima a *S. melongena*, los híbridos son fértiles y tiene un contenido muy alto en polifenoles totales y ácido clorogénico (Knapp et al., 2013). Al igual que *S. insanum*, *S. incanum* es una de las especies más cercanas a la berenjena común (Lester y Hasan, 1991; Meyer et al., 2012), y por tanto una de las especies más interesantes para aumentar la variabilidad de la berenjena común. Los niveles en ácido clorogénico de *S. incanum* son muy elevados (Stommel y Whitaker, 2003), con lo que sería muy útil usarla para mejorar este carácter. Estas tres especies pertenecen al germoplasma primario de *S. melongena* con lo que deberíamos obtener los híbridos con poca dificultad.

No nos ocurrirá lo mismo con *S. dasyphyllum*, que pertenece al germoplasma secundario de la berenjena y, como hemos visto en esta Tesis, es la especie relacionada con la berenjena en la que se ha encontrado una mayor cantidad de ácido clorogénico y una actividad reductora muy alta. Esto la hace una especie muy interesante para incluirla en los planes de mejora de estos compuestos en la berenjena cultivada.

3.2. Potencial de S. aethiopicum para la mejora recíproca con S. melongena

La obtención de semilla híbrida entre *S. aethiopicum* y *S. melongena* es relativamente fácil (Behera y Singh, 2002; Oyelana y Ugborogho, 2008; Khan y Isshiki, 2010) y la germinación de la misma es generalmente alta (Behera y

singh, 2002; Oyelana y Ugborogho, 2008; Gisbert *et al*., 2011). Es por ello que *S. aethiopicum* es una especie candidata de gran interés para la mejora recíproca con *S. melongena*. Dentro de la berenjena escarlata existen distintos grupos de cultivares (Lester, 1986; Lester *et al*., 1986; Lester y Niakan, 1986). En nuestro caso hemos decidido utilizar el grupo Kumba para obtener híbridos ya que este grupo es el que presenta mayor tamaño del fruto dentro de *S. aethiopicum*, lo cual probablemente facilite la labor de recuperar el tamaño de fruto en los retrocruzamientos con *S. melongena*.

En la realización de la familia con la que hemos trabajado se han tenido más dificultades en la utilización de *S. melongena* como parental femenino, tanto en la formación de híbridos como en los retrocruces, con lo que será más conveniente la utilización de *S. melongena* para mejorar las características de *S. aethiopicum* grupo Kumba que viceversa. Hemos conseguido generaciones F1 y BCs, aunque menos individuos hacia *S. melongena*. En el caso de los retrocruces hacia *S. aethiopicum*, al obtener más individuos ha sido posible estudiar la distorsión en la segregación en los retrocruces mediante marcadores SSR, no habiéndose obtenido evidencias de distorsión.

Además del material vegetal, la caracterización de las distintas generaciones con descriptores morfológicos y la herramienta fenómica Tomato Analyzer nos ha proporcionado una información de muchísima utilidad para la mejora integral de estas especies. En la caracterización se ha encontrado que los híbridos entre estas dos especies tienen inflorescencias con un mayor número de flores que los parentales, presentan espinas, los frutos son ligeramente más pequeños y la mayoría presentan la partenocarpia típica de las Solanáceas (Cuartero *et al.*, 1987; Prohens y Nuez, 2001; Kikuchi *et al*., 2008). A su vez, los retrocruces son más variables que las generaciones no segregantes, con lo que la selección en el primer retrocruce puede ser eficiente para caracteres de planta. No ocurre lo mismo en caracteres de fruto, donde el tamaño es similar en el primer retrocruce y en los híbridos.

