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MEJORA GENÉTICA DE LA BERENJENA (*S. melongena* L.)

Tesis Doctoral

Presentada por:

Maria Hurtado Ricart

Directores:

Dr. Jaume Prohens Tomas

Dr. Santiago Vilanova Navarro

Dra. Isabel Andújar Pérez

“Todo trabajo tiene tiene algo divertido,
si encontráis ese algo,
se convierte en un juego”

Mary Poppins

A Olivia, Miguel y mis padres.
A Bimba.

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Marieta

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RESUMEN

La berenjena (*Solanum melongena* L.) es una de las hortalizas más ricas en compuestos fenólicos, lo cual le confiere un alto poder antioxidante y otras propiedades bioactivas beneficiosas para la salud. Ello hace que haya una demanda creciente entre consumidores preocupados por una dieta saludable. Sin embargo, a pesar de ser un cultivo con una gran importancia económica a nivel mundial, es una de las solanáceas menos estudiadas (mucho menos que tomate, pimiento y patata), por lo que es necesario realizar estudios que contribuyan a la mejora genética de la berenjena a nivel comercial de forma que amplíen la diversidad genética y que permitan adaptarse a las demandas de productores y consumidores.

El trabajo realizado en esta Tesis pretende obtener información relevante para los programas de mejora genética de berenjena mediante el estudio de la diversidad genética y el desarrollo y uso de herramientas para la caracterización morfológica, así como el aumento de la diversidad genética en el germoplasma élite de los programas de desarrollo de híbridos de alto valor. Para ello se utiliza material vegetal tanto de la berenjena comercial tipo negra (semi-larga), como material de otros tipos, orígenes y variedades locales.

En una primera parte de la Tesis, nos basamos en el estudio de diversidad genética en *S. melongena* y en la aplicación de nuevas herramientas para realizar una caracterización morfológica precisa y mejorar el proceso de selección en los programas de mejora. Para ello se han realizado estudios de diversidad en tres centros de origen secundarios de distintas regiones (España, Sri Lanka y China), en materiales locales de berenjena con distintas tipologías, y se han utilizado nuevas herramientas fenómicas para la caracterización morfológica del fruto de la berenjena.

Para la mayoría de los caracteres morfológicos se observaron diferencias significativas entre las accesiones de España, Sri Lanka y China, de forma que con la utilización de pocos caracteres se podría asignar correctamente cualquier accesión a su centro de diversidad, lo cual indica un alto grado de diferenciación morfológica. La

diferenciación morfológica viene acompañada por una considerable diferenciación a nivel molecular, determinada por marcadores SSR. Por otro lado, la utilización de un número reducido marcadores moleculares SSRs genómicos ha permitido detectar una considerable variabilidad genética en una colección de variedades tradicionales de diferentes tipologías (Larga, Semi-larga, Redonda y Listada de Gandía), confirmando que España es un centro de origen secundario. Para finalizar esta primera parte, el estudio fenómico de la forma del fruto con el software Tomato Analyzer, permite una considerable mejora en la caracterización con respecto a los descriptores convencionales, ya que se han podido analizar 23 caracteres cuantitativos y se han podido realizar comparaciones de forma directa para encontrar diferencias significativas entre materiales tanto dentro como entre grupos varietales. Ello es de gran utilidad para la caracterización de recursos de germoplasma y de cultivos, así como para la selección y mejora de programas de berenjena.

Como segunda parte de este trabajo, abordamos el desarrollo de material vegetal para el incremento de la base genética de los cultivares de berenjena e implementación de distintos programas de mejora genética. Para ello se plantean y ejecutan diferentes programas de mejora según los objetivos explícitos, incluyendo la realización de un programa de mejora para una variedad local con Indicación Geográfica Protegida (IGP), e incrementando la diversidad y la obtención de nuevos materiales de élite de berenjena tipo negra mediante un programa de mejora genética.

Por una parte, dado que *S. incanum*, una de las especies silvestre más cercana filogenéticamente a *S. melongena*, presenta cantidades de compuestos fenólicos hasta tres veces superiores a las encontradas en *S. melongena,* hemos iniciado un programa de obtención de líneas de introgresión (ILs) de *S. incanum* en el fondo genético de *S. melongena.* El desarrollo de estas ILs es de gran importancia para la mejora de la berenjena, ya que permitirá que caracteres de interés de esta especie se introgresen en el fondo genético de la berenjena. Además estas líneas serán una herramienta de gran utilidad para el estudio de la evolución y domesticación de este cultivo.

En cuanto al trabajo realizado con la variedad local "Berenjena de Almagro" con indicación IGP, y sabiendo que dicha variedad es genéticamente heterogénea, era necesario basarse en un programa de selección individual. El material fue seleccionado tanto por su menor presencia de espinas como por su alto rendimiento en campo. Para mejorar el carácter de espinosidad en la berenjena de Almagro como objetivo principal, hemos realizado un programa de retrocruzamiento con la utilización de la selección H15 como parental recurrente y la utilización de berenjena (una tipo negra y otra andaluza morfológicamente similar a la de Almagro) con ausencia de espinas como parental donante.

Para finalizar esta segunda parte, se realizó un programa de mejora en colaboración con una empresa privada teniendo en cuenta que interesa poder incrementar la diversidad genética, ya que ello permite obtener combinaciones genéticas nuevas y explotar la heterosis. Conociendo de antemano el caso de la berenjena, se crea la necesidad de estudiar mediante la utilización de marcadores moleculares la variabilidad que existe a día de hoy en el mercado. Después de analizar con SSRs 30 variedades comerciales se pudieron separar 3 grandes grupos en los materiales de berenjena negra. La caracterización molecular junto con la morfológica, indican que hay una amplia diversidad genética para determinar la forma del fruto de la berenjena tipo semi-larga y negra, sin recurrir a la utilización del material típicamente usado por los mejoradores, el cual tiene un fondo génetico común al haber sido derivado de las mismas fuentes. Los materiales obtenidos pueden ser de interés para aumentar la heterosis de los híbridos F_1

En definitiva, nos encontramos con un trabajo que muestra que el estudio de la diversidad genética y el desarrollo y utilización de herramientas fenómicas para la caracterización morfológica, además de la obtención de material vegetal nuevo, es de gran utilidad para el desarrollo de nuevas variedades de berenjena, así como para obtener información científico-técnica de interés para otros investigadores y mejoradores. También hemos constatado que la interacción "investigación pública-privada" permite una colaboración sinérgica en la obtención de material vegetal e información de interés en la mejora de hortícolas.

SUMMARY

Eggplant (*Solanum melongena* L.) is one of the vegetables with highest content in phenolic compounds, giving eggplant a high antioxidant power and other bioactive beneficial health properties. This means that there is a growing demand among consumers concerned about a healthy diet. However, despite being a crop with a great economic importance worldwide, it is one of the least-studied *Solanaceae* (much less than tomato, pepper and potato), so it is necessary to carry out studies that contribute to the genetic improvement of eggplant to the commercial level so that the genetic diversity is increased and that is adapted to the demands of producers and consumers.

The work carried out in this Thesis aims to obtain relevant information for the breeding programmes of eggplant through the study of genetic diversity and development and use of tools for the morphological characterization as well as increasing genetic diversity in élite germplasm for the development of programs aimed at obtaining high-value hybrids. For achieving this objective, commercial eggplant (semi-larga) black type, as well as other types, origins and varieties of local material are used.

In the first part of the Thesis, we rely on the study of genetic diversity in *S. melongena* and the application of new tools to perform accurate morphological characterization and improve the process of selection of the breeding programs. This diversity studies have been conducted in three centres of origin side in different regions (Spain, Sri Lanka and China), in local materials of eggplant with different typologies, and new phenomic tools have been used for the morphological characterization of the fruit of the eggplant.

For most of the morphological characters there were no significant differences between the accessions of Spain, Sri Lanka and China, so that with the use of few characters accessions could be assigned correctly to their centre of diversity, which indicates a high degree of morphological differentiation. The morphological

differentiation is accompanied by a considerable differentiation at the molecular level, determined by SSR markers. On the other hand, the use of a few genomic SSRs molecular markers has made it possible to detect a considerable genetic variation in a collection of traditional varieties of different types (long, Semi-larga, Round and Listada de Gandía), confirming that Spain is a secondary center of diversity. To conclude this first part, the phenomic study phenomic of fruit shape using Tomato Analyzer software, allows a considerable improvement in the characterization with respect to conventional descriptors, since we have been able to study 23 quantitative characters and comparisons have been possible to find significant differences between materials both inside and between varietal groups. This is very useful for the characterization of germplasm and genetic resources as well as for the selection and improvement programmes of eggplant.

In the second part of this work, we deal with the development of plant material to increase the genetic base of eggplant cultivars and with the implementation of various programmes for genetic improvement. For this we plan and develop different breeding programs according to different objectives, including the implementation of a programme of improvement of a local variety with protected geographical indication (IGP), and increasing the diversity and new "elite" material of black type eggplant through a program of genetic improvement.

On one side, given that *S. incanum*, one of the species wild closer phylogenetically to *S. melongena*, presents phenolics amounts up to three times higher than those found in *S. melongena*, we have initiated a program for developing introgression lines (ILs) of *S. incanum* in the genetic background of *S. melongena*. The development of these ILs is very important for eggplant breeding, since it will allow that traits of interest of this species become introgressed in the genetic background of the eggplant. In addition, these lines will be a useful tool for the study of the evolution and domestication of this crop.

Regarding the work done with the local variety "Almagro Eggplant" with IGP status, and knowing that the variety is genetically heterogeneous, it was necessary to

rely on a program of individual selection. The material was selected both for its good performance in field and for their low prickliness. In order to improve the prickliness character in Almagro eggplant as main objective, we have performed a backcross program in which we used the H15 selection as recurrent parent and non-prickly eggplants (a black type and other morphologically similar to the Almagro) as donor parents.

To conclude this second part, a breeding programme in collaboration with a private company was performed in order to increase the genetic diversity, so that it may allow obtaining new genetic combinations and exploiting heterosis. The previous knowledge of the eggplant case creates the need to study the variability that exists today in the market through the use of molecular markers. After analyzing 30 commercial varieties with SSRs we could separate 3 large groups of black eggplant materials. The molecular characterization together with the morphological, indicate that there is a wide genetic diversity in the semi-long black type of eggplant, outside the materials typically used by breeders, which have a common genetic background to have been derived from the same sources. The materials obtained may be of interest to increase the heterosis of F_1 hybrids.

In summary, our work that shows that the study of the genetic diversity and the use of phenomics tools for morphological characterization, as well as the development of new plant material, is very useful for obtaining new varieties of eggplant as well as scientific and technical information of interest to other researchers and breeders. We have also found that "public-private research" interaction allows a synergistic collaboration in obtaining plant material and information of interest for vegetables breeding.

RESUM

L'albergina (*Solanum melongena* L.) és una de les hortalisses més riques en compostos fenòlics, el que li confereix un alt poder antioxidant i altres propietats bioactives beneficioses per a la salut. Aquest fet fa que hi haja una demanda creixent entre els consumidors preocupats per una dieta saludable. No obstant això, a pesar de ser un cultiu amb una gran importància econòmica a nivell mundial, és una de les solanàcies menys estudiades (molt menys que tomaca, pimentó i creïlla) , per la qual cosa és necessari realitzar estudis que contribuïsquen a la millora genètica de l'albergina a nivell comercial de manera que amplien la diversitat genètica i que permeten adaptar-se a les demandes de productors i consumidors.

El treball realitzat en esta Tesi pretén obtindre informació rellevant per als programes de millora genètica d'albergina per mitjà de l'estudi de la diversitat genètica i el desenvolupament i ús de ferramentes per a la caracterització morfològica, així com l'augment de la diversitat genètica en el germoplasma elit dels programes de desenvolupament d'híbrids d'alt valor. Per a això s'utilitza material vegetal tant de l'albergina comercial de tipus negra (semi-llarga), com material d'altres tipus, orígens i varietats locals.

En una primera part de la Tesi, ens basem en l'estudi de diversitat genètica en *S. melongena* i en l'aplicació de noves ferramentes per a realitzar una caracterització morfològica precisa i millorar el procés de selecció en els programes de millora. Per a això s'han realitzat estudis de diversitat en tres centres d'origen secundaris de distintes regions (Espanya, Sri Lanka i Xina), en materials locals d'albergina amb distintes tipologies, i s'han utilitzat noves ferramentes fenòmiques per a la caracterització morfològica del fruit de l'albergina.

Per a la majoria dels caràcters morfològics es van observar diferències significatives entre les accessions d'Espanya, Sri Lanka i Xina, de manera que amb la utilització de pocs caràcters es podria assignar correctament qualsevol accessió al seu centre de diversitat, la qual cosa indica un alt grau de diferenciació morfològica. La

diferenciació morfològica ve acompanyada per una considerable diferenciació a nivell molecular, determinada per marcadors SSR. D'altra banda, la utilització d'un número reduït de marcadors moleculars SSRs genòmics ha permés detectar una considerable variabilitat genètica en una col·lecció de varietats tradicionals de diferents tipologies (Llarga, Semi-llarga, Redona i Llistada de Gandia), confirmant que Espanya és un centre d'origen secundari. Per a finalitzar esta primera part, l'estudi fenómico de la forma del fruit amb el programari Tomato Analyzer, permet una considerable millora en la caracterització respecte als descriptors convencionals, ja que s'han pogut analitzar 23 caràcters quantitatius i s'han pogut realitzar comparacions de forma directa per a trobar diferències significatives entre materials tant dins com entre grups varietals. Això és de gran utilitat per a la caracterització de recursos de germoplasma i de cultius, així com per a la selecció i millora de programes d'albergina.

Com a segona part d'este treball, abordem el desenvolupament de material vegetal per a l'increment de la base genètica dels cultivars d'albergina i implementació de distints programes de millora genètica. Per a això es plantegen i executen diferents programes de millora segons els objectius explícits, incloent la realització d'un programa de millora per a una varietat local amb Indicació Geogràfica Protegida (IGP), i incrementant la diversitat i l'obtenció de nous materials d'elit d'albergina tipus negra per mitjà d'un programa de millora genètica.

D'una banda, atés que *S. incanum*, una de les espècies silvestres més pròxima filogenèticament a *S. melongena*, presenta quantitats de compostos fenólics fins a tres vegades superiors a les trobades en *S. melongena*, hem iniciat un programa d'obtenció de línies d'introgresió (ILs) de *S. incanum* en el fons genètic de *S. melongena*. El desenvolupament d'estes ILs és de gran importància per a la millora de l'albergina, ja que permetrà que caràcters d'interés d'esta espècie s'introgresen en el fons genètic de l'albergina. A més estes línies seran una ferramenta de gran utilitat per a l'estudi de l'evolució i domesticació d'este cultiu.

Per el que fa al treball realitzat amb la varietat local "Albargina d'Almagro" amb indicació IGP, i sabent que aquesta varietat és genèticament heterogènia, era

necessari basar-se en un programa de selecció individual. El material va ser seleccionat tant per la seua menor presència d'espines com pel seu alt rendiment en camp. Per a millorar el caràcter d'espinositat en l'albergina d'Almagro com a objectiu principal, hem realitzat un programa de retrocrueuament amb la utilització de la selecció H15 com a parental recurrent i la utilització d'albergina (una tipus negra i una altra andalusa morfològicament semblant a la d'Almagro) amb absència d'espines com a parental donant.

Per a finalitzar esta segona part, es va realitzar un programa de millora en col·laboració amb una empresa privada tenint en compte que interessa poder incrementar la diversitat genètica, ja que això permet obtindre combinacions genètiques noves i explotar l'heterosis. Coneixent per endavant el cas de l'albergina, es crea la necessitat d'estudiar per mitjà de la utilització de marcadors moleculars la variabilitat que existix a hores d'ara en el mercat. Després d'analitzar amb SSRs 30 varietats comercials es van poder separar 3 grans grups en els materials d'albergina negra. La caracterització molecular junt amb la morfològica, indiquen que hi ha una àmplia diversitat genètica per a determinar la forma del fruit de l'albergina tipus semi-llarga i negra, sense recórrer a la utilització del material típicament utilitzat pels milloradors, el qual té un fons génetic comú a l'haver sigut derivat de les mateixes fonts. Els materials obtinguts poden ser d'interés per a augmentar l'heterosis dels híbrids F1.

En definitiva, ens trobem amb un treball que mostra que l'estudi de la diversitat genètica i el desenvolupament i utilització de ferramentes fenómiques per a la caracterització morfològica, a més de l'obtenció de material vegetal nou, és de gran utilitat per al desenvolupament de noves varietats d'albergina, així com per a obtindre informació cientificotècnica d'interés per a altres investigadors i milloradors. També hem constatat que la interacció "investigació pública-privada" permet una col·laboració sinèrgica en l'obtenció de material vegetal i informació d'interés en la millora d'hortícoles.

INTRODUCCIÓN

1. LA BERENJENA, UN CULTIVO CON INTERÉS CRECIENTE

El avance científico está poniendo de manifiesto de forma cada vez más clara la relación existente entre el binomio "Salud" y "Alimentación" (Fulton et al., 2012; Shahidi y Ambigaipalan, 2014). Hasta hace poco, los principales estudios se centraban en el estudio de los nutrientes, es decir, hidratos de carbono, grasas, proteínas, vitaminas y minerales. Sin embargo, los alimentos se caracterizan por ser mezclas complejas, no sólo de nutrientes, sino también de otros componentes que se engloban en un grupo heterogéneo llamado "no nutrientes", como muchos fitoquímicos con acción antioxidante (Kaur y Kapoor, 2001).

Dentro de las especies hortícolas, la berenjena (*Solanum melongena* L.) es una de las más ricas en compuestos fenólicos, lo cual le confiere un alto poder antioxidante (Cao et al. 1996; Stommel y Whitaker, 2003; Prohens et al., 2007; Plazas et al., 2013). Los efectos beneficiosos sobre la salud del ácido clorogénico y compuestos relacionados presentes en la berenjena son numerosos, y además de su potente actividad antioxidante, también neutralizan radicales libres y presentan actividad antitumoral (Sawa et al., 1998, González, MA 2015). Estas interesantes propiedades bioactivas de la berenjena hacen que sea un cultivo con un interés creciente. Además de su contenido en compuestos de interés nutracéutico , la berenjena **contiene vitamina A, B1, B2, C y E**, y es muy rica en minerales como el potasio, calcio, magnesio, hierro y fósforo, además de algunos otros componentes como el ácido fólico, fibra y carbohidratos (Savvas y Lenz, 1996; Lorenz y Maynard, 1998, Raigón et al., 2008; Zaro et al. 2015).

El fruto de berenjena contiene gran cantidad de agua (Raigón et al., 2008), por lo que **tiene muy pocas calorías** y resulta un excelente diurético. Es antioxidante y **previene ciertos tipos de cáncer** y enfermedades cardíacas, reduce el colesterol, y contribuye a prevenir la arteriosclerosis (Plazas et al., 2013). Por otra parte en la India, se considera que las variedades de fruto blanco son buenas para los diabéticos (Choudhury, 1976, González, 2015) y las raíces de la berenjena se utilizan por sus

propiedades antiasmáticas y como analgésico (Chadha, 1993; Choudhury, 1995). También en países como Nigeria o Guinea se usan con fines medicinales.

En la tabla 1 se muestra la composición de la berenjena correspondiente a varios tipos de berenjena, incluyendo la forma morada (cultivar de tipo occidental), verde (cultivar local de la India) y blanca (procedente de Europa).

Tabla 1. Composición por cada 100g de fruto de berenjena para tres tipos diferentes, Morada, Verde y Blanca (Flick et al. 1978; Lorenz y Maynard, 1988; Baixauli, 2001).

	Morada	Verde	Blanca
Composición (por cada 100 g)			
Humedad (g)	93,6	94,20	92,20
Energía (kcal)	26	-	-
Proteína (g)	1,1	-	-
Grasas (g)	0,1	-	-
Carbohidratos (g)	3,6	-	-
Fibra (g)	1	-	-
Ácido oxálico (mg)	18	-	-
Vitaminas (por cada 100g)			
Vitamina A (IU)	70	-	-
Tiamina (mg)	0,09	-	-
Riboflavina (mg)	0,02	-	-
Niacina (mg)	0,6	-	-
Ácido ascórbico (mg)	1,6	-	-
Vitamina B1(mg)	0,04	-	-
Vitamina B2 (mg)	0,05	-	-
Vitamina B6 (mg)	0,09	-	-
Ácido nicotíníco (mg)	0,09	-	-
Minerales (ppm)			
Aluminio	123	76,90	132,50
Calcio	1450,1	1.090,00	1.068,00
Cloro	2060	3.590,00	2.785,00
Cobre	21,8	13,20	14,60
Hierro	164	180,00	157,00
Potasio	17390	28.220,00	27.475,00
Magnesio	1690	1.245,00	1.280,00
Manganeso	11,7	14,60	10,00
Sodio	306	211,00	232,00
Azufre	3800	-	99.510,00
Selenio	2	1,10	1,50

Vanadio	1	1,00	1,00
Zinc	6,1	8,00	5,80
Aminoácidos (mg/100g)			
Lisina	0,769	0,541	0,541
Histidina	0,475	0,338	0,332
Arginina	1,206	0,724	1,033
Ácido aspártico	3,274	2,666	1,969
Treonina	0,776	0,527	0,493
Serina	0,815	0,568	0,562
Ácido glutámico	3,582	2,992	2,405
Prolina	0,784	0,585	0,534
Glicina	0,776	0,542	0,548
Alanina	0,995	0,658	0,677
Valina	1,212	0,807	0,795
Isoleucina	0,722	0,655	0,638
Leucina	1,266	0,95	0,944
Tirosina	0,419	0,287	0,313
Fenilamina	0,869	0,617	0,617

La realización de trabajos de mejora genética de la berenjena, como los de la presente tesis, se debe a que además de su creciente importancia por sus propiedades nutracéuticas, es a **nivel mundial un cultivo de gran importancia económica**. Si juntamos a todo lo comentado anteriormente que es una de las solanáceas menos estudiadas (siendo tomate, pimiento y patata las que más), llegamos a la conclusión que es **necesario realizar estudios que contribuyan a la mejora genética de la berenjena**. Al mismo tiempo, tal como se indica en apartados posteriores es necesario llevar a cabo programas de mejora genética a nivel comercial que amplíen la diversidad genética y que permitan adaptarse a las demandas de productores y consumidores.

2. IMPORTANCIA ECONÓMICA DEL CULTIVO DE LA BERENJENA

Como se ha comentado anteriormente, la berenjena es un cultivo hortícola de gran importancia económica. Es la sexta hortaliza a nivel mundial en volumen de producción (49.418 millones de kilos) en el año 2013 según los datos obtenidos en la FAO (FAOSTAT, 2015), por delante de la berenjena se encuentra el tomate, sandía, cebolla, coles, crucíferas y pepinos. Desde 2004 (31.005 millones de kilos) ha habido un incremento del 59% en cuanto a producción (Figura 1). Con los últimos datos obtenidos, el diferencial en los dos últimos años ha sido del 3'55 por ciento, pasando de los 47.721 millones de kilos producidos en 2012 a los 49.418 correspondientes a 2013.

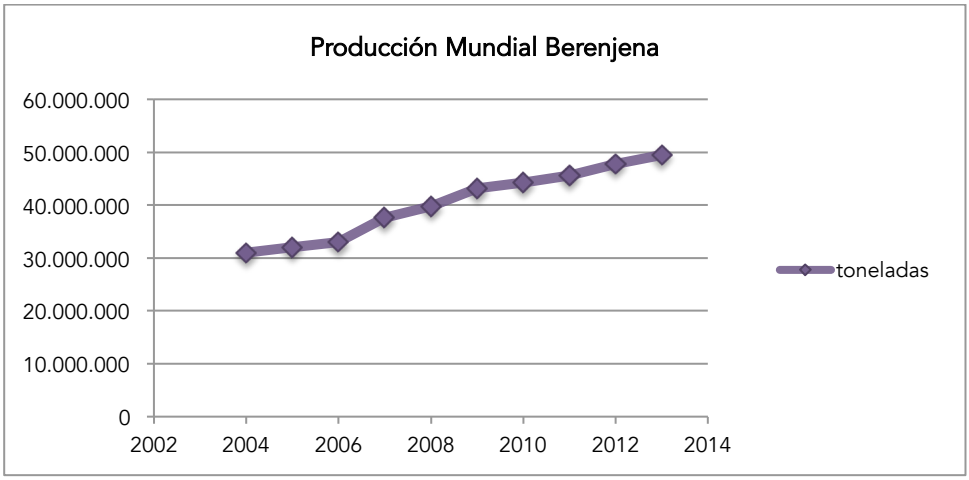

Figura 1. Producción mundial de berenjena en los últimos 10 años.

Como se puede apreciar a continuación (Figura 2), el 82,4% del total de la producción se realiza en el continente asiático, siendo sólo el 3% lo que se produce en Europa.

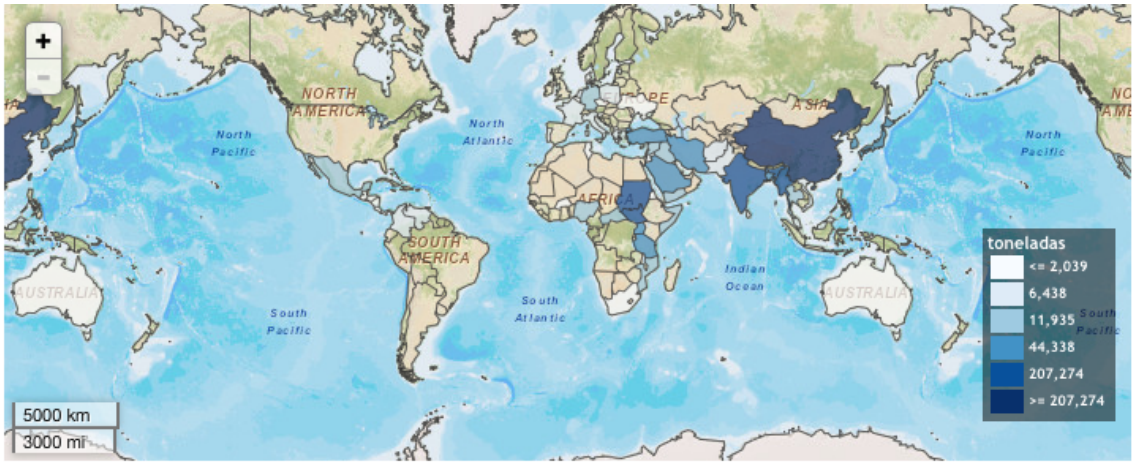

Figura 2. Mapa de las cantidades de producción de berenjena por país (toneladas).

China se impone una vez más en la producción, con 28.455 millones de kilos de berenjena producidos en 2013. Como se puede apreciar en la Tabla 2, le sigue India con 13.444 e Irán en tercera posición, con 1.354 millones de kilos (FAOSTAT, 2015). España ocupa la undécima posición con 206 millones de kilos de berenjena producidos en el año 2013.

Tabla 2. Comparativa de la producción mundial de berenjena entre 2012 y 2013 en toneladas.

País		2013	2012	Diferencia	Dif %
China	1	28.455.760	27.720.910	734.850	2,65
India	2	13.444.000	12.634.000	810.000	6,41
Irán	3	1.354.185	1.300.000	54.185	4,17
Egipto	4	1.194.115	1.193.854	261	0,02
Turquía	5	826.941	799.285	27.656	3,46
Irak	6	510.918	422.336	88.582	20,97
Indonesia	7	509.380	518.827	-9.447	-1,82
Japón	8	321.200	327.400	-6.200	-1,89
Italia	9	220.153	217.690	2.463	1,13
Filipinas	10	219.886	211.854	8.032	3,79
España	11	206.300	245.900	-39.600	-16,1

Turquía produjo en el año 2014 más berenjena que todos los países de la Unión Europea (UE) juntos, hecho que se viene produciendo durante toda la década, según los datos procedentes de la Oficina Europea de Estadística (Eurostat, 2015). Del total relativo a la UE con 776,8 millones de kilos, Italia es el mayor productor comunitario de berenjena. España ocupa la segunda posición con 208,2 millones de kilos. Entre ambos países generan el 66,54 por ciento del total de la berenjena comunitaria (Eurostat, 2015). Rumanía ocupa la tercera posición, con 80'9 millones de kilos. El cuarto mayor productor de berenjena en la UE durante 2014 ha sido Grecia, con 72'2 millones de kilos, apareciendo Holanda en la quinta posición con un total de 51 millones de kilos producidos de esta hortaliza.

El 90,44 % de la berenjena española procede de Almería, ya que la provincia almeriense ha generado en esta campaña que termina (2014/2015) un total de 183,3 millones de kilos (Consejería de Agricultura, Pesca y Desarrollo Rural de la Junta de Andalucía, 2015). Aunque los datos no son exactamente coincidentes en el tiempo, ya que hablamos de un año natural (Eurostat, 2015) y de la campaña (Junta de Andalucía), la berenjena producida en Almería supera en un 269,21 % a la holandesa.

Por lo que respecta al área cultivada, en el total de la UE se dedican al cultivo de berenjenas 22.000 hectáreas. Italia ocupa 8.400 hectáreas para este cultivo, España le dedica 3.700 hectáreas (2.400 en Almería), mientras que en Rumanía se destinan al cultivo de berenjenas 4.900 hectáreas, en Grecia 2.200, y en Holanda 100, según los datos de Eurostat (2015).

2.1 Exportación

España ha aumentado un 15 % sus exportaciones de berenjena en la campaña 2013/2014 (del 1 de septiembre de 2013 al 31 de agosto de 2014), según los datos procedentes del servicio estadístico Estacom (Icex-Agencia Tributaria, 2015). La exportación total de berenjena ha sido de 141,3 millones de kilos en esta campaña, frente a los 122,9 millones de kilos vendidos al exterior en la campaña precedente. El valor total de las exportaciones de esta hortaliza ha sido de 126,69 millones de euros,

con un precio medio de 0,90 euros por kilo.

De los cinco mayores compradores, el país que mejor ha pagado las berenjenas españolas ha sido Holanda con un precio de 1,02 euros por kilo, seguido por Alemania (0,99 euros el kilo) y Reino Unido, que las pagó a 0,96 euros el kilo (Icex-Agencia Tributaria, 2015).

3. TAXONOMÍA

La berenjena (*Solanum melongena*) pertenece a las angiospermas y se encuadra dentro de los siguientes taxones (Sambamurty, 2005):

Clase: *Magnoliopsida*
Subclase: *Lamiidae*
Superorden: *Solananae*
Orden: *Solanales*
Familia: *Solanaceae*
Subfamilia: *Solanoideae*
Tribu: *Solaneae*
Género: *Solanum*
Subgenero: *Leptostemonum*
Sección: *Melongena*
Serie: *Incaniformia*
Especie: *Solanum melongena L.*

La familia *Solanaceae* está compuesta por 83 géneros que engloban unas 1.000-1.400 especies de amplia distribución por todo el mundo, especialmente en zonas templadas y tropicales (D'Arcy, 1975, 1991).

Esta familia se caracteriza por presentar flores pentámeras, con sépalos persistentes, frecuentemente acrescentes. El ovario es súpero, bilocular, raramente pluriocular y con varios óvulos por lóbulo. Los frutos son bayas, drupas o cápsulas, indehiscentes y con varias semillas por lóbulo.

Figura 3. Flor de la berenjena.

El género *Solanum* es el más numeroso dentro de la familia *Solanaceae*. Fue establecido por Linneo (Linneo, 1753) en su obra *Species plantarum*. En él se encuentran plantas herbáceas, arbustivas o arbóreas, usualmente espinosas. La corola es pentagonal o estrellada. Las anteras forman una columna que rodea al estilo y presentan dehiscencia por poros apicales .Los frutos son bayas globulosas.

Solanum melongena pertenece a uno de los grupos no tuberosos del género *Solanum* (Mueller et al., 2005). Se trata de una especie diploide, con un número cromosómico de 2n=24. Se trata de una planta muy termófila, su cero vegetativo se encuentra en 10 ºC.

4. ORIGEN Y DOMESTICACIÓN

Resultados recientes obtenidos a partir de estudios de variabilidad morfológica y molecular indican que la berenjena es el resultado de la domesticación de la especie silvestre *S. insanum* L., la cual se encuentra en el sudeste de Asia (Meyer et al., 2012). Por tanto se trata de una interesante excepción, ya que la mayoría de las *Solanaceae* y en especial muchas de las especies domesticadas del género *Solanum*, son originarias del continente americano.

Solanum insanum y las formas cultivadas de *S. melongena* presentan muchas similitudes morfológicas, teniendo un hábito de crecimiento similar y siendo posible la obtención de híbridos entre las dos especies completamente fértiles y con meiosis regular (Knapp et al. 2013).

Previamente a los estudios de Meyer et al. (2012), Lester y Hasan (1991) sugirieron que la berenjena cultivada fue domesticada a partir de *S. incanum*, especie que se encuentra distribuida en África oriental y Oriente Medio (Knapp et al., 2013). Estos autores postularon que en el Neolítico, o incluso en el Paleolítico, *S. incanum* siguió al ser humano en sus desplazamientos desde el Oriente Medio hasta la India como adventicia. Según la hipótesis de estos autores, en la región indo-birmana, bajo la selección natural y artificial, estas plantas habrían evolucionado. La selección tanto en la India como en otras regiones habría producido los cultivares modernos y al mismo tiempo, se desarrollarían formas adventicias espinosas. Como alternativa, D'Arcy y Pickett (D'Arcy y Pickett, 1991) sugieren que los frutos de especies silvestres emparentadas con la berenjena podrían haber llegado desde África hasta la India arrastrados por las corrientes marinas. Sin embargo, todas las evidencias modernas sugieren que el ancestro silvestre de la berenjena es *S. insanum* y no *S. incanum* (Meyer et al., 2012).

La teoría más arraigada sobre la domesticación de la berenjena es la que considera a la India como centro primario de variación. En esta región se encuentra una abundante variación de formas cultivadas modernas, primitivas y formas adventicias. China y la región mediterránea serían centros secundarios de variación (Bhaduri, 1951; Valivov, 1951; Zeven y Zhukovsky, 1975; Karihaloo y Gottlieb, 1995; Meyer et al., 2012; Cericola et al., 2013). La hipótesis de la domesticación de la berenjena en la región indo-birmana está apoyada por varias pruebas (Nuez et al., 2002). Por ejemplo, no se han encontrado referencias a su cultivo entre los autores griegos y latinos o clásicos, por lo que la berenjena no debía ser conocida en el Imperio Romano (de Candolle, 1883). Sin embargo, sí aparece mencionada en textos sánscritos fechados varios siglos antes de Cristo, utilizándose varios nombres para describirla como *kantapatrika* refiriéndose al carácter espinoso de la planta o *nidralu*

refiriéndose a las propiedades narcóticas o hipnóticas de algunas partes de la planta (Nadkarni, 1927), lo que nos indica que se trataba de una planta ampliamente conocida y utilizada, además muestra la variabilidad de tipos y formas presentes en la India, y sugiere la presencia de formas cultivadas en esta región desde hace mucho tiempo (Khan, 1979).

Se cree que la berenjena habría emigrado desde la zona indo-birmana hacia el Mediterráneo a través de la ruta de la seda, en épocas relativamente recientes. Los árabes habrán contribuido también a su difusión hacia Occidente. En China también fue adoptada como cultivo tempranamente, muestra de ello se peude encontrar en la mención que se hace de ella en tratados botánicos y agrícolas como *El Atlas de Plantas del Sur de China* escrito durante la *Dinastía de Jin Occidental* (265-316 dc). En Japón se tienen referencias de su cultivo desde el siglo VIII d.C. Se tiene constancia de la existencia de variedades locales japonesas de la época Edo (1615-1867), algunas de las cuales todavía se cultivan a pequeña escala (Allard 1996, Daunay, 2007).

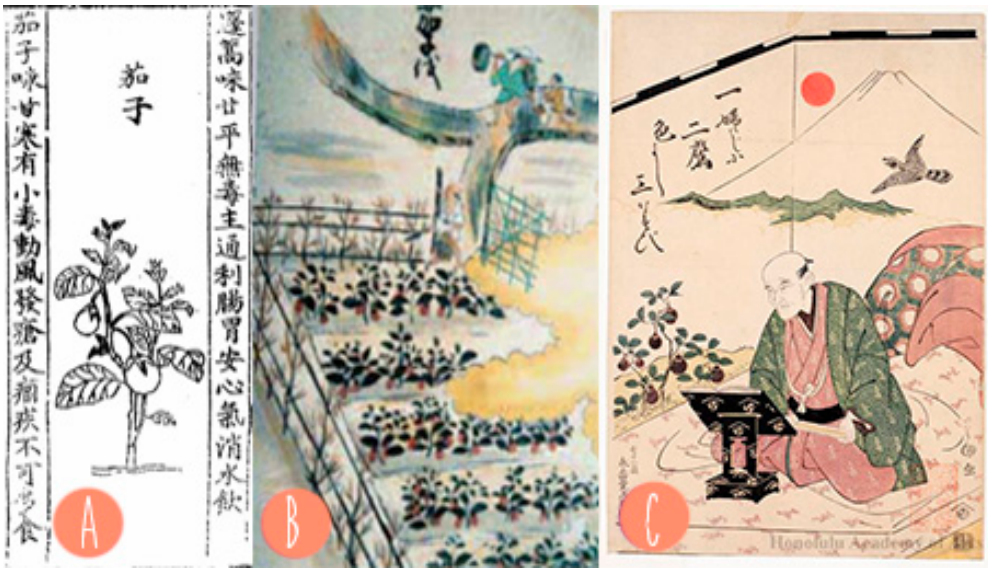

Figura 4. (A) Berenjena China, Hui Sihui *Yinshan Zhengayao* (1330). (B). Campo de berenjenas en Japón a principios del s.XVIII (C) Retrato de Nishimuraya Yohachi , editor de impresión líder de finales del s. XVIII, realizado por Utagawa Tuyokuni I en 1799 (obtenido de Daunay, 2007).

Existen varias referencias bibliográficas que demuestran cuan arraigada estaba la berenjena en la cultura árabe (González, 2015). Por ejemplo, cabe citar el tratado del siglo XII "La agricultura Nabatea", en el siglo X se nombra varias veces en el Calendario de Córdoba de Arib. También de esta época encontramos poemas musulmanes que describen las virtudes de la berenjena. En el siglo XII Abu Zacaría (Abu Zacaría, 1802) indica que en Al-Andalus se encuentran cuatro tipos de berenjena: la local, la cordobesa, la egipcia y la siria.

Como consecuencia de la selección (tanto natural como artificial), la recombinación, la migración resultado de la entrada de material genético nuevo procedente de otras poblaciones, y deriva genética, consistente en la fijación de alelos al azar en poblaciones pequeñas, a partir de los tipos introducidos por los árabes irían surgiendo variedades locales adaptadas a diversas condiciones locales de nuestro país (Prohens et al., 2005). Ya desde la Península Ibérica, la berenjena se habría ido difundiendo, aunque lentamente, a otros países europeos (Nuez et al., 2002). En Italia, las primeras referencias son de finales del siglo XVII en la región de Provenza (Daunay, 1996). En el siglo XIV ya se encontraba en el África Oriental, y los términos utilizados en algunas de las lenguas de la región, por ejemplo *a-bela* en ti o *patansi-jato* en mandiga sugieren que fue introducida en la región por los árabes (Watson,1998).

Figura 5. Copias del libro Tacuinum Sanitatis (Vienna, 1385-1390). (A) Imagen que muestra la parte afrodisiaca de la berenjena. Detalle de cómo una mujer pisa el vestido de la mujer que va de rojo amonestando a los amantes afectados por sentimientos excesivamente románticos. (B) Representación errónea de la berenjena como un árbol.

La introducción en América la debieron realizar los españoles. Entre otros cronistas, el padre Bernabé Cobo (Cobo, 1964) la muestra como una hortaliza habitual en el reino de Perú. Sin embargo, en este continente no ha alcanzado la importancia que tiene en el Viejo Mundo. El hecho de pertenecer a la familia de las Solanáceas probablemente debió restringir su expansión, al menos en Europa. La razón es que muchas Solanáceas silvestres son altamente tóxicas y al igual que el tomate se le dio el nombre de *Lycopersicon* ("melocotón de lobos" en griego), uno de los nombres por los que se conocía la berenjena era por el de *Mala insana* ("manzana insana" en latín) y de hecho en muchos tratados británicos no se aconsejaba su consumo (Daunay, 1996). Incluso entre los árabes, entre los que la berenjena era muy apreciada, algún tratadista, como Ibn Wahsiyya, indica que esta planta puede causar la muerte si se come cruda y la incluye en su tratado de venenos (Watson, 1998, González 2015).

4.1 Complejo berenjena

Este complejo está formado por la especie cultivada *S. melongena*, junto con sus especies silvestres más cercanas, que representan el germoplasma primario de la berenjena, es decir, los materiales que con mayor o menor dificultad, dan híbridos fértiles con la berenjena (Pearce y Lester, 1979; Lester y Hasan, 1991; Daunay et al., 1997; Knapp et al., 2013).

Desde hace años este complejo se ha dividido en 8 grupos (A-H), identificados con letras mayúsculas, basándose en la caracterización morfológica y relaciones de cruzabilidad que corresponden a ubicación geográfica (Figura 6), formas y frutos distintos de la planta (Lester y Hasan, 1991; Daunay, M.C., 1996).

Figura 6. 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.

Pero estudios recientes (Knapp et al., 2013), demuestran después de una caracterización morfológica más exhaustiva en variedades de todo África, que dentro del complejo berenjena existen más grupos de especies diferentes que sólo *S. melongena*, *S. incanum* o *S. insanum* (Tabla 3).

Tabla 3. Diferencias entre las especies que se encuentran dentro de cada grupo del complejo berenjena, según Daunay (1997) y Knapp (2013).

Grupos	Daunay et al. (1997)	Knapp et al. (2013)	Distribución
A	*Solanum incanum*	*Solanum campylacanthum*	Este África
B	*S. incanum*	*S. campylacanthum*	Sudáfrica
C	*S. incanum*	*S. incanum*	Noreste medio de Pakistan
D	*S. incanum*	*S. lichtensteinii*	Sur-Este África (Desiertos).
E	*S. melongena*	*S. insanum*	Asia y Madagascar
F	*S. melongena*	*S. insanum*	Zona más Oriental de *S. insanum*
G	*S. melongena*	*S. melongena*	Sur Este Asia
H	*S. melongena*	*S. melongena*	Todo el Mundo

Dentro del "nuevo" complejo berenjena, encontramos 5 especies de *Solanum* que se distribuyen entre los 8 grupos de Daunay.

1. Solanum campylacanthum.

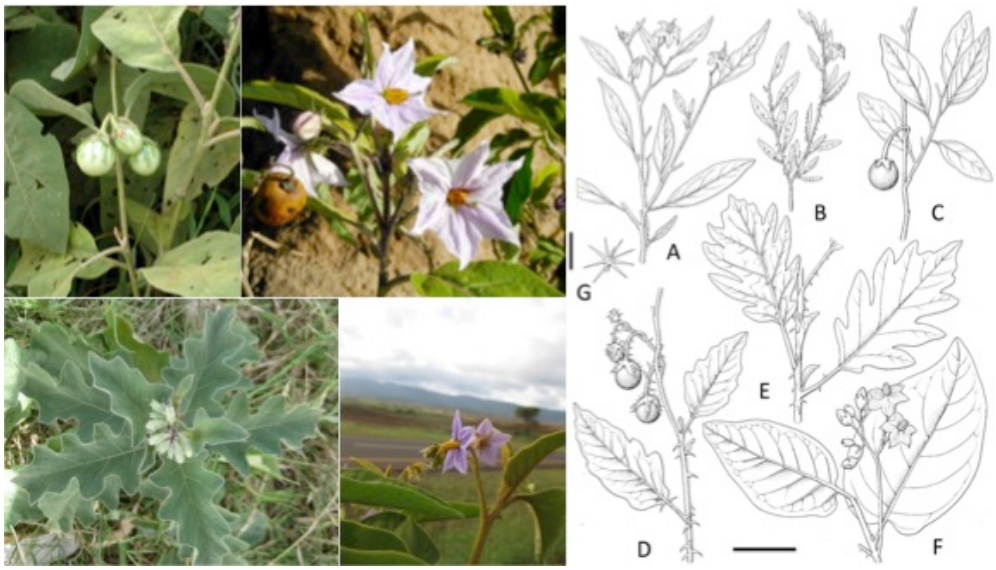

Figura 7. Imágenes de flor, inflorescencia, fruto y planta, y ejemplos de variabilidad de hoja que se puede encontrar dentro de *S. campylacanthum* (imágenes obtenidas de www.africanplants.senckenberg.de)

La gran mayoría de los ancestros silvestres de berenjena que hay en África, erróneamente se ha dicho que pertenecen a *S. incanum*. Pero realmente pertenecen a esta especie. Como se puede apreciar en la Figura 7 , existe gran variabilidad en la hoja de *S. campylacanthum*, es por ello que se llega a creer que son 2 subespecies completamente diferente (Samuels 2012, Daunay et al. 1997). Pero es en el estudio reciente de Knapp (2013) donde se ha demostrado que son la misma especie.

2. Solanum incanum

Figura 8. Imágenes de *S. incanum* en planta, hoja y fruto. (imágenes extraídas de www.africanplants.senckenberg.de)

Solanum incanum (Figura 8*)* se encontraría dentro del Grupo C (Daunay et al., 1997), encontrándose distribuida en las zonas desérticas de Kenia a Pakistán.

3. *S. liechtensteinii*

Figura 9. Imágenes de *S. lichtensteinii* (imágenes extraídas de www.africanplants.senckenberg.de).

Esta especie se encontraría dentro del grupo D del complejo berenjena descrito por Daunay (1997), y su distribución natural es en la región al Sudeste de África.

Solanum incanum y *S. lichtensteinii* son muy similiares morfológicamente, pero se encuentran diferenciadas geográficamente. También se ha observado que en las zonas secas del Sur de África el tamaño de la planta de *S. lichtensteinii* (Figura 9) es más pequeño que el de *S. incanum* (Knapp et al., 2013).

4. Solanum insanum

Figura 10. Imagen e ilustración de *S. insanum*. (extraídas de www.plantillustrations.org).

Lester y Hasan (1991) consideraron que *S. insanum* (Figura 10) era una variedad silvestre de *S. melongena* (*S. melongena* var. *insanum*). Esta idea ha sido seguida por diferentes autores durante años (Mace et al., 1999; Behera et al., 2006; Weese and Bohs, 2010; Tümbilen et al., 2011; Daunay and Hazra, 2012; Samuels, 2012, 2013). Pero en el estudio realizado por Knapp et al. (2013) se considera que el Grupo E y el Grupo F de Daunay (1997) corresponderían a *S. insanum*. Uno de los motivos es porque que *S. insanum* se utiliza medicinalmente en el sur de China y se considera diferente a la *S. melongena* cultivada por la población local. Es por ello que aunque la consideren una especie silvestre, a menudo se la ubicó en muestreos que se hicieron en áreas habitadas por el ser humano (Mutegi et al., 2015).

5. Solanum melongena

Solanum melongena se encuentra distribuida en todas las zonas de clima tropical a templado del planeta. Las formas más primitivas de *S. melongena*, con frutos relativamente pequeños y con abundantes espinas, y que pertenecen al Grupo G se encuentran únicamente en el sudeste de Asia Daunay (1997).

Por tanto la nueva distribución del complejo berenjena, quedaría reflejada en 5

grandes grupos como se puede apreciar en la siguiente Figura (Figura 11).