Un carácter interesante a mejorar en *S. aethiopicum* es el contenido en polifenoles, ya que lo tiene bastante más bajo que *S. melongena* (Stommel y Whitaker, 2003). Se ha visto que los híbridos, al igual que los individuos de retrocruzamiento hacia *S. aethiopicum* presentan niveles bajos, sugiriendo que

el bajo nivel de polifenoles de *S. aethiopicum* es dominante. No ocurre lo mismo con los retrocruces hacia *S. melongena*, en que se encuentran individuos con altos niveles, lo que sugiere que debe ser un carácter controlado por unos pocos genes. Por otra parte, el pardeamiento se ve aumentado en los híbridos interespecíficos, con lo que habría que buscar en las siguientes generaciones segregantes individuos que redujeran la alta actividad enzimáticas de las polifenol oxidasas.

En definitiva, los resultados de esta parte muestran que parece mucho más fácil la utilización de *S. melongena* para la mejora de *S. aethiopicum* que viceversa. No obstante, otras accesiones de *S. aethiopicum*, como las que hemos visto que tienen un alto contenido en clorogénico (Plazas *et al.*, 2014) podrían ser útiles para la hibridación interespecífica y mejora recíproca con *S. melongena*.

3.3. Potencial de S. incanum para la mejora de S .melongena

Mejorar la calidad nutracéutica y la concentración de compuestos bioactivos es un objetivo cada vez más importante en la mejora de hortalizas (Jenks y Bebeli, 2011) y en el caso de *S. melongena*, es nuestro objetivo principal. En estudios previos se comprobó que uno de los cultivos con un contenido más elevado en polifenoles era precisamente *S. incanum*, una de las especies silvestre más cercana filogenéticamente a S*. melongena* (Knapp *et al*., 2013) con cantidades hasta tres veces superiores a las encontradas en *S. melongena* (Stommel y Whitaker, 2003; Ma *et al*., 2011).

Para mejorar este carácter en la berenjena común, la disponibilidad de una fuente de variabilidad en una especie tan cercana es toda una ventaja, ya que obtener descendencia de los híbridos y retrocruces entre ellas debe resultarnos más fácil que utilizando especies más alejadas filogenéticamente (Lester y Hasan, 1991; Daunay, 2012).

Los estudios de caracterización que hemos realizado muestran que las dos especies son muy distintas, tanto para morfología de fruto como de planta. Así, hemos encontrado que los frutos de los híbridos obtenidos tienen características intermedias entre los dos parentales y la planta es mucho más

vigorosa, debido al vigor híbrido. Esta heterosis para caracteres vegetativos se puede explotar para utilizar estos híbridos interespecíficos como portainjertos (Gisbert *et al*., 2011), industria que está en auge debido a la aparición de nuevas enfermedades del suelo a las que las especies cultivadas son susceptibles (Abbasi *et al*., 2014; Soumia *et al*., 2014; Villeneuve *et al*., 2014).

Los híbridos y generaciones BC1P2 y F2 que hemos obtenido han sido completamente fértiles. Una vez evaluadas las características morfológicas y el contenido en polifenoles, se ha encontrado que en la primera generación de retrocruzamiento ya se encuentran individuos con bastante parecido a la berenjena cultivada, algunos de ellos con un alto peso del fruto, lo cual sugiere que en unas pocas generaciones de retrocruzamiento será posible recuperar las características típicas de la especie cultivada. En este proceso de selección también deberemos seleccionar individuos con un bajo pardeamiento, ya que los híbridos tienen un pardeamiento más alto que los padres. En las generaciones segregantes estos valores tienen una amplia variabilidad, lo cual indica que existen posibilidades de selección.

El compuesto fenólico que hemos encontrado en cantidades más elevadas es el ácido clorogénico y está presente en todas nuestras muestras. El que se encuentre en la berenjena común y en su ancestro sugiere que la función de este compuesto pueda estar relacionada con funciones defensivas de la planta, contra pesticidas, patogénos o estreses abióticos (Bradfield y Stamp, 2004; López-Gresa *et al*., 2011). Al igual que otros caracteres de composición nutricional (San José *et al*., 2014) es un carácter influenciado por el ambiente, ya que hemos encontrado variación en las generaciones no segregantes. Además se han encontrado plantas en la F2 y en los retrocruces con alta concentración de ácido clorogénico, bajo pardeamiento, y un tamaño del fruto adecuado para el consumidor lo que hace que esta especie sea muy interesante para incluirla en planes de mejora de *S. melongena*.