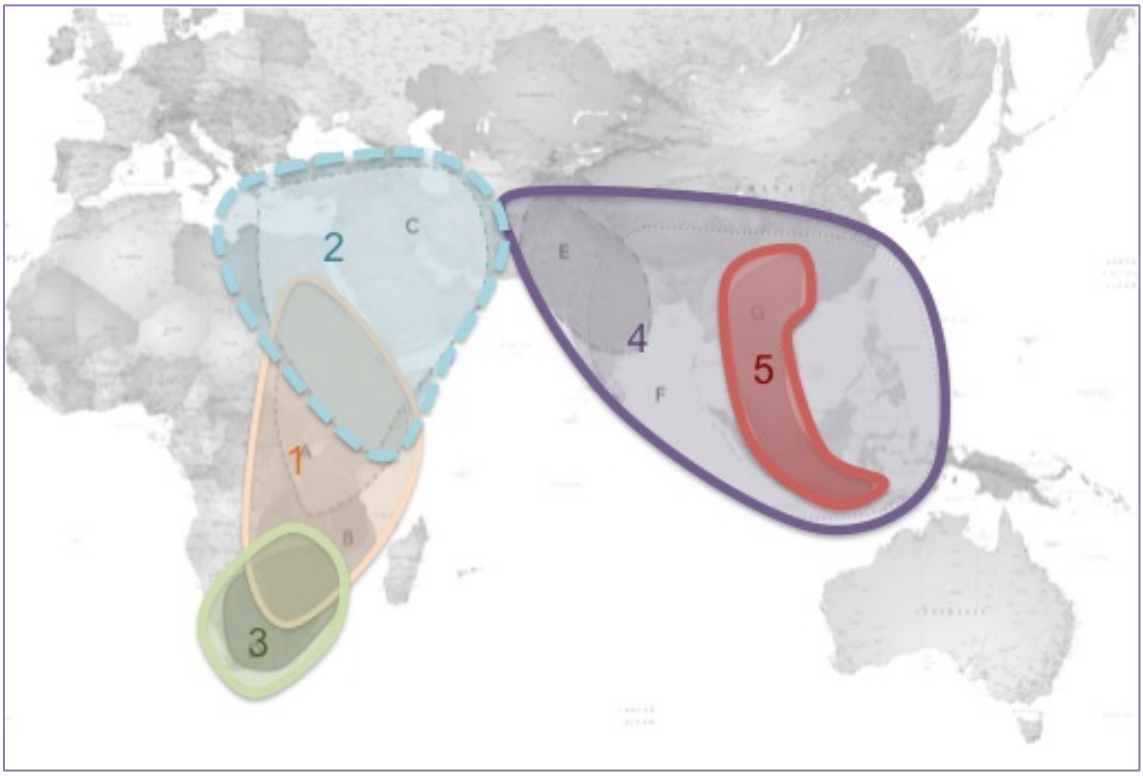

Figura 11. Distribución de la nueva clasificación por especies del complejo berenjena (Knapp et al., 2013). Que se basa en 5 grupos : (1) *S. campylacanthum*. (2) *S. incanum*, (3) *S. lichtensteinii*, (4) *S. insanum* y en el (5) *S. melongena*. Este último grupo además de en la zona marcada en rojo (grupo G), se encontraría distribuida por todo el mundo (Grupo H de Daunay, 1997).

4.1.1 Otras especies.

Además de clasificar las especies africanas dentro de los 8 grupos determinados por Daunay et al. (1997), Knapp et al. (2013) caracterizaron otras 5 especies de *Solanum* (Figura 12) que no han sido clasificadas dentro de ningún grupo anterior debido a su distribución geográfica (Knapp et al., 2013).

- *Solanum aureitomentosum* (Este de África hasta Zambia).
- *Solanum cerasiferum* (Norte de África, de Senegal a Sudán).
- *Solanum linnaeanum* (Sur de África y Mediterráneo).
- *Solanum rigidum* (Islas Cabo de Verde).

- *Solanum umtuma* (Sur de África, zona KwaZulu-Natal).

Figura 12. (A) *S. aureitomentosum* (www.sciencemnhn.fr). (B) *S. cerasiferum* (www.westafricansplants.senckenberg.de). (C) *S. linnaeanum* (www.bioscripts.net). (D) *S. rigidum* (Knapp & Vorontsova, 2013). (E) *S. umtuma* (Vorontsova y Knapp, 2012).

4.1.2 Clasificación intraespecífica de *S. melongena*

Centrándonos en los tipos de berenjena cultivada (grupo H), una de las clasificaciones más utilizadas es la proporcionada por Bailey en 1947 (Figura 13), donde se diferencian tres tipos de variedades botánicas: *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*, que engloba las variedades de frutos largos.

Figura 13. Frutos de la variedad botánica *esculentum* (izquierda), *depressum* (centro) y *serpentinum* (derecha).

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, Prohens et al. (2015) clasificaron las variedades españolas en cuatro grupos de cultivares: redondas, semilargas, largas y Listadas de Gandía (Figura 14). 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 (Vilanova et al., 2012; Cericola et al., 2013).

Figura 14. Clasificación morfológica según Prohens et al. (2015): Semilargas (A), Listada de Gandía (B), Redondas (C), Largas (D).

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) teniendo preferencia por frutos de color negro brillante y de forma semi-larga. 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.

4.2 Especies cultivadas relacionadas

Entre las especies emparentadas con *S. melongena* hay dos africanas cultivadas, *S. aethiopicum* L. y *S. macrocarpon* L. En Europa son poco conocidas, pero en África Occidental se usan tanto por sus frutos como por sus hojas. Estos materiales constituyen unos recursos genéticos de gran interés para la mejora de la berenjena y para la ampliación de la base genética de este cultivo (Plazas, 2014).

Solanum aethiopicum (Figura 15) pertenece taxonómicamente al género *Solanum*, subgénero *Leptostemonum* y sección *Oliganthes* y comprende tres grupos: Shum, Kumba y Gilo (Adeniji, 2012). Estas especies se han ido adaptando según la climatología de cada zona distribuyéndose en áreas distintas. El grupo Shum se encuentra en las partes más húmedas de África, en las zonas semiáridas del occidente de Sahel hasta el norte de Nigeria encontramos berenjenas del grupo Kumba y en las zonas con más lluvia están las del grupo Gilo (Plazas et al., 2014).

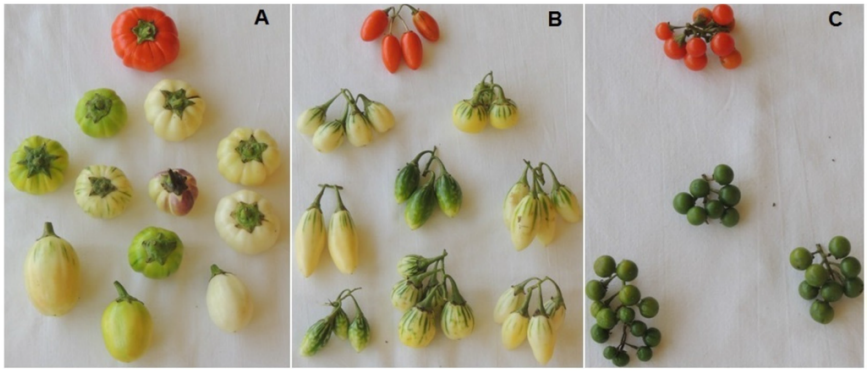

Figura 15. Ejemplos de variablidad dentro del grupo Gilo *(Solanum aethiopicum). N'Drowa* (A), *Kloho (B), Gnangnan* (C) (Kouassi et al., 2014).

La mayoría de los cultivos de *Solanum macrocarpon* (Figura 16) se encuentran en América del Sur, en el Caribe y en el sureste de Asia. En cuanto a África, en muchos países, estas plantas están consideradas como un cultivo menor. Las flores de *S. macrocarpon* se distinguen de *S. aethiopicum* porque tiene los pétalos soldados, además son de color morado claro y con un diámetro de 25-45 mm (Lester et al., 2003). Las hojas, brillantes y glabras, pueden tener diferentes formas y tamaños.

Figura 16. Fruto de *Solanum macrocarpum*. Diferencia entre los pétalos soldados de *S. macrocarpum* (flor izquierda) y los de *S. aethiopicum* (flor derecha).

5. GENERALIDADES DEL CULTIVO

5.1 Botánica

La berenjena es una planta herbácea anual pero que, en climas favorables, puede rebrotar y mantenerse en cultivo más de un año. En este caso, los frutos son de peor calidad que los del primer año. Su sistema radical es fuerte y está muy desarrollado, tanto en profundidad como lateralmente. Posee un tallo semileñoso, cilíndrico, verde o de color violáceo, piloso, rígido y generalmente erecto, de crecimiento indeterminado, pudiendo alcanzar en cultivo al aire libre, una altura de entre 0,5 y 1,5 m (Illescas y Vesperinas, 1989). Es de color verde, aunque en algunas variedades puede tornarse a morado oscuro. En plantas viejas, el tallo se lignifica ligeramente.

Las hojas son sencillas, alternas ovadas u oblongo-ovadas y grandes, con los márgenes ligeramente lobulados en función de la variedad, recubiertas en el envés de una vellosidad de color grisáceo, que puede recubrir todas las partes de la planta (Figura 19). También es frecuente la presencia de espinas en las nerviaciones prominentes o en el pecíolo de las hojas. Sin embargo, en las variedades mejoradas un objetivo de mejora es la ausencia de espinas (Daunay y Hazra, 2012).

Figura 19. Ilustración de 1878 de la planta de berenjena. Dibujado al detalle sus hojas, flores e incluso las semillas.

Las flores, de color blanco o violeta más o menos intenso según variedad, suelen aparecer en forma solitaria o bien formando ramilletes de dos o más, frecuentemente de tres. En algunas variedades aparecen en grupos de hasta 4-5 flores. La corola es rotada, de 2,5 a 4,5 cm de diámetro. Las anteras tienen de 6 a 8 mm. de largo, el estilo puede ser exerto o inserto. El cáliz es persistente, tomentoso y espinoso (Knapp et al., 2013).

El fruto es una baya carnosa de forma muy variable según la variedad, aunque predominan las formas redondas, globosas y alargadas, de colores muy diversos en la madurez comercial, siendo habituales el morado oscuro, el violeta, el negro, el amarillo o el blanco. Cuando el fruto presenta la madurez fisiológica es blanco y de color amarillento a ocre e incluso negro. La pulpa es carnosa, de coloración amarilla, blanca o verde, volviéndose parduzca al contacto con el aire debido a la oxidación. Para el consumo de los frutos deben recolectarse antes de llegar a la madurez fisiológica, cuando aún no se han formado las semillas.

Las semillas son pequeñas, aplastadas, de color marrón y son muy abundantes (Figura 20). En un fruto pueden existir hasta 2.500 semillas y en 1 g. pueden contabilizarse unas 250 semillas. Su poder germinativo medio en condiciones normales es de unos 4-6 años.

Figura 20. Foto al detalle de semilla de berenjena, y de la variabilidad que encontramos en el fruto.

5.2 Exigencias en el medio físico para su cultivo.

La berenjena es la solanácea hortícola más exigente en calor. Según Baixauli (Baixauli, 2001) su desarrollo óptimo se da con temperaturas medias entre 20-25 ºC, siendo la óptima diurna entre 22-26 ºC y la nocturna entre 15-18 ºC. Su crecimiento se paraliza entre 9-10 ºC. La temperatura mínima de germinación está cercana a los 15 ºC, siendo valores óptimos entre 23-28 ºC. Es muy sensible a las heladas. Las altas temperaturas le suelen perjudicar, aunque puede resistir perfectamente niveles térmicos por encima de los 40 ºC (Matarıl et al., 2014). Durante el período de floración le convienen temperaturas situadas entre 20-30 ºC.

En cuanto a la higrometría, las necesidades mayores son durante el período de engorde del fruto; si hubiera carencias hídricas se producirían frutos de menor calibre, con maduración anticipada y menor calidad organoléptica. Una excesiva humedad relativa, de más del 65%, puede provocar ahilado de plantas y falta de cuajado.

Es un cultivo exigente en luz, teniendo mucha influencia en el ahilamiento y la floración, ya que puede producirse malformación de flores y hojas, deficiente fecundación, frutos deformes y pulpa esponjosa. Se considera a la berenjena como una planta de día largo floreciendo cuando el día tiene una duración de 10-12 horas (Mataril et al., 2014).

En cuanto a los suelos, también es una planta exigente, requiere suelos ricos y profundos; soporta más que el pimiento y el tomate los suelos arcillosos (Serrano, 1982), aunque le convienen los de textura media y sin problemas de encharcamiento de aguas. Se adaptan a una gama de pH muy amplia, entre 5,5-8. Tolera niveles medios de salinidad en el suelo. Algunos autores señalan que su cultivo se ve muy perjudicado en suelos ácidos, provocando caída de flores y pérdida de vigor de la planta. En cambio, tiene una buena adaptación a suelos alcalinos.

6. DIVERSIDAD GENÉTICA DE LA BERENJENA

La berenjena es un cultivo con una estrecha base genética (Prohens et al., 2005). Esto puede ser debido a que la domesticación de la berenjena se realizó a partir de un acervo genético limitado, lo cual pudo originar un cuello de botella, que contribuiría a que las variedades de berenjena cultivada presenten una baja diversidad genética (Isshiki et al., 1994, Karihaloo y Gottlieb, 1995; Vorontsova et al., 2013).

El disponer de recursos genéticos con una amplia diversidad genética, adecuadamente regenerados, conservados, caracterizados y documentados es esencial para la mejora genética de este cultivo. El International Board for Plant Genetic Resources (IBPGR), renombrado International Plant Genetic Resources Institute (IPGRI) en 1991 y Bioversity International en 2006, incluyó ya en 1977 a la berenjena en la lista de especies prioritarias cuyos recursos genéticos estaban sufriendo erosión genética (Grubben, 1977).

Estos recursos genéticos podrían verse aprovechados con la realización de cruzamientos dirigidos. El aumento de variabilidad genética generada, será mayor cuanto más alejados genéticamente se encuentren los parentales, siendo los cruzamientos intraespecíficos los más idóneos para obtener materiales comerciales. En berenjena los híbridos de este tipo no presentan incompatibilidad de polen ni infertilidad de los individuos (Daunay y Hazra 2012). La base genética también podría verse ampliada con la inclusión de variedades locales u obsoletas y de especies silvestres relacionadas en los programas de mejora.

El cultivo de la berenjena se ve limitado por varios factores, ya que muchos de los caracteres de interés están controlados oligogénica o poligénicamente, lo que complica su estudio y mejora (Frary et al., 2003; Daunay y Hazra, 2012; Portis et al., 2014). A este respecto, existe un limitado conocimiento sobre el control genético de caracteres de importancia agronómica para la berenjena.

Todo ello hace necesario la caracterización y la mejora en plantas adultas, lo que requiere del uso de invernaderos o campos de cultivo, limitando así el número de individuos a estudiar. Por otra parte, es difícil el estudio de varios caracteres a la vez en una sola progenie. Además, mucho del germoplasma relacionado que podría resultar interesante para la mejora no está suficientemente caracterizado (Daunay et al., 2001b; Robinson et al., 2001).

Para la caracterización de los recursos genéticos del género *Solanum* existe un listado de descriptores publicado por el IPGRI y preparado por el Prof. Richard Lester en el año 1990 (Lester, 1990). Sin embargo, estos descriptores han sido revisados por un grupo de expertos en berenjena dentro de la red europea para recursos genéticos de berenjena (EGGNET) y del Solanaceae Working Group del European Cooperative Programme for Genetic Resources Networks (ECP/GR) y se han producido unos descriptores consensuados para la caracterización primaria de berenjena, los cuales han sido utilizados de forma exitosa en la caracterización de colecciones de germoplasma (Prohens et al., 2005; Plazas et al., 2014).

La diversidad dentro de cultivares avanzados de *S. melongena* no sólo se limita a caracteres de fruto como forma, tamaño y color, también existen muchos otros caracteres en los cuales se puede encontrar una amplia variación como altura de la planta, habito de crecimiento, floración, pigmentación antociánica de algunas partes de la planta (Figura 21), presencia de espinas, lobulado de la hoja etc.

Figura 21. 1. Diferencia en la coloración antociánica en la hoja en diferentes variedades de berenjena. 2. Diferencia en la presencia de espinas en botones flores en diferentes variedades de berenjena. 3. Diferencia en la presencia de espinas en diferentes variedades de berenjena.

También existe variación para importantes caracteres agronómicos como precocidad, rendimiento, amargor del fruto, adaptación climática, resistencias a plagas, enfermedades y estrés abiótico, los cuales varían ampliamente entre accesiones, pero no se ha efectuado aun una caracterización sistemática, por lo cual es preciso una mayor atención en el futuro por parte de mejoradores y conservadores de germoplasma.

El uso de técnicas moleculares puede ayudar a mejorar tanto la eficiencia como la efectividad de los programas de mejora en varios aspectos (Staub et al., 1996; Aranguren-Méndez et al., 2005). El análisis molecular de caracteres cuantitativos complejos permite determinar que loci controlan aspectos fenotípicos de mayor interés, al igual que identificar genes con menores efectos fenotípicos, siendo también útil en programas de mejora de resistencia a plagas y enfermedades. La selección asistida por marcadores, es de gran interés y ayuda para los programas de mejora mediante retrocruzamientos, puesto que permite seleccionar plantas cuando aún están en semillero, lo que reduce el número de plantas adultas a estudiar (Staub et al., 1996; Eathington et al., 2007; Tester y Langridge, 2010,).

Mediante este tipo de técnicas es posible estudiar la diversidad y las relaciones existentes entre las distintas especies relacionadas de *Solanum*, lo que incrementaría el acervo genético a disposición de mejoradores e investigadores para la mejora de la berenjena.

Desde los inicios de la agricultura, se ha aprovechado la variabilidad existente en la naturaleza para realizar selección, de un modo consciente o no, de especies que el hombre tenía a su alrededor, adaptándolas a sus necesidades. Este proceso de mejora se desarrolló por milenios sin un fundamento científico. Fue a partir del siglo XX, cuando se redescubren los trabajos realizados por Gregor Mendel, de manera independiente por Carl Correns en Alemania, Erich von Tschermak en Austria y Hugo de Vries en Holanda, determinando las leyes de la herencia, empezando a consolidarse la mejora sobre una base científica (Eathington et al., 2007).

Los objetivos de mejora fueron evolucionando guiados por la agricultura comercial y tecnificada. Estos mecanismos de mejora basados en la selección, dependen de la eficacia con que se identifican los mejores genotipos en una población. En este sentido, el uso de un marcador genético (entidades heredables asociadas a caracteres económicamente importantes) facilitaría el proceso de selección, ya que se podría realizar una selección temprana de individuos, sin influencias ambientales, reduciendo así el tamaño de la población a evaluar (Staub et

al., 1996; Tester y Langridge, 2010).

7. MEJORA GENÉTICA DE LA BERENJENA

En el sector público la mejora genética de la berenjena, es llevada a cabo principalmente por grupos de investigadores de varios países de Europa, USA, Turquía, Japón, China e India (Daunay et al., 2008). En el sector privado la globalización del mercado de semillas ha impulsado el interés de las empresas por esta especie, la cual es cultivada ampliamente en zonas de clima templado y tropical, principalmente en Asia (Daunay y Hazra, 2012). En lo que respecta al cultivo de la berenjena, durante los últimos tiempos las empresas de semillas han centrado sus esfuerzos en la producción de variedades de híbridos F1 para cultivo bajo invernadero (Muñoz-Falcón et al., 2005, 2007; Hurtado et al. 2015), las cuales poseen algunas características, como desarrollo vegetativo reducido, ausencia de espinas en el cáliz del fruto y buena fructificación bajo condiciones sub-óptimas (Muñoz-Falcón et al., 2007; Daunay et al., 2008).

7.1. Objetivos de mejora

Los principales objetivos en la mejora de la berenjena no difieren notablemente de una zona a otra, ya que en todo el mundo se hacen intentos por mejorar la productividad, la calidad del fruto, la adaptación a las condiciones agroclimáticas y la resistencia a plagas y enfermedades (Daunay et al., 2001a). Sin embargo, en cada país existen diferentes criterios específicos como: apariencia del fruto (forma, tamaño, color, textura), sabor del fruto (mayor amargor o dulzura), condiciones climáticas, o problemas fitopatológicos locales, entre otros (Sekara et al., 2007). Por ejemplo, en la berenjena los mejoradores japoneses buscan variedades altamente productivas y con frutos de color púrpura oscuro, que puedan ser cosechados dos semanas después de la fertilización de la flor. En cambio en Europa los mejoradores prefieren variedades que puedan desarrollar sus frutos por un periodo de tiempo más largo (tres a cinco semanas), y que posean un color negro intenso y brillante, que se mantenga hasta la fecha de cosecha (Daunay et al., 2001a,

Sekara et al., 2007).

En general los objetivos de mejora en la berenjena se enfocan hacia los siguientes aspectos:

Producción

La producción es uno de los objetivos primordiales en los planes de mejora. El desarrollo de híbridos ha sido una de las estrategias más exitosas para la obtención de altos rendimientos en muchos cultivos hortícolas (Virmani et al., 2004). El fenómeno de la heterosis para rendimiento, es una característica conocida desde hace mucho tiempo en el cultivo de la berenjena (Nagi y Kida, 1926; Kakizaki, 1931; Sambanbam, 1962; Kalloo, 1993; Sidhu et al., 2004; Rodriguez Burruezo et al., 2008; Vorontsova et al., 2013). Ya en los años 40 Odland y Noll (1948), observaron que los híbridos F1 de berenjena excedían el rendimiento medio de los parentales en un 62% y que estos altos rendimientos eran debidos principalmente al mayor número de frutos por plantas producidos por los híbridos.

Figura 22 Invernaderos en Almería dedicados a la producción de Berenjena.

La utilización de materiales no explotados en los programas de mejora puede dar lugar a la identificación de materiales con buena capacidad productiva y/o que sean parentales con una buena aptitud combinatoria y puedan dar lugar a híbridos con una mayor producción (Rodríguez-Burruezo et al., 2008). Por otra parte, un aspecto importante para la obtención de altos rendimientos durante la época otoñal-invernal en invernadero (Figura 22), es el uso de la partenocarpia natural, que permite el cuajado aun cuando el polen presenta un alto grado de esterilidad debido a las temperaturas frías en invierno, o cuando la polinización es deficiente debido a la falta de liberación del polen de las flores (Pessarakli y Dris, 2004; Donzella et al., 2000). Con respecto a esto, Fuzhong et al. (2005) señalan que las temperaturas que inducen la partenocarpia natural en la berenjena están alrededor de los 12ºC, y que bajo estas condiciones las variedades partenocárpicas son claramente superiores a las variedades no partenocárpicas en lo que a rendimiento se refiere.

Calidad aparente

La obtención de frutos de calidad es imprescindible para el éxito de una nueva variedad. En este sentido, la calidad del fruto de berenjena depende de varios caracteres que deben ser tomados en cuenta. Uno de los caracteres más importantes en la calidad aparente del fruto de berenjena es el color, el cual depende de la ausencia, presencia y distribución en la piel de las antocianinas y la clorofila (Daunay et al., 2004). Las antocianinas son las responsables de la coloración violácea o negra de los frutos de berenjena, y dependiendo del número y la orientación de los grupos hidroxilos y metoxilos de las moléculas de antocianinas, el color de la epidermis del fruto puede ser percibida como más roja o más violeta (Daunay et al., 2008). Las antocianinas se encuentran localizadas en las vacuolas de las células de la epidermis del fruto. Su ausencia o presencia está controlada por un QTL dominante llamado *fap10.1*, el cual explica un 86-93% de la variación total (Doganlar et al., 2002; Barchi et al., 2012). Así mismo, la intensidad de la coloración antocianica del fruto está controlada por varios genes (Cericola et al., 2014).

Además de la intensidad del color y presencia o ausencia de antocianos, es importante indicar que la síntesis de los antocianos de la piel de los frutos de berenjena puede depender de la incidencia de la luz solar (Figura 18). La presencia de más o menos antocianos bajo el cáliz está controlada mono- génicamente por un gen llamado *Puc* (*Purple under calyx)* (Tigchelaar et al., 1968). Por otra parte, la distribución de la coloración antociánica en los frutos berenjena puede ser uniforme, irregular o estriada (Figura 23). La herencia de este carácter aún no ha sido establecida.

Figura 23. Variabilidad en apariencia del fruto tanto por distribución de los antocianos, forma fruto, etc.

El segundo pigmento implicado en la coloración de los frutos de berenjena es la clorofila, la cual es responsable del color verde de la piel de los frutos de berenjena. La presencia o ausencia de clorofila en la epidermis del fruto es controlada por un gen dominante llamado *G* (Tigchelaar et al., 1968). La distribución de la clorofila en la piel puede ser uniforme o reticulada (Figura 23). En este sentido, Tigchelaar et al. (1968) demostró que la distribución irregular de la clorofila era controlada de forma dominante por un gen llamado *Gv (Green variegated)*, y Doganlar et al. (2002) identificó un QTL (*fst4.1*) que explicaba un 49-67% de la variación de la variación fenotípica.

Otro carácter importante en la calidad del fruto de berenjena es la ausencia de espinas en el cáliz. Las espinas en el cáliz del fruto representan un problema ya que pueden llegar a producir daños en la piel de los frutos durante el almacenamiento poscosecha, además de dificultar las labores de recolecta y manejo poscosecha de los mismos (Daunay et al., 2008). La presencia de espinas en el cáliz del fruto, está asociada a un QTL llamado *ftcp6.1*, el cual se encuentra en el cromosoma 6 y explica un 51% de la variación para este carácter (Doganlar et al., 2002; Cericola et al., 2014).

Además de los dos caracteres mencionados anteriormente existen otras atributos importantes para la calidad aparente del fruto de berenjena, entre los que se encuentran: la forma y tamaño, la uniformidad dentro y entre plantas (cuanto más alta sea la uniformidad mejor), firmeza del fruto (debe ser alta), pardeamiento de la carne (una vez cortado el fruto debe ser lo menor posible), amargor de la carne (lo menor posible). Para estos caracteres existe una importante variación lo cual hace posible su selección y mejora (Prohens et al., 2005b; Plazas et al., 2013).

Calidad nutracéutica

En los últimos años, se están planteando programas de mejora que, además de tener en cuenta caracteres de calidad aparente como los mencionados anteriormente, tengan en cuenta la mejora de la calidad nutritiva y nutracéutica (Prohens et al., 2005a, Plazas, 2014). Algunos constituyentes bioquímicos como las antocianinas, los compuestos fenólicos y los glicoalcaloides, juegan un papel importante en la calidad de la berenjena (Chadha, 1993; Sánchez-Mata et al., 2010). Además, en los mercados actuales existe un interés creciente por parte de los consumidores en productos vegetales más sanos y que protejan frente a enfermedades. Es por ello que los programas de mejora genética van paulatinamente incorporando la mejora del contenido en compuestos beneficiosos para la salud humana y que prevengan enfermedades (calidad nutracéutica) entre sus objetivos (Kaushik et al., 2015).

Entre los objetivos más importantes de mejora de la calidad nutracéutica en hortalizas, se encuentra el de incrementar el contenido en sustancias con poder antioxidante, como el contenido en ácido ascórbico o los polifenoles (Gramazio et al., 2013). El ácido ascórbico es un potente antioxidante y existen diferencias significativas entre accesiones de berenjena en el contenido de vitamina C (San José et al., 2008), pero los contenidos relativamente bajos de ácido ascórbico en los frutos de berenjena y el hecho de que los frutos de berenjena se consumen cocinados limitan su interés como antioxidantes (Hanson et al., 2006; Prohens et al., 2007). Por otra parte, la berenjena contiene altos contenidos de compuestos fenólicos, lo cual le confiere un alto poder antioxidante (Cao et al., 1996; Stommel y Whitaker, 2003; Plazas et al., 2013). Los polifenoles están adquiriendo un interés creciente por sus múltiples efectos beneficiosos, habiéndose demostrado que muchos de ellos tienen un poder antioxidante similar al de la vitamina C (Stommel y Whitaker, 2003; Singh et al., 2009). Además, al contrario que la vitamina C, los polifenoles tienen una gran estabilidad térmica, por lo que la degradación de los mismos es mínima incluso después de ser sometidos a altas temperaturas (Dao y Friedman, 1992, Zaro et al., 2015)

De esta forma, la selección de accesiones de berenjena con altas concentraciones de compuestos fenólicos como una manera de desarrollar nuevas variedades con una mejor calidad nutracéutica seria de interés. Por otra parte en varios estudios (Stommel y Whitaker, 2003; Rodríguez-Burruezo et al., 2005; Hanson et al., 2006; Prohens et al., 2007; Plazas et al., 2013) se ha encontrado una importante variación entre diferentes materiales de berenjena en el contenidos de polifenoles, lo cual indica que la mejora de este carácter es posible. Sin embargo, la oxidación de los polifenoles en contacto con el aire mediada por la polifenol oxidasa, produce el pardeamiento de la carne, lo cual es una característica negativa para la calidad aparente del fruto (Macheix et al., 1990). La actividad polifenol oxidasa puede variar entre distintas variedades de berenjena (Dogan et al., 2002). En este sentido, Prohens et al. (2007) encontraron una correlación moderada entre el contenido en polifenoles y el grado de pardeamiento, por lo cual los autores señalan que es posible seleccionar y desarrollar nuevas variedades con altos contenidos en polifenoles y un

grado de pardemiento moderado. (En la Figura 24 podemos ver el Colorítmetro que se utiliza para la medición del color de la carne recién hecho el corte y 10 minutos después, y dando la diferencia de coordenadas el pardeamiento de la carne en cada fruto). De esta manera los programas de mejora genética para aumentar el contenido en polifenoles del fruto de berenjena, también deben tener en cuenta que los valores de pardeamiento se encuentren en valores admisibles (Plazas et al., 2015).

Figura 24. Colorímetro que se utiliza para la medición del pardeamiento de la carne del fruto.

El modo de herencia, así como la vía de biosíntesis de los polifenoles de la carne de los frutos de la berenjena es aún poco conocida, y un mejor conocimiento de estos aspectos permitiría efectuar una mejora más efectiva de este carácter. A este respecto, se están realizando trabajos dirigidos a estudiar el modo de herencia y la expresión de los genes asociados con el contenido en polifenoles y la actividad polifenol oxidasa en la carne de los frutos de berenjena (Prohens et al., 2013; Gramazio et al., 2013).

Diversificación

La mayoría de variedades comerciales corresponden a un solo tipo de coloración (negro intenso) que difieren únicamente en la forma (Marín, 2015). En los tiempos actuales existe un interés en los mercados europeos por nuevos tipos de berenjena de diferentes colores y características (Daunay and Hazra, 2012), al igual

que ha ocurrido en tomate y pimiento. En otros países, como Estados Unidos, es común encontrar estos tipos de variedades. Por otra parte, como ocurre en muchos otros cultivos (Cooper et al., 2001), un incremento de la diversidad en el fondo genético usado por los mejoradores, así como la identificación de nuevas fuentes de variación para caracteres de interés entre los materiales locales, puede ser de gran utilidad en la obtención de nuevas variedades.

Resistencia o tolerancia a plagas y enfermedades

Al contrario que en otros cultivos hortícolas como el tomate o el pimiento, la resistencia a enfermedades no es un aspecto clave en la oferta varietal de berenjena y de hecho, la mayoría de variedades anunciadas en catálogos comerciales no hacen referencia a resistencia a enfermedades (Marín, 2015), mientras que, por ejemplo, en tomate, las resistencias a enfermedades son un factor esencial en el éxito de una variedad (Díez y Nuez, 2008). La berenjena exhibe resistencia parcial a varias plagas y enfermedades, pero a menudo a niveles bastantes bajos (Collonnier et al., 2001a, Mennella et al., 2010). En Europa la principal enfermedad que afecta a la berenjena es la verticilosis (*Verticillium dahliae*), la cual produce un marchitamiento de la planta, pero raramente llega a provocar su muerte, aunque reduce apreciablemente el rendimiento (Bletsos et al., 2003; Sunseri et al., 2003, Mennella et al., 2010, Villenueve et al., 2014). La lucha contra la verticilosis es difícil debido a que no existen altos niveles de resistencia entre el germoplasma de berenjena disponible (Daunay et al., 2008; Mennella et al., 2010). Un método de control efectivo contra el *Verticillium* es el injerto sobre patrones tolerantes como *S. torvum*, el cual es una práctica comúnmente usada en países de Asia especialmente en Japón (Villenueve et al., 2014).

En los países de clima tropical la principal enfermedad que afecta el cultivo de la berenjena es la marchitez bacteriana producida por *Ralstonia solanacearum,* la cual puede llegar a causar pérdidas de entre 50 a 100% de la producción (Collonnier et al., 2001b). Se han encontrado fuentes de resistencias en varios materiales de berenjena según Chaudhary (2000) y al mismo tiempo distintos niveles de resistencia (Yu et al., 2015), habiéndose propuesto que la resistencia a *R, solanacearum* es monogénica.

También se han identificado resistencias a esta enfermedad en especies relacionadas con la berenjena como *S. torvum* (Gousset et al., 2005), *S. aethiopicum* (Collonnier et al., 2001b) y *S. sisymbriifolium* (Mondal et al., 1991). Otra enfermedad de importancia sobre todo en Japón, es la marchitez producida por el hongo *Fusarium oxysporum*. En Europa esta enfermedad afecta a cultivos tanto en invernadero como al aire libre (van Steekelenburg, 1976; Urrutia Herrada et al., 2004; Altinok, 2005). Yamakawa y Mochizuki (1979) encontraron diferentes variedades de berenjena con varios niveles de resistencia, pero ninguna de ellas ofrecía una resistencia completa. Por otra parte Mochizuki et al. (1997), describió una resistencia monogénica dominante en la línea *LS174.* Dentro de las especies relacionadas con la berenjena se ha encontrado resistencia en S. *integrifolium, S. indicum, S. incanum, S. torvum, S. sisymbriifolium, S. aethiopicum* y *S. macrocarpom* (Daunay et al., 2008).

Otro problema fúngico, especialmente en invernadero o condiciones de alta humedad, es la pudrición del fruto por *Botrytis.* Las variedades más sensibles son aquellas en que la corola de la flor no se desprende después del cuajado, lo cual favorece la acumulación de humedad en la zona de unión del cáliz con el fruto. En este sentido, la mayoría de las variedades modernas desprenden pronto la corola después del cuajado (Prohens et al., 2005a).

Varios virus transmitidos por insectos (CMV, AMV, PVY, TSWV, EDMV) o por contacto (TMV, ToMV), pueden infectar a la berenjena, pero en la práctica su ocurrencia es mucho más limitada que en otras solanáceas como el tomate y el pimiento (Daunay et al., 2008). Aunque la mayoría de variedades pueden resultar infectadas por el virus del mosaico del tomate (ToMV), esta especie muestra un comportamiento tolerante, no apreciándose daños económicos (Prohens et al., 2005a).

Entre las plagas que afectan a el cultivo de la berenjena algunas de las más importantes son la mosca blanca (*Bemisia tabaci*), la araña roja (*Tetranychus urticae*), los pulgones (*Aphis sp*), el escarabajo de la patata (*Leptinotarsa decemlineata*), el minador (*Lyriomiza trifolii)* y los nematodos (*Meloidogyne sp*). La mayoría de estas

plagas se pueden controlar de manera eficiente con la aplicación de agroquímicos (Baixauli, 2001). Varias especies silvestres relacionadas con la berenjena poseen resistencia a insectos, ácaros o nematodos (Robinson et al., 2001), pero éstas aún no han sido evaluadas apropiadamente debido a las dificultades técnicas que esto conlleva (Daunay et al., 2008).

Otros caracteres de interés en la mejora

Un carácter de interés sobre todo para la producción de semilla hibrida es la androesterilidad, que permite obviar la emasculación en el proceso de hibridación, lo cual reduce el trabajo y los costos de producción (Khan y Isshiki, 2008). La androesterilidad en berenjena ha sido señalada por varios autores (Phatak y Jaworski, 1989; Phatak et al., 1991; Isshiki y Kawajiri, 2002; Khan y Isshiki, 2008; Khan et al., 2014) y se han utilizado dos especies relacionadas con la berenjena, *S.aethiopicum* (Fang et al., 1985) y *S. violaceum* (Isshiki y Kawajiri, 2002) para desarrollar líneas de berenjena con androesterilidad citoplasmática.

Varios caracteres de tipo productivo son de interés en la berenjena. Aspectos como la precocidad en la fructificación, periodos de producción más largos o cortos, la arquitectura y hábito de crecimiento de la planta, entre otros, son relevantes en el éxito de una variedad. Sin embargo, el control genético de muchos de estos caracteres es aún desconocido (Daunay et al., 2008). Otro carácter de interés en la mejora del cultivo de la berenjena es la ausencia de amargor de la carne del fruto, el cual es debido a la presencia de saponinas y glicoalcaloides (Daunay et al., 2001b). Este carácter está influenciado por varios factores, tanto climáticos como de cultivo. Además, dependiendo del mercado y los consumidores se pueden demandar frutos más o menos amargos (Daunay et al., 2008).

7.2. Implicaciones de la biología reproductiva de la berenjena en la mejora

El conocimiento de la biología reproductiva es esencial para la elaboración de programas de mejora (Pessarakli, 2004). En este sentido, la planta de berenjena presenta flores con un número de pétalos, sépalos y estambres que oscila entre 5 y 9. Tanto el pedúnculo como el cáliz de las variedades tradicionales poseen abundantes espinas, aunque actualmente se tiende al cultivo de variedades que eliminan este carácter desfavorable (Nuez et al., 2002). La heterostilia (diferencia en el tamaño y posición de los elementos sexuales de la flor) es un fenómeno común en las variedades primitivas (Sidhu et al., 2004; Salas et al., 2012). Así, la mayor parte de las variedades primitivas florecen en ramilletes de tres a cinco flores, una de las cuales es hermafrodita y de pedúnculo corto y continuo desde el tallo hasta el cáliz, y da lugar a un fruto comercial, mientras que el resto de las flores abortan o dan lugar a un fruto pequeño y de peor calidad. Normalmente la primera flor o inflorescencia aparece en el vértice de la primera bifurcación o tallo principal de la planta (Prohens et al., 2005a). Como consecuencia de la selección, la mayoría de las variedades modernas de berenjena poseen inflorescencias con una única flor.

La berenjena es una especie normalmente autógama (Salas et al., 2012). De esta forma, los materiales locales suelen ser bastante homogéneos y con un alto grado de homocigosis (Vilanova et al., 2012). No obstante, en algunos casos puede presentar cierto porcentaje de alogamia. Sambandam (1964) estimó que el porcentaje de polinización entre flores de plantas oscilaba entre 1.9-10.9% en cultivo al aire libre. Asimismo, encontró que entre plantas separadas a más de 50 metros el porcentaje de polinización cruzada era nulo. Sidhu et al. (2004), en materiales asiáticos, señala que el porcentaje de alogamia en berenjena puede llegar hasta un 60-70% en presencia de insectos polinizadores. En nuestras condiciones hemos comprobado que la tasa de alogamia en cultivo al aire libre es de un 2-3%. En cultivo en invernadero, en ausencia de polinizadores, la fecundación es fundamentalmente autógama. En cambio, cuando se introducen polinizadores, como los abejorros, las posibilidades de alogamia se

incrementan ya que cada abejorro puede visitar varias flores por día (Abak et al., 1998).

La obtención de semilla comercial se suele realizar cultivando los materiales en condiciones aisladas, en el caso de líneas puras o variedades de polinización abierta, o mediante polinización manual en el caso de los híbridos. La relativa facilidad con que se pueden realizar los cruces, junto con la gran cantidad de semilla por fruto que se puede obtener, son una ventaja en para la producción de semilla hibrida de berenjena (Sidhu et al., 2004).

7.3 Métodos de mejora

La mayor parte de variedades de berenjena se han obtenido utilizando la variación intraespecífica. La variación interespecífica ha sido utilizada para obtener líneas o materiales de premejora (Chadha, 1993, Plazas et al., 2014; Rotino et al., 2014.).

Los métodos de selección utilizados han sido diferentes para cada tipo de variedad (polinización libre, líneas puras y híbridos F1) y dependen de la naturaleza genética de los caracteres objeto de mejora (monogénico, oligogénico, poligénico), de la influencia ambiental sobre los mismos y del modo de acción génica de los genes implicados (Daunay y Hazra, 2012).

En las variedades de polinización abierta se realiza generalmente una selección masal que permite eliminar las plantas con peores características y se mantiene un cierto grado de variación dentro de la población. Un caso típico sería el de la variedad población "Almagro", utilizada para las berenjenas de encurtido típicas de esa región (Muñoz-Falcón et al., 2008). En estos casos, en que no se controla la polinización, es esencial asegurar un aislamiento de la población a seleccionar de otros materiales de berenjena.

Para la obtención de líneas puras, ya sea para su utilización como variedades o como parentales de híbridos se siguen distintos métodos, como la selección individual dentro de poblaciones genéticamente heterogéneas, la hibridación con selección genealógica o la selección recurrente (Daunay et al., 1997). En los casos en que se pretende subsanar una deficiencia específica de un material de élite, se ha utilizado la mejora por retrocruzamiento. Es de destacar que en berenjena se ha utilizado de forma bastante habitual la obtención de líneas puras a partir de la diploidización de plantas haploides obtenidas mediante cultivo de anteras o polen (Collonnier et al., 2001a; Salas et al., 2011:).

Los híbridos de berenjena presentan la ventaja de ser heteróticos para caracteres de producción (Sidhu et al., 2004; Rodríguez-Burruezo et al., 2008). Esta es una característica que se conoce desde hace tiempo y que ha permitido obtener variedades con rendimientos muy elevados al cruzar líneas puras no emparentadas genéticamente.

7.4 Las nuevas herramientas de la biotecnología

En el cultivo de la berenjena, la utilización de nuevas herramientas de la biotecnología, como el cultivo "in vitro", la fusión somática, los marcadores moleculares y la transformación genética puede ser de gran utilidad en la mejora de la berenjena (Huang y Cao, 2012). A este respecto, los marcadores moleculares, especialmente los AFLP, RAPD y microsatélites han permitido identificar parte de la variación existente en este cultivo y especies relacionadas (Doganlar et al., 2014) además de ayudar a cuantificar distancias génicas entre materiales a fin de explotar de manera más eficientes el fenómeno de heterosis para la obtención de híbridos altamente productivos (Rodríguez-Burruezo et al., 2008).

Los cruzamientos entre berenjenas y determinadas especies silvestres relacionadas de interés en la mejora se han visto relativamente limitados por la incompatibilidad sexual entre éstas (Rotino et al., 2014). Sin embargo, la capacidad de la berenjena para responder de buena manera al cultivo de tejidos, ha permitido la

aplicación de la biotecnología, particularmente en la explotación de la variación somaclonal, hibridación somática y transformación genética (Huang y Cao, 2012). En este sentido, se han obtenido plantas transgénicas con resistencias a ataque de insectos y enfermedades (Arpaia et al., 1997; Schuler et al., 1998; Kumar et al., 1998), resistencia a estrés abiótico (Prabhavati et al., 2007) y para la producción de frutos partenocarpicos (Donzella et al., 2000; Acciarri et al., 2001). Otra técnica usualmente utilizada en berenjena es el cultivo de anteras (Figura 25) para la obtención de plantas haploides (Salas et al., 2011). La androgénesis "in vitro" es usada frecuentemente por los mejoradores para producir de manera rápida líneas puras a partir materiales heterocigotos y, con éstos, producir híbridos F1 comerciales (Corral-Martínez et al., 2008; Salas et al., 2011).

Figura 25 . Comparación de flores (imagen de la izquierda) y anteras (imagen de la derecha) entre un individuo haploide y otro diploide. H: Haploide. D: Diploide. (Salas et al., 2011).

En la actualidad ya se dispone de un borrador de la secuencia del genoma de la berenjena (Hirakawa et al., 2014). Esto es de gran utilidad ya que proporciona innumerables marcadores y permite realizar estudios de sintenia con otras solanáceas más estudiadas, como el tomate.

7.5 Las variedades locales en la mejora

Las variedades tradicionales son el resultado de un proceso continuo de evolución basado en la selección, adaptación e interacción entre la planta, el agricultor y el medioambiente a lo largo de cientos de años. En este sentido, estas variedades tienen un valor intrínseco como patrimonio etnobotánico de un país, y como tal deberían ser conservadas (Zeven, 2002). No obstante, como recurso fitogenético constituyen un excelente material de trabajo para el mejorador. Lamentablemente muchas de las variedades tradicionales de berenjena se están perdiendo, al verse reemplazadas por nuevas variedades mejoradas, hibridas en su mayor parte, y que permiten unas producciones más altas (Prohens y Nuez, 2001).

La pérdida de las variedades tradicionales no sólo significa la pérdida de unos recursos genéticos de gran valor para la obtención de nuevas variedades en el presente o en el futuro. Junto con ellas también se pierde la experiencia y profundos conocimientos etnobotánicos asociados a su utilización (Nuez y Fernández de Córdova, 1994; Cebolla-Cornejo et al., 2001). Además, no hay que olvidar que al presentar adaptación a ambientes específicos, las variedades tradicionales permiten reducir la necesidad de insumos adicionales, por lo que pueden ser muy útiles para la agricultura ecológica (Nuez y Ruiz, 1999).

En este contexto, las variedades locales pueden actuar bien como fuentes de variación, o bien como receptores de variación. Como fuentes de variación, pueden ser aprovechados para la mejora de ciertas características de los cultivares modernos. Como receptores de variación, las propias variedades locales pueden ser objeto de mejora, introduciendo caracteres que mejoren su competitividad frente a los cultivares modernos. En ambos casos, el objetivo reside en aprovechar las dos características más importantes de las variedades locales: la calidad interna y la adaptación a las condiciones agroclimáticas locales (Cebolla-Cornejo 2005).

7.6. Protección de las variedades locales

Como se ha comentado anteriormente, las variedades tradicionales presentan unos atributos de calidad destacados, lo cual conduce en muchos casos a la obtención de mayores precios de venta en el mercado. Desde 1992, en la Unión Europea, los productos agrícolas que presenten características específicas asociadas a una alta calidad y que a su vez sean originarias de una cierta región, pueden ser protegidas de imitaciones por una Denominación de Origen Protegida (DOP) o una Indicación Geográfica Protegida (IGP) (Figura 25) (Martín, 2009). La principal diferencia entre la DOP y la IGP es que en la DOP el producto final debe ser producido, transformado y elaborado en una zona geográfica determinada, con unos conocimientos específicos reconocidos y comprobados, mientras que la IGP solo se requiere que al menos una de las etapas de producción, transformación o elaboración esté vinculada con una determinada zona geográfica. Tanto la DOP como la IGP implican que la calidad y/o características esenciales del producto sean atribuidas a su origen (MAGRAMA, 2015).

Figura 26. Logotipo distintivo de la IGP y DOP.

La principal ventaja de la protección mediante DOP o la IGP es que los productos que poseen esta categoría usualmente adquieren un valor añadido en los mercados (Garcia y Albisu, 2001; Babcock y Clemens, 2004; Martín, 2009). En la Unión Europa existen un total de 169 DOP e IGP en la categoría de "frutos, cereales y hortalizas". Muchos de los productos protegidos son originarios de países del sur de Europa (Italia: 53; España: 33; Francia: 27; Grecia: 22; Portugal: 21; Austria: 3;

Alemania: 3; República Checa: 2; Holanda: 2; Dinamarca: 1; Finlandia: 1; Reino Unido: 1), lo cual probablemente refleja la alta diversidad de variedades tradicionales producidas en estos países. En cuanto al cultivo e de la berenjena a nivel europeo la única variedad protegida por una de las categorías antes mencionadas es la llamada "Berenjena de Almagro" la cual cuenta con una IGP, otorgada en 1994 (Figura 27) (Castro, 2005).

El uso de variedades locales es a menudo un requisito para la obtención de una POD o una IGP (Trichopolou et al., 2007). En estos casos la autenticidad del material vegetal debe ser garantizado, dado que en algunos casos puede llegar a ocurrir que variedades que no cumplan con los requisitos de la variedad protegida puedan ser introducidas de forma voluntaria o involuntaria en los campos de cultivo (Rao et al., 2006). Por lo tanto en estos casos es importante disponer de herramientas que permitan distinguir de forma efectiva a las falsas variedades de la auténticas. A este respecto la caracterización morfológica y agronómica de las variedades tradicionales, junto con el uso de nuevas tecnologías como los marcadores moleculares, pueden ser útiles en el estudio de la diversidad y relaciones de los materiales locales, así como en el establecimiento de huellas genéticas que permitan su identificación.