Esto confirma que *S. incanum* se muestra como una alternativa prometedora para el desarrollo de nuevas variedades de berenjena con alto contenido en compuestos bioactivos y que las características típicas de *S. melongena* se podrían recuperar en unas pocas generaciones de retrocruzamiento.

4. Perspectivas de futuro

El trabajo presentado está enfocado hacia la mejora del contenido en compuesto bioactivos de la berenjena desde una perspectiva integral y supone un avance importante respecto al conocimiento que se tenía hasta el momento. Ello abre una nueva vía para el desarrollo de nuevas estrategias para conseguir el desarrollo de variedades modernas con una mejora sustancial de sus propiedades bioactivas, y por tanto, con un considerable valor añadido.

Uno de los trabajos que actualmente está en marcha y que nos facilitará muchísimo el trabajo en el futuro a la hora de la selección es tener mapeados los genes implicados en la ruta de ácido clorogénico y los genes que codifican para las PPOs. Hasta la fecha, Gramazio *et al.* (2013) basándose en la sintenia con tomate, han posicionado en un mapa genético interespecífico entre *S. melongena* y *S. incanum* (Prohens *et al.*, 2013), seis genes candidatos que codifican enzimas implicadas en la ruta biosíntetica del ácido clorogénico. Esta información puede ser de gran utilidad para la selección asistida por marcadores moleculares en programas de mejora.

Paralelamente en el mismo mapa genético se han mapeado cinco enzimas polifenol oxidasas (PPO1, PPO2, PPO3, PPO4, PPO5) implicadas en procesos de oxidación enzimaticas que producen un pardeamiento de la carne de berenjena y una consecuente pérdida de la calidad aparente (Shetty *et al*., 2011). Los marcadores desarrollados en este trabajo serán empleados en diferentes estrategias para la mejora de la calidad nutracéutica de la berenjena a través de la selección asistida por marcadores (MAS). Una de las estrategias a seguir es la búsqueda de variantes alélicas de los genes implicados en la síntesis del ácido clorogénico, que permitan una mayor acumulación de este último, así como de variantes alélicas de polifenol oxidasas de baja actividad en colecciones de germoplasma mediante plataformas de EcoTILLING o TILLING (Pérez-de-Castro *et al*., 2012). Los alelos más favorables, dependiendo de su procedencia, se podrán combinar mediante piramidación o introgresar a través de procesos de retrocruzamiento.

El interés despertado por el desarrollo de la familia entre *S. melongena* y *S. incanum* y sus implicaciones en la mejora de la berenjena común han llevado al desarrollo de otras líneas de investigación similares con la especies que hemos estudiado en este trabajo; como serían el desarrollo de líneas de introgresión con otras especies, como *S. dasyphyllum*, con un alto contenido en polifenoles, con *S. melongena*, así como poblaciones multiparentales (MAGIC) (Cavanagh *et al*., 2008; Pascual *et al*., 2014).

La obtención de plantas transgénicas o cisgénicas de berenjena con elevada concentración en ácido clorogénico y bajo pardemiento también es una posibilidad, tal como se ha visto en tomate (Niggeweg *et al*., 2004). Un ejemplo del uso práctico de plantas de berenjena transformadas genéticamente es el caso de la berenjena Bt en India (Kolady y Lesser, 2012). Sin embargo, a efectos prácticos a nivel comercial este tipo de materiales tiene pocas perspectivas de éxito en Europa, al menos a corto-medio plazo (Fresco, 2013).

En definitiva, este trabajo contribuye a la utilización de la diversidad intraespecífica e interespecífica en el desarrollo de nuevas variedades de berenjena con mejores propiedades bioactivas y aporta información relevante para el uso de nuevas estrategias de mejora que contribuirán al desarrollo de una nueva generación de variedades con propiedades bioactivas mejoradas. Esta aproximación puede servir como modelo para la mejora de la calidad de otras hortalizas.