Figura 27. Cartel Publicitario de Berenjena de Almagro con IGP.

Otros mecanismos de protección de las variedades tradicionales lo constituyen las variedades de conservación, que permiten que sea reconocido el patrimonio hortícola y se regule la producción de semilla. Por otra parte, para evitar el expolio, en determinados casos es recomendable desarrollar títulos de obtención vegetal que representan la máxima protección legal de las variedades. Para llegar a desarrollar esta segunda aproximación es necesario desarrollar cierto nivel de mejora. En este caso, se puede aprovechar para aumentar la uniformidad y capacidad productiva de estas variedades, el desarrollo de híbridos o incluso para introducir genes de resistencia a enfermedades, consiguiendo que estas dejen de ser consideradas "cultivos de riesgo".

También se han utilizado "marcas registradas" asociadas a productos de calidad ® mediante estrategias de marketing. La ventaja de emplear marcas registradas se basa en la facilidad del control de los derechos del propietario de la marca, ya que las infracciones son fácilmente perseguibles.

Todo ello llevaría a promover un mercado de calidad alrededor de estas variedades libres de falsificaciones que permita estabilizar unos precios de venta elevados que compensen la menor capacidad productiva de estas variedades. Para que esta estrategia tenga éxito es necesario asociar la variedad tradicional con elevada calidad interna, por lo que se hace necesario acometer una exhaustiva caracterización de la misma para seleccionar los mejores materiales para el mercado.

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OBJETIVOS

El objetivo principal de esta Tesis Doctoral es obtener información relevante para los programas de mejora genética de berenjena mediante el estudio de la diversidad genética y el desarrollo y uso de herramientas para la caracterización morfológica, así como el aumento de la diversidad genética en el germoplasma de élite de los programas de desarrollo de híbridos de alto valor. Para ello utilizaremos material vegetal tanto de la berenjena tipo negra (semi-larga), como material de otros tipos, orígenes y variedades locales. De ese modo, toda la información obtenida, además del material vegetal obtenido mediante un programa de mejora, serán de utilidad para el desarrollo de nuevas variedades de berenjena y la utilización de nuevas técnicas de selección por parte del mejorador.

Para cumplir este objetivo principal se ha dividido este estudio en 2 bloques diferenciados, cada uno de los cuales consta de tres apartados:

1. Estudio de diversidad genética en *S. melongena* y la aplicación de nuevas herramientas para realizar una caracterización morfológica precisa para mejorar el proceso de selección en los programas de mejora.

1.1. Estudio de diversidad de berenjena en tres centros de origen secundarios de distintas regiones: España, Sri Lanka y China.

1.2. Estudio de diversidad de variedades locales de berenjena con distintas tipologías.

1.3. Caracterización morfológica de la berenjena mediante el uso de nuevas herramientas fenómicas.

2. Desarrollo de material vegetal para el incremento de la base genética de los cultivares de berenjena e implementación de distintos programas de mejora genética.

2.1. Planteamiento e inicio de diferentes programas de mejora con distintos objetivos.

2.2. Realización de un programa de mejora para una variedad local (berenjena de Almagro) con Indicación Geográfica Protegida.

2.3. Incremento de la diversidad y obtención de nuevos materiales de élite de berenjena tipo negra mediante un programa de mejora genética.

RESULTADOS

DIVERSIDAD GENÉTICA Y HERRAMIENTAS

Diversity and Relationships of Eggplants from Three Geographically Distant Secondary Centers of Diversity

Maria Hurtado[1], Santiago Vilanova[1], Mariola Plazas[1], Pietro Gramazio[1], H. Hemal Fonseka[2], Ramya Fonseka[3], Jaime Prohens[1*]

1 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain,

2 Horticultural Crop Research and Development Institute, Gannoruwa, Sri Lanka,

3 Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka

E-mail: jprohens@btc.upv.es

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Abstract

Eggplant (*Solanum melongena* L.) was domesticated in the Indo-Birmanian region, which is also the primary center of diversity for this crop. From there eggplant spread to other regions, and diversity accumulated in several secondary centers of diversity. We have assessed the diversity and relationships of 52 accessions of eggplant from three geographically distant secondary centers of diversity (China, Spain, and Sri Lanka) using 28 morphological descriptors and 12 highly polymorphic genomic SSRs. A wide variation was found for most morphological traits, and significant differences among the three centers of diversity were detected for 22 of these traits. The PCA analysis showed that eggplants from the three origins were morphologically differentiated, and accessions from each of the three secondary centers of diversity presented a typical combination of morphological characteristics. In this respect, discriminant analysis showed that accessions could be correctly classified to their origin using only six traits. The SSR characterization identified 110 alleles and allowed obtaining a unique genetic fingerprint for each accession. Many alleles were found to be private to each origin, but no universal alleles were found for any of the origins. The PCA analysis showed that the genetic differentiation among origins was less clear than for morphological traits, although the analysis of the population structure shows that accessions mostly group according to the origin, but also provides evidence of migration among the three secondary centers of diversity. The genetic diversity (H_T) within each origin was high, ranging between H_T=0.5400 (Sri Lanka) and H_T=0.4943 (China), while the standardized genetic differentiation (G'_{ST}) among origins was moderate (G'_{ST}=0.2657). The correlation between morphological and SSR distances was non-significant (r=0.044), indicating that both data are complementary for the conservation of germplasm and breeding of eggplant. These results are relevant for the management of genetic resources, breeding programmes, and evolutionary studies of eggplant.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is the most important *Solanum* crop native to the Old World, and it ranks as one of the most important vegetable crops in the world with an annual worldwide production of more than $41 \cdot 10^6$ t (2010; FAO data). This results in an average production of 6.1 kg/person/year or 16.7 g/person/day. Eggplant is one of the vegetables with highest antioxidant capacity [1], which is a consequence of its high content in phenolics [2]. Despite the economic and nutritional importance of eggplant, breeding efforts in this crop have been limited [3]. The use of exotic germplasm in breeding programmes can be of great relevance for the improvement of the crop and for addressing future breeding challenges. In this respect, Muñoz-Falcón et al. [4] found that the genetic diversity of modern cultivars of black eggplants was reduced, and that incorporation of black fruit materials from different origins could increase the genetic base of this cultivar type and contribute to better exploitation of the heterosis resulting from the crosses of genetically distant materials [5].

Assessment of the diversity and relationships of the cultivated species facilitates the establishment of conservation strategies, the use of genetic resources in breeding programmes, and the study of the crop evolution. The domestication and evolution of eggplant has been the subject of a number of studies in which historical [6,7], morphological [8], and molecular [9,10] data have been used. There is general consensus that eggplant was domesticated in South-East Asia from the wild relative *Solanum incanum* L, and morphological [8], and molecular [9-14] data, as well as the high fertility of F1 hybrids with *S. melongena* [3,8] support this hypothesis. It is unknown how *S. incanum*, which is naturally distributed in Africa and the Middle East, reached the Indo-Birmanian region, although it has been speculated that it could have arrived there unintentionally as a weed or dragged by ocean currents from Africa to India, or intentionally because of the value of its berries for tanning hides [8,15]. In any case, as occurred with tomato [16], the domestication of eggplant outside of the area of natural distribution of its wild ancestor resulted in an important genetic bottleneck [3,8].

Diffusion of the crop from its primary center of diversity, situated in the Indo-Birmanian region [17,18], where primitive cultivars and weedy forms exist [3,8,10], to other areas resulted in the diversification of the crop due to micro-evolutive forces like mutation, selection (natural and artificial), genetic drift, or gene flow, and to recombination, and led to the accumulation of diversity in several secondary centers of diversity [10,19]. For example, the cultivation of eggplant in China has been documented since more than 2000 years ago [7]; its introduction into Europe, through Spain, was brought about by the Arabs before the 10th century [19]. The evolution of the crop in geographically distant secondary centers of diversity led to the differentiation of cultivar types specific to different regions of the world [3]. In this respect, two large groups of eggplant cultivars are considered by breeders: "Occidental" or "Western" eggplants (Middle East, Africa, Europe, and America) and "Oriental" or "Asian" eggplants (Eastern and Southeastern Asia) [3,6,10]. Within each of these large groups, several cultivar types are distinguished [3].

Although a number of studies have been devoted to the study of the diversity of eggplant materials from specific countries or regions, like India [18], Spain [19], China [20,21], Turkey [22], or Asia [10,23], up to now no comprehensive assessment has been performed on the comparative diversity and regional differentiation of eggplant materials from different regions of the world. The study of the diversity of geographically distant centers of diversity, where eggplant was introduced through different routes, can provide information of interest for understanding the structure of variation in eggplant, as well as for the conservation of genetic resources and breeding of this crop. Similar studies have been undertaken in other crops, such as lentil (*Lens culinaris* Med.) [24], coffee (*Coffea arabica* L.) [25], maize (*Zea mays* L.) [26] and sorghum (*Sorghum bicolor* (L.)) [27].

The characterization of eggplant with both morphological descriptors and molecular markers has proved useful for the study of the diversity and relationships of different varietal groups of eggplant, as they sample different levels of diversity [4,19,28,29]. Availability of characterization data for traits of agronomic interest is essential for breeding programs. Morphological descriptors for the characterization of

eggplant are available as a result of the European Eggplant Genetic Resources Network (EGGNET) [30]. These descriptors have been used and validated in a number of characterizations of eggplant genetic resources and breeding materials [4,19,28,29,31]. Also, molecular markers are useful to study the genetic diversity of eggplant [4,10-12,14,20-23,28,29,32-35]. SSRs have revealed as the best presently available markers to study the relationships of different groups of cultivars of eggplant, as they have wide genome coverage, are highly variable, have a highly repeatability, are easy to use, and are amenable to high throughput [36,37]. For example, Muñoz-Falcón et al. [28,29,38] have found that SSRs are much better than AFLPs at resolving the relationships between and within cultivar groups as well as for assigning correctly accessions to their cultivar groups. Also, Demir et al. [22], when comparing SSRs with RAPDs, found that the former were more adequate than the latter to study the diversity and relationships of local landraces. Several hundred SSR markers are available in eggplant [14,23,32-35,39], and among them genomic SSRs have proved to be more polymorphic than EST-SSRs [38].

Here we assess the morphological and the molecular (SSR) diversity of a collection of eggplants from three geographically distant secondary centers of diversity: China, Spain, and Sri Lanka. Our objective is to obtain information on the diversity, relationships, and differentiation among accessions of these three different origins. These results will be of interest for the conservation of genetic resources, breeding, and study of the evolution of the cultivated eggplant.

RESULTS

Morphological characterization

The collection of eggplants studied, as well as each of the three groups of accessions originating from China, Spain, or Sri Lanka, displayed a wide variation for most of the morphological traits studied (Table 1). Although for the 28 morphological traits considered there is overlap in the range of variation of individual accessions among the three groups, significant differences among means of the three origins are

found for 22 of the morphological traits considered (Table 1). In this respect, the number of significant differences among origin means for the traits studied has been of 12 for China vs. Spain accessions, 20 for China vs. Sri Lanka accessions, and 15 for Spain vs. Sri Lanka accessions.

Chinese accessions are less vigorous than those of Spain or Sri Lanka, so that the Chinese accessions have lower plant height (P-Height) and smaller leaves (L-Pedicel, L-Length, L-Breadth) than either the Spanish or Sri Lankan eggplants (Table 1). Regarding anthocyanic pigmentation of the vegetative parts of the plant (P-Anthocyans, L-Anthocyans, Fr-CAnthocyans), the highest pigmentation is found in the Chinese eggplants, the lowest in the Sri Lankan ones, while in the Spanish accessions it is intermediate. While the plant prickliness (P-Prickles) has been in general low, Sri Lankan accessions have presented, as a mean, a lower prickliness (P-Prickles) than either the Chinese or Spanish eggplants. Leaves of Sri Lankan accessions had a smaller leaf blade breadth/width ratio (L-Ratio) than the Chinese of Spanish eggplants, and the Chinese accessions had a lower leaf blade lobing (L-Lobing) than the Spanish or Sri Lankan eggplants (Table 1).

For flower traits, Chinese eggplants had, in general, a smaller number of flowers per inflorescence (Fl-Number) than either the Spanish or Sri Lankan eggplants (Table 1). Spanish eggplants had more petals per flower (Fl-Petals) than that of the Chinese or Sri Lankan accessions.

Table 1. Mean and range for the traits measured in the eggplant accessions originating from China, Spain, and Sri Lanka used for the present study, and probability of the *F*-statistic, obtained from ANOVA analyses, for differences among means.

Trait	Code	China		Spain		Sri Lanka		Prob. *F*
		Mean[a]	Range	Mean[a]	Range	Mean[a]	Range	
No. of accessions		20		14		18		
Plant growth habit	P-Habit	2.1a	1.0-4.2	1.6a	0.7-5.0	2.1a	1.0-5.0	0.394
Plant height (cm)	P-Height	81.2a	48.3-108.2	105.4b	45.0-149.5	95.2b	61.2-120.3	0.006
Shoot tips anthocyanins intensity	P-Anthocyans	6.2b	1.0-9.0	5.1ab	3.0-9.0	4.0a	1.0-7.0	0.010
Stem prickles	P-Prickles	2.3b	0.0-3.0	2.6b	0.0-3.0	0.7a	0.0-3.0	<0.001
Leaf pedicel length (cm)	L-Pedicel	4.9a	2.9-6.2	7.2c	5.1-9.7	5.6b	4.3-6.7	<0.001
Leaf blade length (cm)	L-Length	15.9a	11.6-20.2	20.7c	17.6-28.2	19.2b	17.3-21.9	<0.001
Leaf blade breadth (cm)	L-Breadth	9.4a	5.8-13.3	12.2b	9.4-15.1	12.3b	9.8-14.6	<0.001
Leaf blade length/breadth ratio	L-Ratio	1.71b	1.43-2.01	1.70b	1.53-1.88	1.58a	1.35-1.86	0.005
Leaf blade lobing	L-Lobing	3.5a	1.0-7.0	4.9b	3.0-7.0	4.8b	3.0-7.0	0.009
Leaf anthocyanins intensity	L-Anthocyans	6.9c	0.0-9.0	4.6b	0.0-9.0	2.4a	0.0-9.0	<0.001
Flowers per inflorescence	Fl-Number	1.4a	1.0-4.0	2.5b	1.0-6.0	2.8b	1.5-4.0	0.001
Flower diameter (cm)	Fl-Diameter	3.9a	3.0-5.2	4.1a	2.5-5.9	3.5a	2.5-5.7	0.162
Petals per flower	Fl-Petals	5.5a	5.0-7.0	6.1b	5.7-7.0	5.3a	5.0-6.0	<0.001
Corolla colour	Fl-Corolla	3.4a	1.0-5.0	2.9a	0.0-5.0	3.2a	1.0-7.0	0.606
Fruit longitudinal perimeter (cm)	Fr-Perimeter	30.2a	22.9-46.4	26.8a	17.6-38.7	29.7a	20.6-36.4	0.253
Fruit length (cm)	Fr-Length	13.3a	6.9-22.7	12.5a	6.4-18.9	16.2b	7.6-20.7	0.019
Fruit breadth (cm)	Fr-Breadth	7.6b	3.0-13.5	7.6b	5.5-10.0	5.2a	3.5-8.6	0.002
Fruit length/breadth ratio	Fr-Ratio	2.26a	0.58-5.00	1.70a	0.85-2.99	3.42b	0.89-4.73	<0.001
Fruit weight (g)	Fr-Weight	426b	139-982	332ab	61-685	209a	97-392	0.030
Relative fruit calyx length	Fr-CLength	2.6a	1.0-5.0	5.7b	1.0-9.0	3.1a	1.0-9.0	<0.001
Fruit calyx anthocyanins intensity	Fr-	7.6c	0.0-9.0	3.6b	0.0-9.0	0.6a	0.0-9.0	<0.001

	CAnthocyans							
Fruit colour intensity under calyx	Fr-UnderC	0.7a	0.0-5.0	2.1b	0.0-5.0	4.2c	3.0-5.0	<0.001
Fruit skin chlorophyll	Fr-Chlorophyll	4.3b	0.0-9.0	4.4b	0.0-9.0	0.0a	0.0-0.0	<0.001
Fruit skin L* primary colour	Fr-L*	25.8a	19.6-58.5	34.1a	21.3-54.7	52.5b	22.3-74.8	<0.001
Fruit skin a* primary colour	Fr-a*	8.4a	-17.9-19.2	6.7a	-2.2-16.0	8.7a	-0.8-16.7	0.649
Fruit skin b* primary colour	Fr-b*	2.6a	-2.1-25.14	4.4a	0.3-14.1	4.8a	-1.08-14.01	0.335
Fruit skin gloss (gloss units)	Fr-Gloss	7.1a	1.9-11.9	10.6b	5.6-17.9	13.7c	8.9-18.5	<0.001
Fruit flesh browning	Fr-Browning	4.0a	1.1-8.4	4.9ab	1.6-9.4	6.3b	0.9-13.2	0.035

[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

The fruits of Sri Lankan accessions, in general, have higher fruit length (Fr-Length) and lower fruit breadth (Fr-Breadth) and, in consequence, are more elongated (Fr-Ratio) than those of China or Spain (Table 1). Spanish eggplants have a larger part of the fruit covered by the calyx (Fr-CLength) than those of China or Sri Lanka, while the intensity of the fruit skin covered by the calyx (Fr-UnderC) is higher in Sri Lankan accessions, followed by the Spanish ones, and finally by the Chinese accessions,. Contrary to what occurred in a number of Chinese and Spanish accessions, the Sri Lankan eggplants studied did not present chlorophylls in the fruit skin (Fr-Chlorophyll) and therefore presented lower mean values than Chinese or Spanish accessions for this trait (Table 1). Regarding the skin colour, Sri Lankan materials had, on average higher L* (Fr-L*) values (indicating closer to white) than Chinese or Spanish accessions. Fruits of Sri Lankan accessions were, on average, more glossy (Fr-Gloss) than those of the Spanish accessions, which, in turn were also more glossy than the Chinese accessions. Finally, Sri Lankan accessions had a significantly higher flesh browning (Fr-Browning) than Chinese accessions (Table 1).

According to the scree plot method, a total of five principal components were found to be relevant in the morphological PCA study. These five principal components account for 63.4% of the total variation. However, given that the first and second components account, respectively, for 22.9% and 16.2% of the total variation (Figure 1), and that in the graphical analyses no relevant changes are introduced by including the third, fourth or fifth principal components, we just present and discuss the data referring to the first and second components. The first component was positively correlated to plant vigour traits (L-Breadth, L-Length, L-Pedicel, Fl-Number, and P-Height) as well as to traits related to elongated fruits (Fr-Length, Fr-Ratio, and Fr-Perimeter), and to those associated to the light skin colour, like a high luminosity (Fr-L*), high gloss (Fr-Gloss), and also to a high intensity of the color intensity under calyx (Fr-UnderC) (Figure 1). Negative correlations for this first component were found for traits associated to broad fruits (Fr-Breadth) as well as to traits related to the dark pigmentation (due to content in anthocyanins) of the different plant parts (L-Anthocyanins, Fr-CAnthocyanins, P-Anthocyanins, and Fl-Corolla), or of the fruit

caused by the presence of chlorophylls (Fr-Chlorophyll) (Figure 1). Negative correlations of this first component were also found with the leaf breadth/width ratio (L-Ratio), presence of prickles in the plant (P-Prickles), or fruit flesh browning (Fr-Browning). The second component was positively correlated to fruit and flower size (Fr-Weight, Fl-Petals, and Fl-Diameter), to plant vigour traits (L-Breadth, L-Length, L-Pedicel, Fl-Number, and P-Height), to fruit breadth (Fr-Breadth), as well as to the content of chlorophyll in the fruit skin (Fr-Chlorophyll) (Figure 1). This second component was negatively correlated to traits related to elongated fruits (Fr-Ratio, Fr-Length, and Fr-Perimeter) and to prostrate plant habit (P-Habit) (Figure 1).

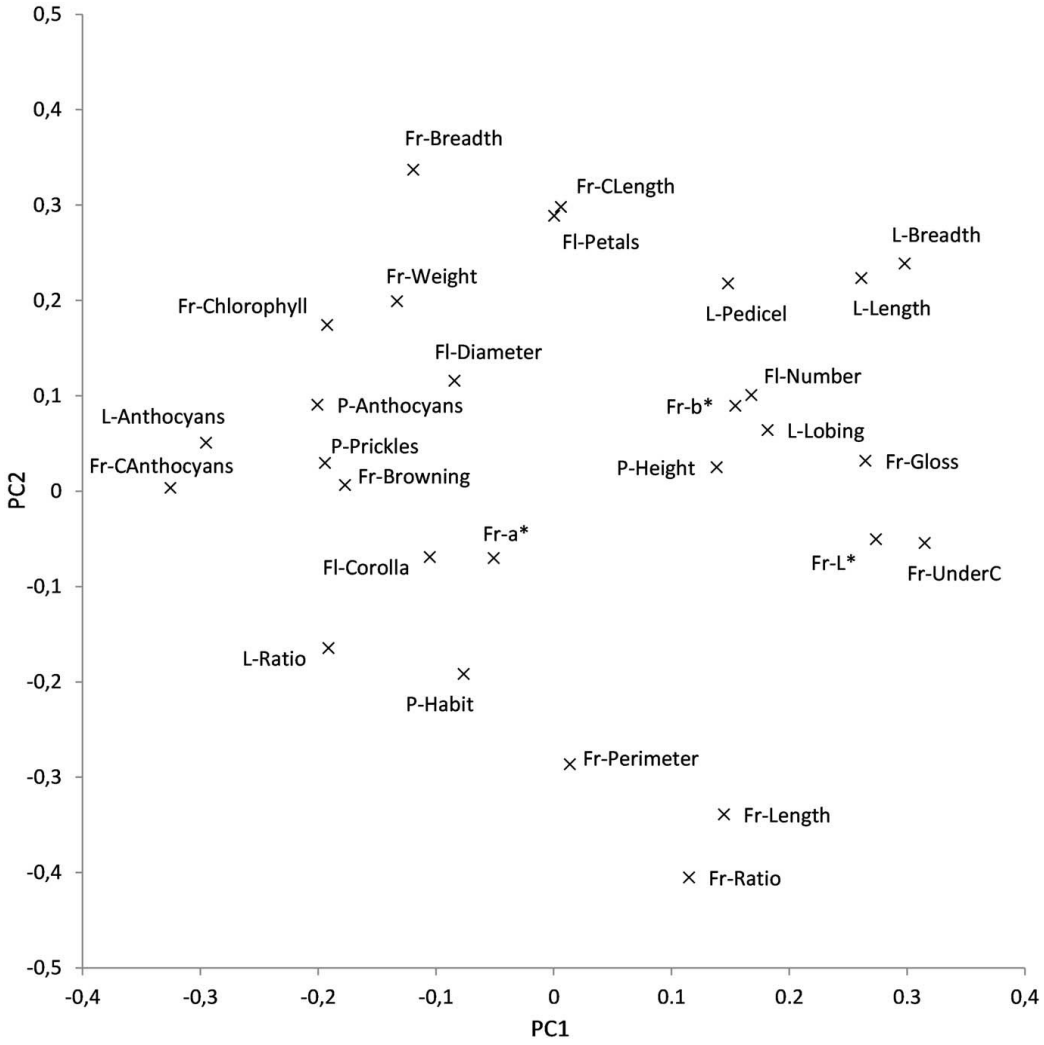


Figure 1. PCA relationships between morphological traits. The two first components (PC1 and PC2) of the principal components analysis account for 22.9% and 16.2% of the total variation, respectively. Results were obtained after the characterization of 52 *S. melongena* accessions from China, Spain, and Sri Lanka using 28 morphological descriptors (see text and Table 2).