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Conclusiones

CONCLUSIONES

1. De acuerdo con los ensayos realizados en berenjena común (*S. melongena*) se ha encontrado una baja correlación entre el contenido en polifenoles y el pardeamiento de la carne del fruto, con lo que se abre la posibilidad de seleccionar plantas con mejores propiedades bioactivas y con un bajo pardeamiento.

2. En la misma colección de berenjena común, en base al perfil de contenido en polifenoles, ácido clorogénico, actividad antioxidante y pardeamiento, se han identificado cuatro grupos con comportamientos distintos. Esto permitirá seleccionar los materiales más adecuados para la mejora de la calidad funcional y aparente.

3. La caracterización de una colección de berenjena escarlata (*S. aethiopicum*) y gboma (*S. macrocarpon*) usando descriptores convencionales (EGGNET) y fenómicos (Tomato Analyzer) nos ha permitido obtener una información relevante sobre la diversidad y las relaciones genéticas entre los dos cultivos. La herramienta fenómica Tomato Analyzer permite diferenciar los grupos de cultivares en los que hay muy pocas diferencias en cuanto a características de planta. La combinación de estos dos tipos distintos de descriptores conjuntamente proporcionan una herramienta muy útil para estudiar estos dos complejos e identificar materiales adecuados para una mejora integral.

4. La alta diversidad morfológica de las berenjenas escarlata y gboma se ve correspondida por una considerable variación en el contenido en ácido clorogénico y actividad reductora. La heredabilidad encontrada en estos caracteres ha sido alta, con lo que la selección para los mismos será eficiente. Para muchas de las muestras estudiadas estos dos caracteres se encuentran correlacionados. Los valores más elevados para la actividad reductora y el contenido en ácido clorogénico más alto lo hemos encontrado en *S. dasyphyllum*, especie silvestre que se considera el ancestro de *S. macrocarpon*.

5. Las pruebas realizadas con cultivos celulares de macrófagos muestran que los extractos de berenjena escarlata y gboma, y en particular las de esta

última, inhiben la producción de óxido nítrico, mostrando una importante actividad biológica. Los materiales con mayor contenido en ácido clorogénico presentan mayor actividad biológica, confirmando que este compuesto es responsable, en gran medida, de las propiedades bioactivas de la berenjena.

6. Los resultados muestran que es posible obtener individuos procedentes de retrocruzamientos de híbridos interespecíficos entre *S. melongena* y *S. aethiopicum* hacia las dos especies, y en particular hacia *S. aethiopicum*, con características adecuadas para la mejora. Existe una amplia variación para características morfológicas, contenido en polifenoles y pardeamiento en los retrocruzamientos, lo cual es de interés para la mejora recíproca. Además se ha comprobado con un conjunto de marcadores que no existe distorsión en la segregación.

7. Los resultados de la hibridación interespecífica entre *S. melongena* y *S. aethiopicum* muestran que la primera es una fuente de variabilidad para *S. aethiopicum*, para caracteres como el contenido en polifenoles, la partenocarpia, el color purpura del fruto, y el bajo contenido en saponinas.

8. Los datos obtenidos en el primer retrocruzamiento de *S. incanum* hacia berenjena común muestran que las características deseables de la especie cultivada pueden recuperarse rápidamente, incorporándose al mismo tiempo el alto contenido en polifenoles de *S. incanum.*

9. En esta misma familia se ha encontrado que el ácido clorogénico es el mayoritario entre los polifenoles y que los valores de heredabilidad son relativamente elevados. Esto refuerza el interés de *S. incanum* como fuente de variación para la mejora de la calidad funcional de la berenjena.

10. A partir de la exploración de la variabilidad intra e interespecífica en las accesiones y cruzamientos realizados, se han identificado y obtenido materiales destinados a mejorar las propiedades bioactivas, tanto en la berenjena común (*S. melongena*) como en las berenjenas africanas (*S. aethiopicum* y *S. macrocarpon*).


Te QUIERO
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