The projections of the accessions on the PCA graph show that with the exception of two accessions (C02 and L11) the accessions of China, Spain and Sri Lanka plot in different parts of the PCA graph (Figure 2). The Chinese accessions are, in general, characterized by negative values of the first component, with low values of traits related to plant vigour (L-Breadth, L-Length, L-Pedicel, Fl-Number, and P-Height), dark fruits (low Fr-L* and Fr-Gloss, and high Fr-Chlorophyll), and high pigmentation of the plant (high L-Anthocyanins, Fr-CAnthocyanins, P-Anthocyanins,

and Fl-Corolla), as well as for a wide range of values for the second component. Spanish accessions present intermediate-high values for both the first and second components, and therefore are associated to high plant vigour (high L-Breadth, L-Length, L-Pedicel, Fl-Number, and P-Height), large flower and fruit size (high Fl-Petals, Fl-Diameter, Fr-Breadth, Fr-Weight), and negatively to elongated fruits (low Fr-Ratio, Fr-Length, and Fr-Perimeter). Sri Lankan accessions present positive values of the first component and relatively low values of the second component, and therefore are associated to elongated fruits and small flowers and fruits (high Fr-Ratio, Fr-Length, and Fr-Perimeter, and low Fl-Petals, Fl-Diameter, Fr-Breadth, and Fr-Weight) with light skin fruit (high Fr-L* and Fr-Gloss and low Fr-Chlorophyll) and low plant pigmentation (L-Anthocyanins, Fr-CAnthocyanins, P-Anthocyanins, and Fl-Corolla) (Figure 2). The outlier Chinese accession C02 is, together with C01, the only accession from this origin that does not have anthocyanins in the fruit (high Fr-L* and Fr-UnderC values), and is also characterized by large leaves (high L-Pedicel, L-Length, L-Breadth) and high fruit flesh browning (Fr-Browning), which results in high values of the first component. Also, the outlier Sri Lankan accession L11 is the only one in this group that does not present elongated fruits (high L-Breadth and low Fr-Length and Fr-Ratio) and therefore has high values for the second component of the PCA and is intermingled with the Spanish accessions (Figure 2).

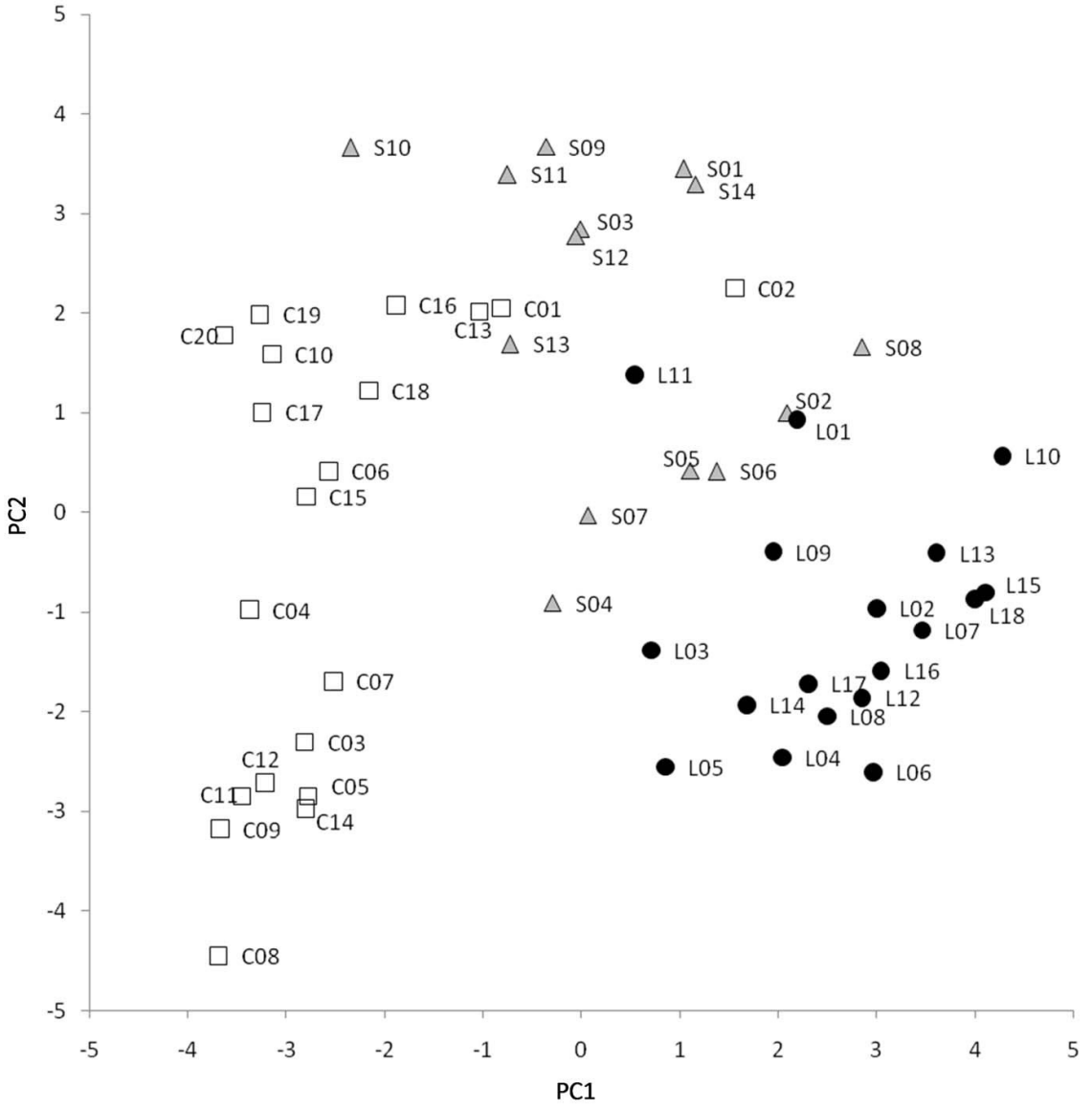


Figure 2. PCA morphology-based relationships between accessions. A total of 52 *S. melongena* accessions from China (white squares), Spain (grey triangles), and Sri Lanka (black circles) (Table 1) were morphologically evaluated with 28 morphological traits (see text and Table 2) and were represented on the two first components (PC1 and PC2) of the principal components analysis (22.9% and 16.2% of the total variation explained by the first and second component, respectively).

The discriminant analysis of the 28 morphological traits revealed that 100% of the accessions were correctly classified to their country of origin. The forward stepwise discriminant analysis showed that a correct classification of all the accessions could be achieved using a minimum set of six traits: L-Length, Fr-L*, Fr-UnderC, Fr-b*, P-Prickles, and Fr-Weight. The two discriminating functions for this model were highly significant ($P<0.001$). For all these traits, with the exception of Fr-b*, important and highly significant ($P<0.001$) differences were found among origins (Table 1).

SSR characterization

The twelve SSR loci evaluated were polymorphic in the materials evaluated and amplified 110 alleles (average of 9.2 alleles per locus) (Table 2). The number of alleles per locus ranged between five for CSM54 and CSM73 and 16 for CSM30. PIC values ranged between 0.251 for CSM43 and 0.722 for CSM30, with an average value of 0.574. A unique genetic fingerprint was obtained for each of the individual accessions.

Table 2. SSR markers, number of alleles per locus for all the samples and for each origin (China, Spain, and Sri Lanka), number of private alleles (i.e., present in one or more accessions of each origin), and PIC value for each SSR locus.

SSR locus	All samples	China		Spain		Sri Lanka		PIC
	Alleles (n)	Alleles (n)	Private alleles (n)	Alleles (n)	Private alleles (n)	Alleles (n)	Private alleles (n)	
CSM7	6	4	1	3	0	4	1	0.489
CSM27	11	4	1	4	2	8	4	0.569
CSM30	16	9	4	7	3	6	4	0.722
CSM31	12	7	4	4	2	6	3	0.626
CSM32	12	5	2	6	2	6	4	0.598
CSM36	6	3	1	2	0	5	3	0.351
CSM43	6	1	0	3	2	4	3	0.251
CSM44	11	3	1	5	2	7	5	0.606
CSM52	6	3	0	4	1	4	1	0.550
CSM54	5	5	3	2	0	2	0	0.422
CSM73	5	1	0	4	2	4	1	0.331
CSM78	14	7	3	6	2	7	5	0.720
Mean	*9.2*	*4.4*	*1.7*	*4.2*	*1.5*	*5.25*	*2.8*	*0.574*
Total	110	52	20	50	18	63	34	---

When considering the number of alleles present in accessions from different origins, the Sri Lanka materials presented the highest number of alleles (63), followed by Chinese (52), and Spanish (50) accessions, which corresponds, respectively, to 57.3%, 47.3%, and 45.5% of the total number of alleles detected (Table 2). However, no private and universal SSR alleles were found for any of the three origins. For the materials of Sri Lanka, a total of 34 alleles were private (i.e., unique to one or more accessions of this group), while for China and Spain the number of private alleles was of 20 and 18, respectively (Table 2). Most of these private alleles specific to each origin were present in low frequencies, so that when considering each of the origins separately the average value of the frequency of the private alleles was of 0.077, with a maximum frequency value of 0.308. Eighteen alleles were present in all the groups, while 9 were exclusive of China and Spain, 5 of China and Sri Lanka, and 5 to Spain and Sri Lanka (Figure 3).

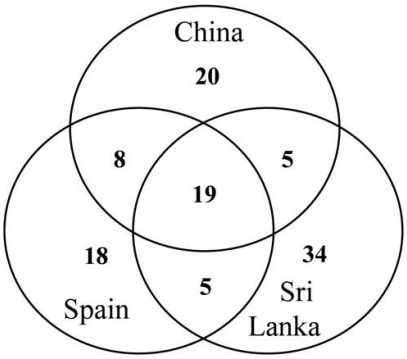
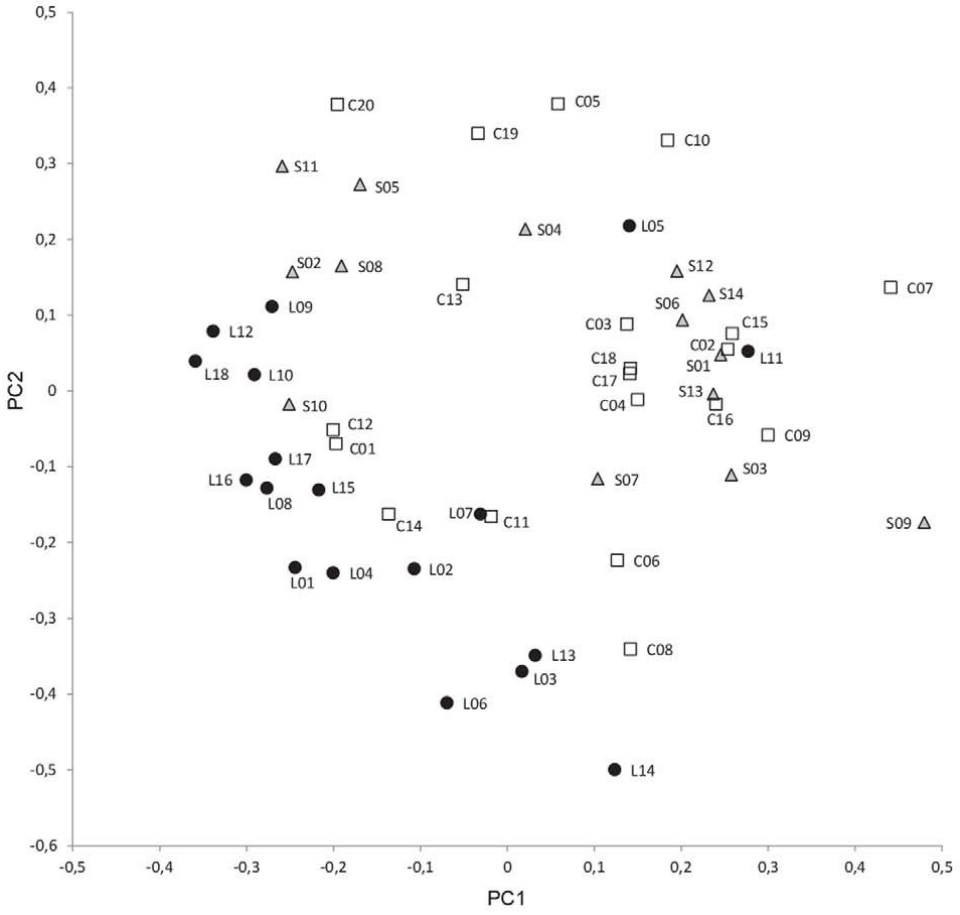


Figure 3. SSR alleles in the different origins. The figures indicate the number of SSR alleles private to each of the secondary centers of diversity, as well as the number of alleles shared by accessions from two or the three different origins.

The first and second components of the PCA analysis performed with SSR data account for 22.0% and 19.1% of the total variation, respectively. Although in this analysis accessions of the different origins are intermingled (Figure 4), accessions from China and Sri Lanka are mostly distributed in different areas of the plot. With the exception of L05 and L11, accessions from Sri Lanka have a combination of low and moderate values for the first and second components; on the other hand, with the exception of C01, C11, C12, and C14, accessions from China present positive values of either the first or second components (Figure 4). Accessions from Spain are scattered all over the graph with the exception of the bottom part of the plot (i.e., the area with values <-0.2 for the second component). Inclusion of a third component in the analysis (17.9% of the total variation explained by this component) shows that no accessions from Spain present low values for the third component (all accessions have values >-0.15), while no accessions from Sri Lanka present high values for this component (all accessions have values <0.15). On the other hand, accessions for China present a wide range of values for the third component (Figure 4).


PC2
PC1
C20
C05
C19
C10
S11
S05
S04
L05
S02
S08
S12
C13
S14
C07
S06
L09
C03
C15
L12
C18
C02
L11
L18
L10
C17
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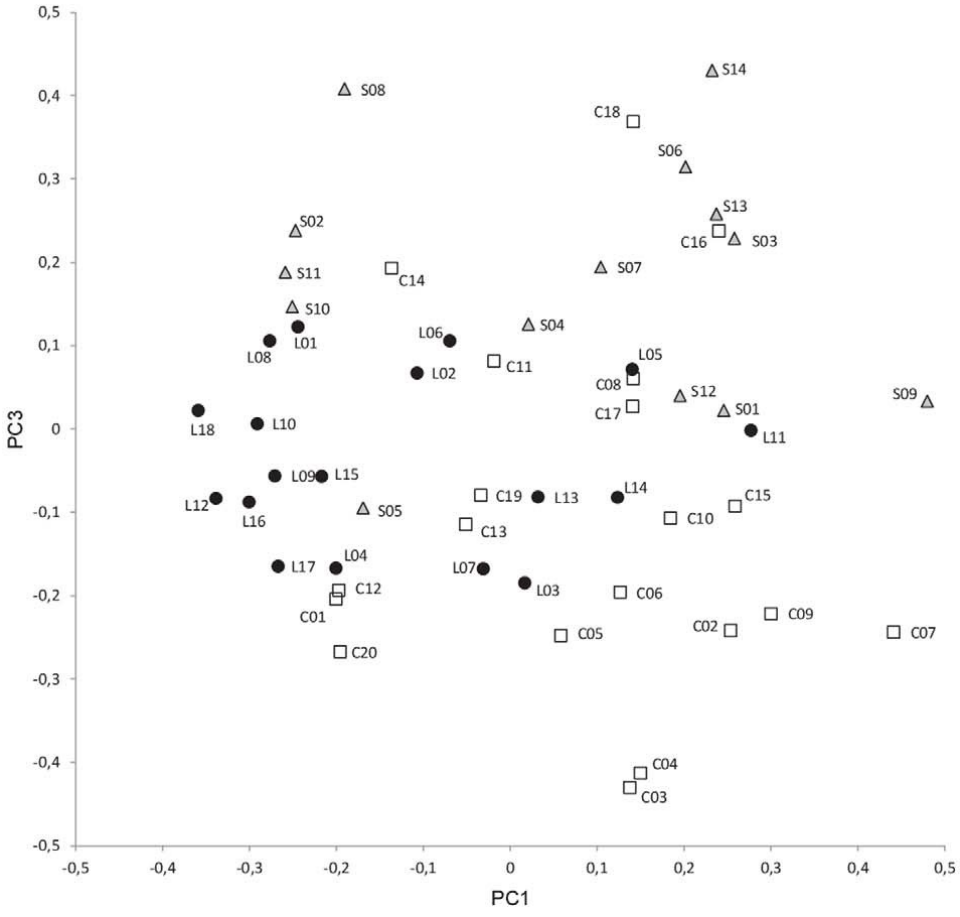
C08
L03
L06
L14


PC3
PC1
S14
S08
C18
S06
S13
S02
C16
S03
S11
C14
S07
S10
S04
L06
L01
L08
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L04
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C20
C04
C03

Figure 4. PCA SSR-based relationships between accessions. A total of 52 *S. melongena* accessions from China (white squares), Spain (grey triangles), and Sri Lanka (black circles) (see Table 1) were evaluated using 12 polymorphic SSRs (see text and Tables 3 and 5) and were represented on the three first components (PC1, PC2 and PC3) of the principal components analysis (22.0%, 19.1%, and 17.9% of the total variation explained by the first, second, and third principal components, respectively). Scatterplots show the projections of the accessions on the first and second principal components (above) and on the first and third principal components (below).

The Evanno´s test indicated that the most informative number of populations (*K*) was 3. The inferred population structure for *K*=3 obtained with the STRUCTURE software showed that most of the accessions of each origin are assumed to belong to the same population (Figure 5). Therefore, we have named these populations as CH, SP, and SL, corresponding to populations containing mostly Chinese, Spanish, and Sri Lankan accessions, respectively. In this respect, when the highest value for the membership coefficient (q_i) is used to assign one accession to a population, for the Chinese accessions, 14 out of the 20 accessions are assigned to the CH population; for the rest of Chinese accessions, C14, C16, C17, and C18 are assigned to the SP population, and C01 and C11 to the SL population. In the case of the Spanish accessions, 10 out of the 14 accessions are assigned to the SP population; three of the rest of Spanish accessions (S01, S09, and S12) to the CH population, while the remaining one (S10) to the SL population. Finally, the 15 out of the 18 Sri Lankan accessions are assigned to the SL population; two out of three of the rest of accessions (L05 and L11) to the CH population, and the last one (L02) to the SP population (Figure 5). For most of the accessions (69.2%), the membership coefficient q_i to one of the populations was higher than 0.8, while the rest (30.8%) could be considered as admixed ($q_i \leq 0.8$). Seven of the admixed accessions were from China (C01, C08, C12, C10, C14, C15, and C19), five from Spain (S03, S05, S09, S10, and S12), and four from Sri Lanka (L02, L03, L07, and L10) (Figure 5).

The three origins present a high level of total genetic diversity (H_T), which ranges from H_T=0.4943 for Chinese accessions to H_T=0.5400 for Sri Lankan accessions (Table 3). The partition of the total diversity shows that most of the genetic diversity is

found within each of the groups (D_{ST}=0.0481 and H_S=0.4909), which results in a moderate value of the relative magnitude of genetic differentiation (G_{ST}=0.0892). However, the standardized G_{ST} (G'_{ST}) reached a much higher value (G'_{ST}=0.2657) (Table 3). The values of heterozigosity for individual accessions were low, with an average value of 0.052, with non-significant differences among origins (P>0.05).

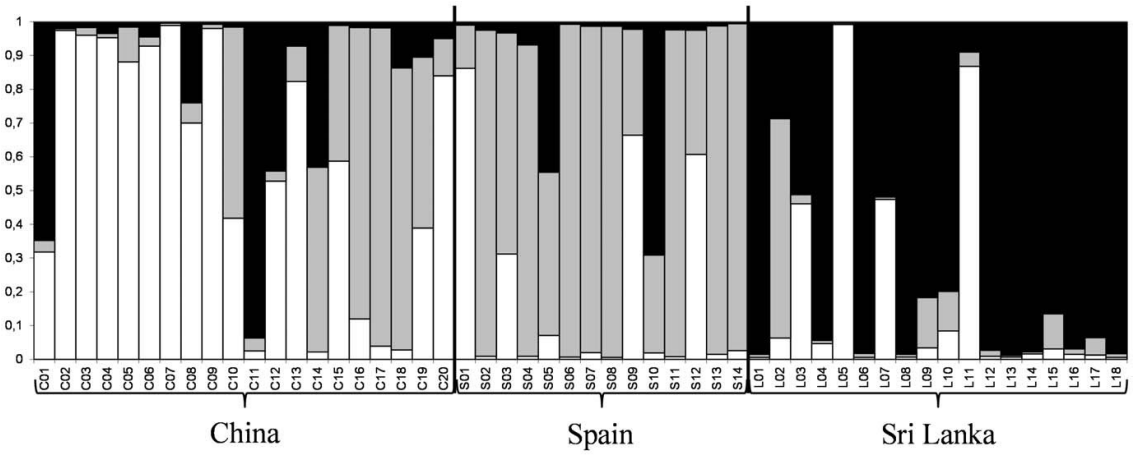

Figure 5. Assignment tests of accessions to populations. The estimated number of populations (parameter *K*) was set to 3 in the STRUCTURE software (Pritchard et al., 2000). Accessions are organized according to their origin (China, Spain, and Sri Lanka). The three populations are named according to the origin of the majority of the accessions constituting each population (CH for China, SP for Spain, and SL for Sri Lanka). Each accession is represented by a horizontal line, which is partitioned into colored segments (white for the CH population, grey for the SP population, and black for the SL population).

Table 3. Total genetic diversity (H_T), among groups genetic diversity (D_{ST}), within groups genetic diversity (H_S), relative magnitude of genetic differentiation (G_{ST}) and standarized G_{ST} (G'_{ST}), estimated from SSR data for the eggplant accessions according to their origin.

Group	Sample size	H_T	D_{ST}	H_S	G_{ST}	G'_{ST}
Total	52	0.5390	0.0481	0.4909	0.0892	0.2697
Origins						
China	20	0.4943				
Spain	14	0.5286				
Sri Lanka	18	0.5400				

The lowest genetic distance (and consequently highest genetic identity) values are found between the Chinese and Sri Lankan origins, with a value of 0.1204 (Table 4). The genetic distance values of Spanish and Chinese origins and of Spanish and Sri Lankan eggplants are similar, with values of 0.1613 and 0.1604, respectively (Table 4). Correlations obtained from the Mantel test between the morphological and SSR distances were low (r=0.044) and non-significant (t=0.5729; P=0.7166).

Table 4. SSR-based estimates of genetic distances (above the diagonal) and genetic identities (below the diagonal) between different eggplant origins.

Origin	China	Spain	Sri Lanka
China		0.1613	0.1204
Spain	0.8510		0.1604
Sri Lanka	0.8813	0.8514	

DISCUSSION

Morphological and molecular data are not only complementary for the study of the diversity and relationships of eggplants from these distant centers of diversity, but also synergistic, as information of interest becomes conspicuous when comparing both type of data. Several studies are available on the relationships between morphological and molecular data in eggplant, but these studies have been restricted to specific cultivar groups [4,28-29], to study Spanish accessions [19], or to compare both type of data for predicting the performance of hybrids [5]. In this respect, our study is the first one to study simultaneously the morphological and molecular diversity of a significant number of eggplant accessions from geographically distant centers of diversity of eggplant.

Morphological diversity

The morphological data show that within each of the origins, a considerable diversity exists, confirming what had already been observed within Spanish eggplants [19]. For most morphological traits significant differences among origins are found for most traits, and eggplants from the different origins mostly plot in different areas of

the PCA graph, showing that accessions from each of the three secondary centers of diversity are morphologically differentiated and present a typical combination of traits [28,29]. In this way, a combination of just six morphological traits is enough to correctly assign any of the accessions to its origin. Amazingly, in this study we have found that the number of differences between eggplants from China and Sri Lanka, has been much greater than those between any of the former origins and Spanish eggplants. These results indicate that although cultivated eggplants have often been classified into "Western" or "Occidental" and "Asian" or "Oriental" cultivar groups [3,6,10,35], the real situation, in particular of the "Asian" eggplants may be more complex, with several groups with important morphological differences. For example, in general, the Chinese accessions used in our study have more anthocyanin pigmentation, are more prickly, have darker fruits, with more chlorophyll in the skin, and less vigorous, with smaller leaves, and less elongated and glossy fruits than Sri Lankan accessions. The few outlier accessions that in the morphological PCA group with accessions from other origins (C02 and L11) present a few morphological characteristics that differ greatly from the rest of accessions of their origin (e.g. lack of anthocyanin pigmentation, large leaves, and high fruit flesh browning in C02, and round fruits in L11). However, for the rest of traits present values similar to those of the Chinese (C02) and Sri Lankan (L11) accessions and therefore, when using the discriminant traits are correctly classified to their origins.

The wide range found for most morphological traits within each of the origins suggests that genetic drift may not have been an important force in the morphological differentiation of eggplant within each of the three secondary centers of diversity [40]. On the other hand, the morphological differentiation found among origins may indicate that natural selection and/or, more likely, artificial selection may have been responsible for the combination of morphological differences characteristic of accessions from different secondary centers of diversity [41]. Some of the traits for which significant differences exists among origins (e.g., prickliness, anthocyanin pigmentation, fruit chlorophyll, presence of pigmentation under the calyx) are monogenic or oligogenic and present a high degree of penetration and expression

[3,42]; therefore, the frequencies of phenotypes change quickly in response to selection. In consequence, the fact that for these traits the means are different, even though a wide range of variation is present in each origin, suggests that artificial selection, very likely linked to different uses or to different management practices, may have been responsible for the morphological differentiation among origins. This suggests that ethnobotanical aspects may have had an important role in the differentiation of eggplant landraces from separate regions

The important differences among origins for morphological traits may be relevant for eggplant breeding, and indicates that sources of variation of interest for several relevant agronomic traits of materials from a center of diversity may be found in exotic materials from other centers of diversity. For example, when considering some traits of interest for breeding modern cultivars [3], in general Chinese accessions seem to be a good source of variation for large fruits and low fruit flesh browning, Spanish ones for vigorous (e.g., high with large leaves) plants, and Sri Lankan accessions for low prickliness and high glossiness.

Molecular diversity

The high morphological variation in the materials studied is matched by high levels of molecular diversity. In this sense, we have found that the 12 genomic SSR loci were polymorphic and allowed the detection of a total of 110 SSR alleles (9.2 alleles/polymorphic locus). These 12 SSR loci were developed by Vilanova et al. [35] and were selected for the present study for its high polymorphism. However, the level of polymorphism found here has been higher than that found in the original work of Vilanova et al. [35], in which a total of 92 alleles (7.7 alleles/polymorphic locus) were detected in 22 eggplant materials from different cultivar types and origins. In other studies, the level of SSR polymorphism has been much lower. For example, Muñoz-Falcón et al. [38] in a study of 42 eggplant accessions from different origins and cultivar groups used 49 SSR loci (17 corresponding to genomic SSRs and 32 to EST-SSRs) of which only 21 were polymorphic and which allowed the detection of 85 alleles (4.1 alleles/polymorphic locus). In a similar study, Muñoz-Falcón et al. [4] found

polymorphism for 11 out of 14 SSR loci (2 genomic SSRs and 12 EST-SSRs), giving a total of 61 alleles (4.7 alleles/polymorphic locus) in a collection of 44 eggplants. Stàgel et al. [32] found that only 11 out of 38 EST-SSR loci were polymorphic in a collection of 38 eggplant cultivars of different origins and allowed the detection of 39 alleles (3.5 alleles/polymorphic locus). Ge et al. [23] in a study of 88 EST-SSRs of 42 Asian accessions found polymorphism for 79 of them, with a total of 323 polymorphic alleles (4.1 alleles/polymorphic locus). Also, when we consider each of the three origins, a high diversity is found, and the number of alleles per polymorphic locus is of 4.3, 4.2, and 5.3 for accessions from China, Spain, and Sri Lanka, respectively, which is a confirmation that these three countries can be considered as secondary centers of diversity [19-21]. The fact that a high number of private alleles are found in each of the origins we have studied, although most of them are present at low frequencies, has important implications for the conservation of the genetic diversity. For example, it suggests that the establishment of core collections for this crop should include accessions from different origins so that most of the allelic diversity is represented [43].

The multivariate analysis of SSR data shows a less clear picture of the differentiation among centers of diversity than the analysis of morphological data, with a higher degree of admixture among accessions of different origins in the SSR PCA plots than in the morphological PCA graph. In any case, the combination of the first and second components separate most of the Sri Lankan accessions from most of the Chinese accessions, while the third component differentiates mostly the Spanish and Sri Lankan accessions. Therefore, despite the fact that some of the accessions are intermingled, differentiation is observed among the three origins in the PCA analysis. The Bayesian-based analysis without a priori assignment of accessions to populations resulted in the creation of three populations, each of which was formed mostly by accessions from a single origin, which suggests an important differentiation among origins [25,26]. The results of the PCA analysis show a good agreement with the population structure analysis. In this respect, accessions from one origin that in the PCA analysis plotted closer to most of the accessions of another origin, were assigned

to the population formed mostly by accessions of this latter origin in the population structure analysis. More than two thirds of the accessions were clearly assigned to one of the populations, as they presented a membership coefficient q_i to one of the populations higher than 0.8 [25,44]; the rest of accessions could be considered as admixed, although in most cases were basically an admixture of only two populations. Accessions from one origin (e.g., China) assigned in the population structure analysis to populations mostly composed by accessions from another origin (e.g. Spain or Sri Lanka) were mostly not admixed accessions, which may indicate that introduction of these materials have taken place in relatively recent times. Also, the fact that eggplant is fundamentally autogamous [45] may have helped to maintain the genetic integrity of the materials introduced from another center of diversity.

The analysis of the genetic diversity with the total diversity (H_T) parameter [46] shows that the total diversity present in each of the origins is high and similar to the total diversity present in the whole collection, which is an indication of the high diversity present in each origin. This result is somewhat surprising, as the diversity present in a small country, like Sri Lanka, is even higher than the diversity found in a large country, like China. Given that Sri Lanka is close to India, which is part of the primary center of diversity [3,9,10], may have allowed the accumulation of a high diversity in Sri Lanka.

Despite the fact that the genetic diversity among groups (D_{ST}) is considerably lower than the genetic diversity within groups (H_S), the standardized genetic differentiation (G'_{ST}), which is more adequate than G_{ST} for SSR data [47] is greater than 0.25, which is a moderate value and shows that there is a considerable differentiation among the three origins [25,47,48]. When considering the genetic relationships among the three origins, the lowest genetic distance is found between Chinese and Sri Lankan accessions, while the genetic distance between Spanish and Chinese or Sri Lankan accessions are similar. Given that China and Sri Lanka are geographically closer than to Spain probably has contributed to a greater exchange of materials and genetic flux among these two countries, resulting in lower genetic distances.

The heterozygosity values observed were low, and similar to the values found by others in eggplant landraces of the black type [4]. This result was expected, given the high degree of autogamy of eggplant [3,45], which results in the fixation of alleles in homozygosis in populations in which there is no artificial control of pollination.

Comparison of morphological and molecular diversity

The correlations between morphological and SSR molecular data have not been significant, which is contrast to the moderate value (r=0.38) found by Muñoz-Falcón et al. [4] when comparing morphological and SSR data in a study of black eggplants which included modern F1 hybrids, old non hybrid cultivars, Spanish landraces, and Non-Spanish landraces. Significant correlations between morphological data and AFLPs were also found in the former study, and also by Prohens et al. [19] in a study of the diversity of Spanish eggplants. The correlations between morphological and molecular data are usually very variable and dependent, among others, on the plant material, morphological descriptors, and molecular markers used. In this respect, in other solanaceous crops, like pepper [49] or potato [50] no significant correlations were found among both types of data.

The lack of correlations in our collection suggests that both types of data sample different levels of diversity and, therefore, both of them should be considered for the management and conservation of germplasm [51]. In this respect, morphological markers in eggplant sample traits for which the variation is usually controlled by a few genes or QTLs [3,42], while genomic SSRs detect differences in the length of short tandem repeated sequences mostly situated in non-coding regions of the genome [36,37]. Wendel and Doyle [52] suggested that the lack of correlation between morphological and molecular data may be caused by the fact that both types of markers follow different evolutionary paths.

CONCLUSIONS

The results show that eggplant accessions from the three geographically distant secondary centers of diversity studied (China, Spain, and Sri Lanka) present a wide morphological and molecular diversity. At the morphological level, a clear differentiation exists among the different origins, indicating that different selection criteria have been applied in each secondary center of diversity, leading to a typical syndrome of traits for each origin. The molecular data also show that considerable differentiation exists at the molecular level among the three origins, although in this case there is evidence of migration between the different origins, as revealed by the study of the population structure. The lack of correlation between morphological and molecular diversity shows that both types of data provide complementary information and that both of them should be taken into account in the management of germplasm and formation on core collections [43,51]. The results also have importance for eggplant breeding, as it shows that sources of variation of interest can be found in the materials evaluated, and also suggests that crossing among individuals assigned to different populations may result in heterotic hybrids [5]. Finally, these results are also important for understanding the evolution and domestication of eggplant [9,10].

MATERIALS AND METHODS

Permissions

No specific permits were required for the described field studies, which took place in an experimental field plot at the Universitat Politècnica de Valencia. This field plot is used by the authors of this paper affiliated to the aforementioned institution (MH, SV, MP, PG, and JP) for field trials for characterization of germplasm of cultivated species.

Plant materials

Fifty-two accessions of *S. melongena*, of which 20 originated in China (C codes), 14 in Spain (S codes), and 18 in Sri Lanka (L codes) were used for the present study (Table 5). The materials correspond to local landraces or to selections within the local landraces and were chosen trying to represent the diversity of local materials of *S. melongena* of each country. The criteria used for choosing the accessions was based on previous available morphological and genetic data (for the Spanish accessions) [4,19,28,35], on distribution across geographic range, and also on availability of seeds. The plant material used is part of the collection of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (Valencia, Spain).

For each accession five plants raised from seed were grown in an open-air field plot (39°28'55" N; 0°20'11" W) in Valencia (Spain) following a completely randomized experimental design. Plants were spaced 1 m between the rows and 0.8 m apart in the row. The standard horticultural practices for eggplant production in the area of Valencia were followed.

Table 5. Plant materials used for the study of morphological and molecular (SSR) variation of a collection of eggplants from China, Spain, and Sri Lanka.

Accession name	Code	Place of origin
China		
Qi Xian Hei You Guan	C01	Qi
Tuo Cheng Qing Qie	C02	Tuocheng
Zao Xian Qie	C03	Acheng
Qie Zie	C04	Aihui
Zi Chang Qie	C05	Hubei
Tuan Qie	C06	Zhuix
Wu Xian Qie	C07	Anxiang
He Xian Qie	C08	Chenzhou
Yong Ji Xian Qie	C09	Yongji
Zi Yuan Qie	C10	Taoan
Niu Jiao Qie	C11	Changzhou
Niu Jiao Qie	C12	Jiangyin
Hong He Bao Qie	C13	Yichun
Yu Jiang Chang Xian Qie	C14	Yujiang
Bao Tou Niu Xin Qie	C15	Baotou
Tian Jin Da Min Qie	C16	Yinchuan
Tian Jin Er Min Qie	C17	Yinchuan
Cao Xian Yuan Zie Qie	C18	Cao
De Zhou Duan Ba Hong Qie	C19	Dehou
ASI-S-1	C20	Beijing
Spain		
AN-S-24	S01	Jaén
V-S-10	S02	Alicante
AN-S-3	S03	Córdoba
MU-S-7	S04	Murcia
B-S-3	S05	Baleares
MU-S-4	S06	Murcia
V-S-3	S07	Valencia
V-S-2	S08	Gandía
V-S-9	S09	Alicante
MU-S-8	S10	Murcia
MU-S-6	S11	Murcia
NC056471	S12	Murcia
H15	S13	Almagro
AN-S-23	S14	Jaén
Sri Lanka		
BW11	L01	Bombuwela
SM 164	L02	Gannoruwa
Thinnevelly Purple	L03	Jaffna
8890	L04	Matara
SA7MTE2	L05	Unknown
5124	L06	Gampaha

7517	L07	Colombo
1624	L08	Galle
558	L09	Nuwara Eliya
799	L10	Matale
8891	L11	Matara
1139	L12	Puttlam
2287	L13	Unknown
Ridiyagama	L14	Hambantota
Farmer Lenairi	L15	Anuradhapura
Kaluthavelly	L16	Batticaloa
Ampara	L17	Ampara
Welimada	L18	Welimada

Morphological characterization

Individual plants were characterized using 23 primary descriptors, most of which were developed by EGGNET [4,19,30]. These descriptors include plant (P), leaf (L), flower (Fl), and fruit (Fr) characteristics. Thirteen traits corresponding to these primary descriptors are quantitative: plant height (cm; P-Height), leaf pedicel length (cm; L-Pedicel), leaf blade length (cm; L-Length), leaf blade breadth (cm; L-Breadth), leaf blade length/breadth ratio (L-Ratio), flowers per inflorescence (Fl-Number), flower diameter (cm; Fl-Diameter), petals per flower (Fl-Petals), fruit longitudinal section perimeter (cm; Fr-Perimeter), fruit length (cm, Fr-Length), fruit breadth (Fr-Breadth), fruit length/breadth ratio (Fr-Ratio), fruit weight (g; F-Weight). The other ten traits are measured in a scale with predetermined values (Table 6), and correspond to EGGNET descriptors [4,19,30]. Apart from these primary descriptors, the skin colour and brightness, as well as the fruit flesh browning were also measured objectively in at least three fruits per plant. For the skin colour the L* (Fr-L*), a* (Fr-a*), and b* (Fr-b*) Hunter colour coordinates of the predominant (primary) colour of the skin were assessed with a Minolta CR300 (Minolta Co., Osaka, Japan) chromameter. The skin gloss (gloss units; Fr-Gloss) was measured with a Novo-Curve Elcometer 400 (Elcometer, Manchester, UK) glossmeter. Finally, for the fruit flesh browning (Fr-Browning), the flesh colour in the central part of a transversal section of the fruit was measured with the CR300 chromameter at 0 min and 10 min after being cut, and the

flesh browning was measured as the difference between the Hunter colour coordinate L* (luminosity) at 0 and 10 min [53], so that the higher the value of the difference the greater the browning.

Table 6. Morphological traits measured in a scale with pre-determined values of the descriptor states, and description of the scale used for the study of morphological variation in the eggplant accessions studied [4,19,30].

Traits	Codes	Range (scale)
Plant growth habit	P-Habit	1-9 (1 = upright; 9 = prostrate)
Shoot tip anthocyanins intensity	P-Anthocyans	0-9 (0 = absent; 9 = very strong)
Stem prickles	P-Prickles	0-9 (0 = absent; 9 = very many)
Leaf blade lobing	L-Lobing	1-9 (1 = very weak; 9 = very strong)
Leaf anthocyanins intensity	L-Anthocyans	0-9 (0 = absent; 9 = very strong)
Corolla colour	Fl-Colour	0-7 (0=White; 7=blue)
Relative fruit calyx length	Fr-CLength	1-9 (1 = very short (<10%); 9 = very long (>10%))
Fruit calyx anthocyanins intensity	Fr-CAnthocyans	0-9 (0 = absent; 9 = very strong)
Fruit color intensity under calyx	Fr-UnderC	0-9 (0 = none; 9 = very strong)
Fruit skin chlorophyll	Fr-Clorophyll	0-9 (0 = none; 9 = very strong intensity)

Molecular characterization

Genomic DNA was extracted from a mixture of young leaf from the five plants morphologically evaluated according to the CTAB method procedure [54]. The quality of DNA was checked on 1% agarose gels and the DNA concentrations estimated using a Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, Delaware, USA) spectrophotometer.

For the present study we used twelve simple sequence repeat (SSR) markers (Table 7) that proved to be highly polymorphic and that presented high PIC values in eggplant [35]. The map position of nine out of these 12 SSRs is known (Table 7). SSRs were amplified following the M13-tail method described by Schuelke [55] to facilitate the incorporation of a dye label during PCR. Amplifications were performed in a total volume of 12 µl, with 10 ng DNA, 1mM $MgCl_2$, 0.05 µM of forward primer, 0.25 µM of reverse primer, 0.2 µM fluorescent-labelled M-13 primer, 0.2 mM dNTPs and 1 unit of *Taq* polymerase in 1X PCR buffer. Conditions of the PCR amplification were as follows: 1 cycle for 2 minutes at 94ºC, 35 cycles of 15 s at 94ºC, 30 s at appropriated annealing temperature (Table 7), 45 s at 72ºC, followed by 10 min extension at 72ºC. SSR alleles were resolved on an ABI PRISM 3100 DNA (Applied Biosystems, Carlsbad, California, USA) genetic analyzer using GeneScan 3.7 (Applied Biosystems) software and precisely sized using GeneScan 500 LIZ molecular size standards with Genotyper 3.7 (Applied Biosystems) software.

Table 7. Primer sequences, expected size, annealing temperature, linkage group, and map position [35,39] of the twelve SSR markers used for molecular characterization of the materials studied.

SSR locus	Repeat	Primer sequence (5'-3')	Annealing temperature	Linkage group
CSM7	CT	F- CGACGATCACCTTGATAACG R- CCTAAATGCAGAGTTTCCAAAG	58.6	Unknown
CSM27	GA	F- TGTTTGGAGGTGAGGGAAAG R- TCCAACTCACCGGAAAAATC	60.0	3[a]
CSM30	CT	F- CACTGTTCCTGGTTGCTGTG R- TTTAGCTTTAGCCCATCTACCG	60.1	9[b]
CSM31	AG	F- CAACCGATATGCTCAGATGC R- GCCCTATGGTCATGTTTTGC	59.8	1[c]
CSM32	AG	F- TCGAAAGTACAGCGGAGAAAG R- GGGGGTTTGATTTTCATTTTC	59.6	4
CSM36	GA	F- CCTCAATGGCAGTAGGTCAGA R- GTTCTTTGAGCCTCCAGTGC	60.1	9[b]
CSM43	AG	F- ATTTTAACCCCGGGAAAATG R- ACCGCTTCTAGGTTTTGCAC	59.6	1[c]
CSM44	AG	F- CGTCGTTGTAACCCATCATC R- TTGCCAAATTCCTTGTGTTC	58.7	3[a]
CSM52	TC	F- CTTGGGTCACAAAAGGTTCC R- TCACCGAAAAAGATCCAACC	59.7	Unknown
CSM54	GA	F- ATGTGCCTCCATTCTGCAAG R- TGGGTGGGATGCTGAGTAAG	61.1	9[b]
CSM73	CT	F- TTCAACATAGCCTGGACCATT R- AATGCAGGGTTTGGACTTCA	60.0	Unknown
CSM78	CT	F- AGGGAGGAGCTCTCGTGTG R- CAATAACGTAGCTTAATTACTCCCAAG	60.2	10

[a]Markers CSM27 and CSM44 are positioned at 10.8 cM and 88.7 cM, respectively, in linkage group 3.

[b]Markers CSM30, CSM36 and CSM54 are positioned at 29.3 cM, 71.2 cM, and 0.0 cM, respectively, in linkage group 9.

[c]Markers CSM31 and CSM43 are positioned at 182.0 cM and 161.8 cM, respectively, in linkage group 1.

Data analysis

The range and mean values for the morphological traits for each of the groups of Chinese, Spanish, and Sri Lankan eggplants were calculated from the means of each accession. Analyses of variance were used to detect differences for the traits studied among the three groups of accessions. Kolmogorov-Smirnov and Bartlett tests were performed to test, respectively, the normality of data and homogeneity of variances among different origins. Principal components analysis (PCA) were performed for standardised morphological and agronomic data using pairwise Euclidean distances among accessions. Eigenvalues were calculated for each of the principal components, and relevant components were identified using the spree plot method [56]. Discriminant analysis was used to study the percentage of cases correctly classified, and the forward stepwise procedure was used for selecting the minimum subset of variables for discriminating among the three groups.

For the SSR data, pairwise genetic similarities were estimated using the Dice (Sorensen) similarity coefficient. Principal components analyses (PCA) were performed using the pairwise genetic similarities. Possible population structure associated to origin and likelihood of assignment of each accession to population was estimated using the Bayesian-based model procedure implemented in the software STRUCTURE v2.3.3 [57]. The analysis was carried out using a burning period of 10000 iterations. We tested a continuous series of *K*, from 1 to 10, in 10 independent runs [58]. No prior knowledge about the population of origin was introduced. The most informative *K* was identified using the statistic ΔK [59]. Subsequently, population structure was inferred for K=3 and using 50000 iterations. Genetic diversity was estimated with the total diversity (H_T) [46]. Total diversity was partitioned into diversity among origins (D_{ST}), and within origins (H_S). The relative magnitude of genetic differentiation among origins (G_{ST}) was calculated as the ratio D_{ST}/H_T [46]. The standarized G_{ST} (G'_{ST}), which standardizes the observed G_{ST} value with the maximum possible value that G_{ST} could obtain given the amount of observed diversity, was calculated as $(G_{ST}(1+H_T))/(1-H_S)$ [47]. For each accession, heterozygosity values for SSRs were calculated as $1-\sum(p_i^2)$, where p_i is the frequency of the ith allele [60]. Genetic distances and identities among

groups were calculated according to Nei [61]. Correlations between morphological and SSR distance matrices were investigated using a Mantel [62] test.

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    a. Estudio de diversidad de berenjena de variedades locales con distintas tipologías.

Genetic Diversity and Relationships in Local Varieties of Eggplant from Different Cultivar Groups as Assessed by Genomic SSR Markers

Santiago VILANOVA, Maria HURTADO, Adriana CARDONA, Mariola PLAZAS, Pietro GRAMAZIO, Francisco J. HERRAIZ, Isabel ANDÚJAR, Jaime PROHENS*

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain; jprohens@btc.upv.es

(*corresponding author)

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ABSTRACT.

Spain is a secondary center of diversity for eggplant (*Solanum melongena*). Spanish landraces of eggplant are normally classified in four cultivar groups: Round, Listada de Gandía, Semi-Long, and Long. We have used 19 genomic SSRs for the molecular characterization of 30 eggplant accessions corresponding to the four cultivar groups. Sixteen SSRs of which 15 were polymorphic could be amplified and 65 polymorphic alleles, with a range of two to 11 alleles/locus, were detected. The polymorphism information content (*PIC*) of SSR markers ranged from 0.07 to 0.77, with an average value of *PIC*=0.50. The mean observed heterozygosity (H_o) presented a very low value H_o=0.01, while the mean expected heterozygosity (H_e) had a value of H_e=0.57. Multivariate cluster analyses revealed that a considerable diversity exists within each of the cultivar groups. Listada de Gandía and Long cultivar groups were clearly separated from each other in different branches of phenogram. The principal coordinates analysis (PCoA) confirmed that each of the cultivar groups is genetically diverse and, with the exception of the Round group, they plot in different areas of the PCoA graph. Overall, the results indicate that Spanish eggplant landraces present a high degree of homozygosis, considerable intra-cultivar group diversity, and a certain degree of genetic differentiation. This information is of interest for selection and breeding of eggplant as well as for germplasm conservation.

Keywords: breeding, cultivar groups, fruit shape, landraces, multivariate analysis, *Solanum melongena*

INTRODUCTION

Eggplant (*Solanum melongena* L.) was domesticated in Southeast Asia (Meyer *et al.* 2012). From there it spread to other tropical and subtropical areas of the world, where selection and other microevolutive forces led to the development of a wide array of locally adapted landraces of eggplant and to the emergence of secondary centers of diversity (Cericola *et al.*, 2013; Hurtado *et al.*, 2012; Prohens *et al.*, 2005; Tümbilen *et al.*, 2011). Differentiation between eggplants of Southeast Asia on one side and those of the Middle East, Africa and Mediterranean region of Europe on the other have led to the recognition of two major groups of eggplant cultivar groups denominated, respectively, Oriental and Occidental eggplants (Cericola *et al.*, 2013; Vilanova *et al.*, 2012).

The Mediterranean region of Spain is considered as a secondary center of diversity (Hurtado *et al.*, 2012; Prohens *et al.*, 2005) in which four cultivar groups are normally recognized: Round, Listada de Gandía, Semi-Long, and Long (Hurtado *et al.*, 2013; Muñoz-Falcón *et al.*, 2011; Prohens *et al.*, 2005). These four cultivar groups are distinguished mostly by the fruit length/width ratio (i.e., the Round, Semi-Long, and Long cultivar groups) (Cericola *et al.*, 2013; Hurtado *et al.*, 2013; Prohens *et al.*, 2005; Tümbilen *et al.*, 2011), and also for the presence of purple stripes on a white background combined with a fruit shape intermediate between the Round and Semi-Long types in the case of Listada de Gandía (Hurtado *et al.*, 2013; Muñoz-Falcón *et al.*, 2011).

Development of new eggplant varieties addressing old and new breeding objectives (Barchi *et al.*, 2012; Lebeau *et al.*, 2013; Plazas *et al.*, 2013a; Sunseri *et al.*, 2003) requires of genetic diversity. Normally development of new commercial varieties, is based on intra-varietal group crossings, except in the case of the Semi-Long type, which can also be obtained by crossings between Round and Long types. Increasing the genetic base of new eggplant cultivars can be achieved

through the incorporation of local landraces in the commercial breeding programmes (Muñoz-Falcón *et al.*, 2009a). Therefore, the study of genetic diversity and relationships of collections of local varieties provides information of relevance for the breeding programmes.

Several molecular studies (Cericola *et al.*, 2013; Prohens *et al.*, 2005; Tümbilen *et al.*, 2011) have shown that eggplant cultivar groups are genetically diverse. In the case of Spanish varieties, Prohens *et al.* (2005) studied the AFLP diversity in a collection of eggplants from the Round, Listada de Gandía, Semi-Long, and Long types and found considerable intra-group diversity and genetic differentiation among groups. However, SSRs have proved as more powerful than AFLPs to study the relationships amongst closely related eggplant materials (Muñoz-Falcón *et al.*, 2009b). Furthermore, unlike AFLPs, which are dominant, SSRs are co-dominant, which allows determining the levels of observed heterozygosity. In particular, in eggplant, genomic SSRs have proved as much more informative than EST-SSRs (Muñoz-Falcón *et al.*, 2011). Therefore, in order to obtain information of interest for eggplant breeding and germplasm conservation, we studied the diversity, heterozygosity and relationships of 30 eggplant varieties from the Region of Valencia, situated in the Mediterranean region of Spain using genomic SSR markers.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 30 accessions corresponding to four cultivar groups (Round, Listada de Gandía, Semi-Long, and Long) were used (Tab. 1). All the accessions used, except the commercial selection Listada Clemente (Semillas Clemente, Vitoria, Spain), and the breeding line LF3-24 (INRA, France) are local landraces from the region of Valencia (Spain). A number of these accessions have been recently characterized by fruit shape (Hurtado et al., 2013), and phenolics content (Plazas *et al.*, 2013b).

For each accession, genomic DNA was extracted from 75 mg of young leaf tissue using the CTAB method (Doyle and Doyle, 1987). DNA concentration was quantified, after electrophoresis on a 1.0% agarose gel, using a Nanodrop ND-1000 (Nanodrop Technologies, Wilminton, Delaware, USA) spectrophotometer. Samples were adjusted to a DNA concentration of 20 ng/ml. The quality of DNA was evaluated through the 260/280 nm and 260/230 nm absorbance ratios (Sambrook *et al*., 1989).

Tab. 1. Accessions used in the present work, fruit weight and fruit length/width ratio (mean±SE), grouped according to their varietal group

Accession[a]	Code	Fruit weight	Fruit length/width ratio
		Round	
B-31	B31	225±17	1.09±0.05
B-32	B32	346±33	1.63±0.05
V-S-9	VS9	233±18	0.83±0.02
V-S-13	VS13	180±12	0.97±0.03
		Listada de Gandía	
07-A25-01	07A	330±26	2.31±0.08
IVIA-025	I025	330±16	1.70±0.06
IVIA-347	I347	300±26	1.35±0.06
Listada Clemente	LC	209±23	2.47±0.07
V-S-1	VS1	239±32	1.59±0.07
V-S-2	VS2	278±15	1.60±0.05
V-S-7	VS7	346±20	1.47±0.06
V-S-8	VS8	288±24	2.36±0.10
V-S-10	VS10	169±12	1.56±0.07
V-S-11	VS11	182±9	1.87±0.05
V-S-15	VS15	228±12	1.86±0.03
		Semi-long	
B-33	B33	171±8	2.93±0.07
B-36	B36	176±16	2.99±0.12
V-S-14	VS14	250±16	2.88±0.09
V-S-16	VS16	290±19	2.61±0.35
V-S-17	VS17	228±20	2.99±0.12
V-S-18	VS18	168±14	3.47±0.11
		Long	
B-35	B35	218±15	3.98±0.22
LF3-24	LF3	181±13	3.99±0.12
V-S-3	VS3	164±12	3.92±0.13
V-S-4	VS4	206±18	3.84±0.14
V-S-5	VS5	232±19	4.43±0.13
V-S-6	VS6	223±13	4.02±0.13
V-S-12	VS12	200±12	5.36±0.11
V-S-19	VS19	252±15	4.52±0.11
V-S-21	VS21	301±24	3.88±0.23

[a]All accessions are local landraces of the Region of Valencia (Spain), except Listada Clemente, which is a commercial selection (Semillas Clemente, Vitoria, Spain) of the Listada de Gandía type, and LF3-24, which is a breeding line from INRA (France).

Molecular characterization

Nineteen genomic highly polymorphic SSR markers developed by Vilanova *et al*. (2012) were used to screen the 30 eggplant accessions (Tab. 2). SSRs were tested following the M13-tail PCR method of Schuelke *et al*. (2000) to facilitate the incorporation of a dye label during the PCR. An M13-tailed forward primer was used in combination with a standard M13 primer dye-labeled with FAM, NED, PET, or VIC fluorophores at its 5'-end.

PCR amplifications were performed in a total volume of 12 ml with 20 ng of DNA, 1.5 mM $MgCl_2$, 0.05 mM of forward primer, 0.25 mM of reverse primer, 0.2 mM of fluorescent M-13 primer, 0.2 mM dNTPs, and 0.04 units of Taq DNA polymerase. Amplifications were carried out in an Eppendorf Mastercycler ep gradient S (Eppendorf AG, Hamburg, Germany) thermocycler. Two different protocols for amplification (T or TD) were used depending on the marker (Tab. 2). The T protocol consisted in an initial step at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 1 min at the appropriated annealing temperature (Tab. 2), 72°C for 2 min, and final 10 min extension at 72°C. The TD protocol consisted in an initial step at 94 °C for 2 min, 7 cycles of 94 °C for 15 s, 55-48°C (beginning with 55 °C in the first cycle and reducing 1°C in each of the subsequent cycles) for 30 s, 72°C for 45 s, and final 10 min extension at 72°C; subsequently, other 28 cycles are performed with the following conditions: 94 °C for 15 s, 48 °C for 30 s, 72°C for 45 s, and final 10 min extension at 72°C.

PCR products were separated on an ABI Prism 3100 Avant (Applied Biosystems, Foster City, California, USA) genetic analyzer using GeneScan 3.7 (Applied Biosystems) software. SSR alleles were precisely sized using GeneScan 500 Liz (Applied Biosystems) molecular size standards with Genotyper 3.7 software (Applied Biosystems).

Tab. 2. SSR markers used in the present study along with their repeat motif, amplification protocol, annealing temperature, and linkage group in which they map (Vilanova et al., 2010, 2012)

SSR locus	Repeat motif	Protocol[a]	Annealing temperature	Linkage group
CSM4	$(GA)_{15}$	TD	55-48	8
CSM7	$(CT)_{10}$	TD	55-48	Unknown
CSM12	$(AG)_{12}$	T	51	6
CSM27	$(GA)_{23}$	T	51	3
CSM29	$(AG)_{17}$	TD	55-48	12
CSM30	$(CT)_{20}$	T	51	9
CSM31	$(AG)_{28}$	T	51	1
CSM32	$(AG)_{23}$	T	51	4
CSM36	$(GA)_{27}$	T	51	9
CSM40	$(CT)_{45}$	T	51	4
CSM43	$(AG)_{14}$	TD	55-48	1
CSM44	$(AG)_{14}$	T	51	3
CSM45	$(AG)_{16}$	TD	55-48	5
CSM52	$(TC)_{12}$	T	50	Unknown
CSM54	$(GA)_{19}$	T	51	9
CSM57	$(CT)_{8}$	T	51	Unknown
CSM62	$(GA)_{27}$	T	51	Unknown
CSM74	$(GA)_{26}$	TD	55-48	12
CSM78	$(CT)_{19}$	TD	55-48	10

[a]See text for technical details of the amplification protocols.

Data analysis

For each SSR locus, the number of polymorphic alleles (N_a), frequency of the predominant allele (*f*), and effective number of alleles (N_e) was determined using the PowerMaker software (Liu and Muse, 2005). The polymorphism information content (*PIC*) was calculated as $PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{j=i+1}^{n} 2p_i^2 p_j^2$, where *n* is the total number of alleles detected, p_i is the frequency of the *i*th allele, and p_j is the frequency of the *j*th allele (Botstein *et al.*, 1980). Also, the observed heterozygosity (H_o), expected heterozygosity (H_e), calculated as $H_e = 1 - \sum_{i=1}^{n} p_i^2$, were determined. Nei and Li (1979) genetic similarities were calculated and a neighbor-joining phenogram was built using genetic distances with the PowerMaker software (Liu and Muse, 2005) and plotted using TreeView software (Page, 1996). Genetic distances were also used to graphically represent genetic relationships among accessions by principal coordinates analysis (PCoA) using GenAlEx 6.5 software (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

SSR characterization and diversity

The 19 SSR markers could be amplified, but for three of them (CSM36, CSM44, and CSM78) the PCR products could not be successfully resolved. The 16 remaining SSR markers were polymorphic with the exception of CSM32, for which only one allele was detected. A total of 65 alleles were detected for the 15 polymorphic SSR loci, with an average number of alleles/locus (N_a) of 4.33 and a range between two (CSM52) and 11 (CSM31) alleles (Tab. 3). The frequency of the predominant allele (f_p) ranged between 0.36 (CSM31) and 0.97 (CSM74), although with the exception of the latter SSR locus (CSM74) in all cases the f_p values have been below 0.65 (Tab. 3). The effective number of alleles (N_e) ranged between 1.07 (CSM74) and 4.85 (CSM31), and with the exception of CSM74 and CSM52, which are the only polymorphic markers with N_a=2 (and therefore N_e<2), all loci had N_e>2 (Tab. 3).

The average value for the *PIC* value of the SSR markers tested was of 0.50, but the *PIC* value of individual SSR markers ranged between 0.07 (CSM74) and 0.77 (CSM31) (Tab. 3). The mean value for the observed heterozygosity (H_o) was very low (H_o=0.01), corresponding to H_o=0.00 values for 13 out of the 15 polymorphic SSR loci and to low values (H_o=0.04) for the two remaining loci (CSM4 and CSM57). Conversely, the mean value for the expected heterozygosity (H_e) was much higher (H_e=0.57), with values for individual SSR loci ranging from 0.06 (CSM74) to 0.79 (CSM31).

Tab. 3. SSR polymorphic markers, number of alleles per locus (N_a), frequency of the predominant allele (*f*), number of effective alleles per locus (N_e), polymorphism information content (*PIC*), observed heterozygosity (H_o), and expected heterozygosity (H_e) in the studied collection of 30 eggplant accessions

SSR locus	N_a	*f*	N_e	*PIC*	H_o	H_e
CSM4	6	0.43	3.03	0.61	0.04	0.67
CSM7	3	0.55	2.21	0.46	0.00	0.55
CSM12	3	0.50	2.14	0.42	0.00	0.53
CSM27	4	0.57	2.47	0.54	0.00	0.59
CSM29	3	0.56	2.31	0.49	0.00	0.57
CSM30	3	0.54	2.12	0.42	0.00	0.53
CSM31	11	0.36	4.85	0.77	0.00	0.79
CSM40	6	0.39	3.32	0.65	0.00	0.70
CSM43	3	0.59	2.04	0.41	0.00	0.51
CSM45	3	0.52	2.14	0.42	0.00	0.53
CSM52	2	0.58	1.95	0.37	0.00	0.49
CSM54	5	0.37	3.74	0.69	0.00	0.73
CSM57	5	0.63	2.25	0.52	0.04	0.55
CSM62	6	0.38	3.38	0.65	0.00	0.70
CSM74	2	0.97	1.07	0.06	0.00	0.06
Mean	4.33	0.53	2.60	0.50	0.01	0.57

Multivariate analyses

The cluster analysis performed showed that the four cultivar groups present a considerable diversity (Fig. 1). However, a clear separation of the Long and Listada de Gandía accessions in different basal branches of the phenogram was observed (Fig. 1). All the Listada de Gandía accessions, except accession VS11, are clustered together in one of the sub-branches of the phenogram. Also, all Long accessions, except B35, are clustered together in another sub-branch in which there are also a Round (B32) and a Semi-Long (B33) accessions. Semi-Long, and particularly, the Round accessions, do not present this pattern of clustering, and are scattered in different branches of the phenogram.

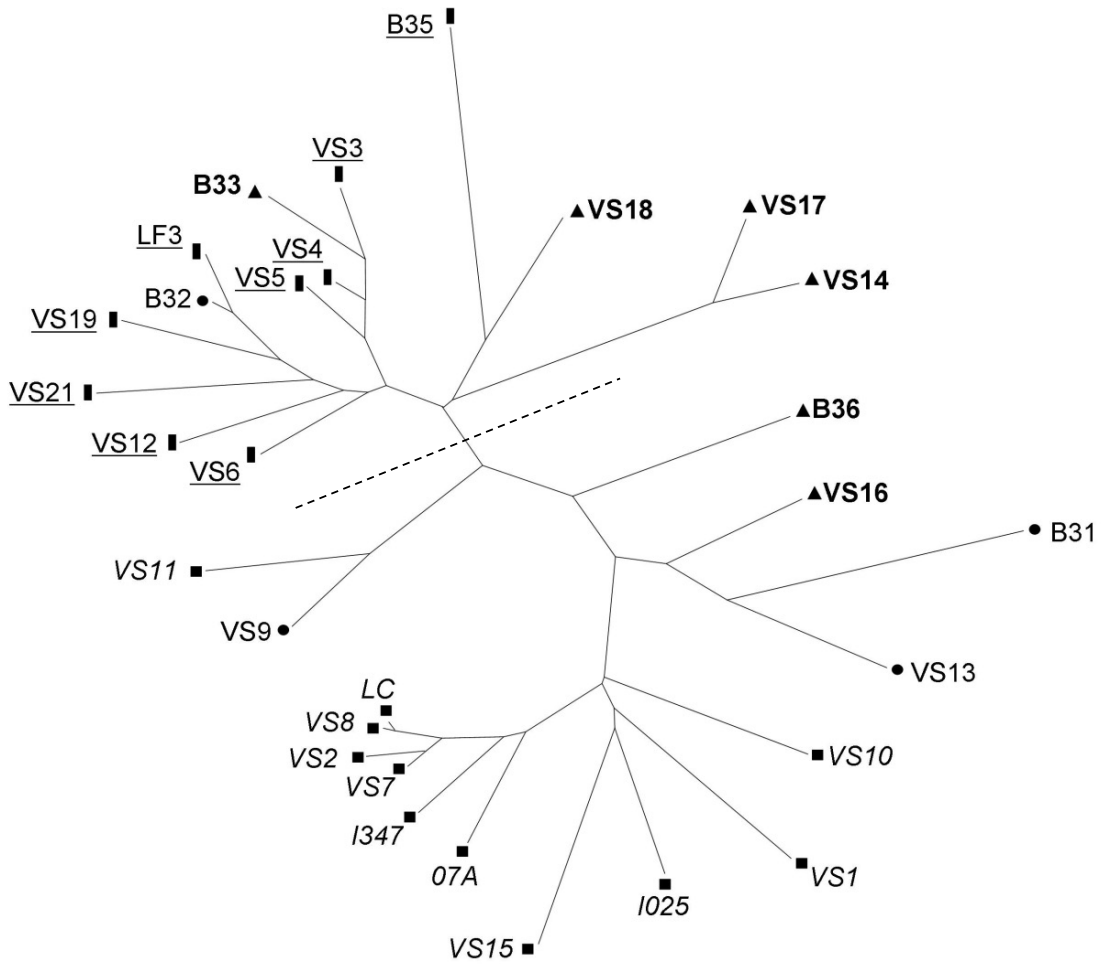

Fig. 1. Unrooted neighbor-joining phenogram of 30 eggplant accessions based on 15 polymorphic SSR markers. Phenetic relationships were derived from genetic distances (Nei and Li, 1979). The different groups of accessions are represented by different symbols and font types: Round (□ and normal font); Listada de Gandía (□ and italics font); Semi-long (□ and bold font); Long (□ and underlined font). The dashed line separates the phenogram branches that contain all Long accessions (above) and all the Listada de Gandía accessions (below)

When considering the principal coordinates analysis (PCoA), the first and second principal coordinates account, respectively, for 47 % and 13 % of the total variation. The representation of accessions in the PCoA graph shows that the cultivar groups are diverse, although each of them plot in different parts of the graph (Fig. 2). In this respect, all Listada de Gandía accessions present positive values of the first coordinate and either low positive or negative values of the second coordinate and plot together, not being intermingled with other cultivar groups. Only the odd VS11 accession plots closer to a Round accession (VS9) than to the other Listada de Gandía accessions. All Long accessions present negative values for the first coordinate and either low positive or negative values for the second coordinate (Fig. 2). Also, all Semi-Long accessions plot in the same area of the PCoA graph, with either low positive or negative values for the first coordinate and positive values for the second coordinate. Conversely to the other cultivar groups, the four Round accessions are scattered in different parts of the PCoA graph, with two accessions presenting positive values for both the first and second coordinates and situated close to Semi-Long accessions, and two accessions with negative values for both coordinates, with one accession (B32) close to Long accessions and the other (VS9) close to the odd VS11 Listada de Gandía accession (Fig. 2).

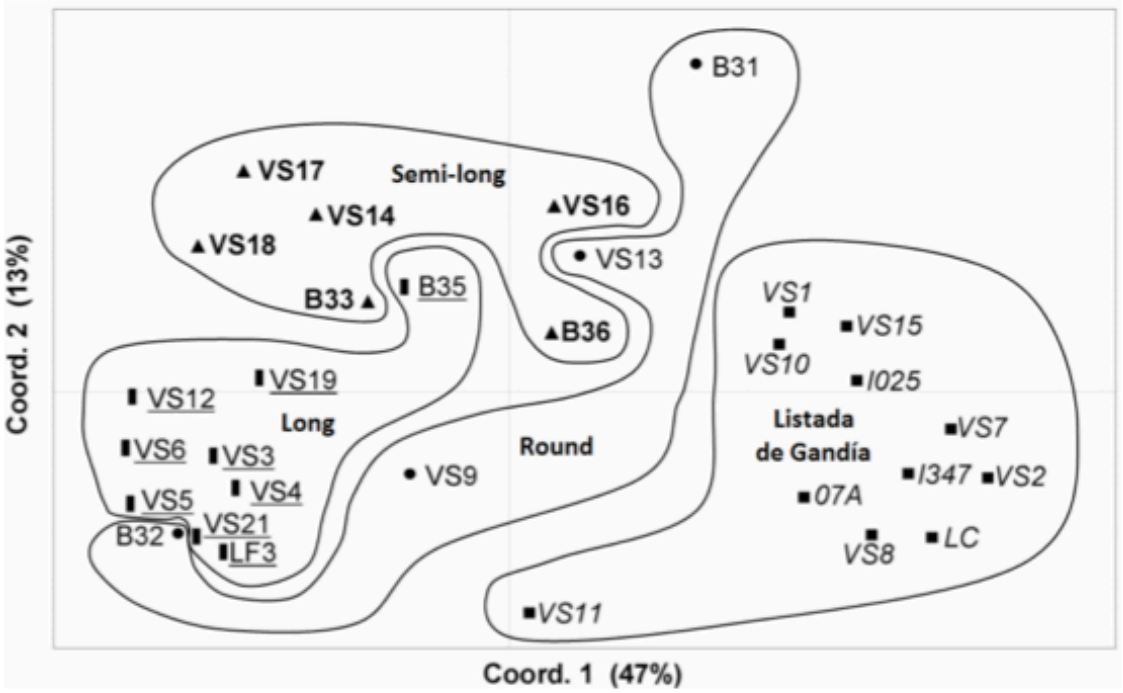

Fig. 2. Relationships between the 30 eggplant accessions based on 15 polymorphic SSRs according to the first and second principal coordinates obtained from a principal coordinates analysis (PCoA) (47% and 13% of the total variation accounted by the first and second coordinates, respectively). The different groups of accessions are represented by different symbols and font types: Round (▲ and normal font); Listada de Gandía (■ and italics font); Semi-long (▲ and bold font); long (▮ and underlined font)

DISCUSSION

Genomic SSRs that previously proved to be highly polymorphic in eggplant (Vilanova *et al.*, 2012) have been found to be of great value for evaluating the genetic diversity and relationships in a collection of eggplants from different cultivar groups. The screening of this collection has revealed a high degree of polymorphism for the SSR markers tested, confirming that the Mediterranean region is a secondary center of diversity for eggplant (Cericola *et al.*, 2013; Hurtado *et al.*, 2012; Prohens *et al.*, 2005). Muñoz-Falcón *et al.* (2011) have shown that in eggplant genomic SSR markers are more polymorphic than EST-SSRs. Our results seem to confirm these results and that the genomic SSRs developed from an enriched genomic library by Vilanova *et al.* (2012) are particularly useful for the study of relationships in germplasm collections.

The same SSRs used by us were tested by Vilanova *et al.* (2012) in a collection of 22 *S. melongena* from different origins, including Occidental and Oriental types of eggplant. For the 15 polymorphic loci used in our study these authors found an average number of alleles per locus (6.47) higher than ours (4.33). Hurtado *et al.* (2012) in a study of 52 accessions from China, Spain, and Sri Lanka shared seven of the SSR markers that have been polymorphic in our study and also found a higher average number of alleles per locus (8.86) than we found for these seven SSR markers in our study (4.43). Also, Cericola et al. (2013) in a wide study of 238 eggplant materials from different origins shared four SSRs with our study. For these four SSRs, Cericola et al. (2013) found on average 7 alleles/locus, while these same alleles in our collection presented a mean of 5.5 alleles/locus. The higher diversity found by Vilanova *et al.* (2012), Hurtado *et al.* (2012), and Cericola *et al.* (2013) probably is a consequence of the fact that our collection comes from a single geographic area (Region of Valencia, in the Mediterranean coast of Spain). In fact, when considering only the 14 Spanish accessions used in the Hurtado *et al.* (2012) study the number of alleles per locus (3.86) is similar to ours (4.43). Amazingly, for the CSM32 loci, which has been monomorphic in our collection, Hurtado *et al.* (2012) and Vilanova *et al.* (2012) found 12 and 8 alleles, respectively, indicating that selection or genetic drift in the materials of the collection we have evaluated have led to fixation of one specific allele.

SSR loci are highly informative when they present a great number of alleles (N_a), and have low values for the predominant allele frequency (f_p), i.e., with f_p values close to the theoretical minimum of $f_p=1/N_a$ (Botstein *et al.*, 1980; Powell *et al.*, 1996). In our case, some markers have presented a high number of alleles and presented relatively low values for the predominant allele (f_p), resulting in high values for the number of effective alleles (N_e) polymorphic information content (*PIC*), and expected heterozygosity (H_e) confirming that highly relevant information on the diversity and relationships of eggplant can be obtained with a relatively low number of genomic SSR markers (Hurtado *et al.*, 2012; Muñoz-Falcón *et al.*, 2009, 2011).

The low values of observed heterozigosity (H_o) were expected as eggplant is fundamentally autogamous (Pessarakli and Dris, 2004), and the materials

used are non-hybrid. In this respect, Muñoz-Falcón (2009) also found very low values for H_o in eggplant landraces (H_o<0.03), but substantially higher values (H_o=0.38) for commercial F1 hybrids. Cericola *et al*. (2013) also found that most eggplant landraces used in their study had low heterozygosity values, and only 38 out of 238 materials had H_o>0.10. The high level of homozygosity in eggplant landraces shows that pure lines can easily be derived by individual selection from these materials.

The multivariate analysis with SSR markers shows that, as already found by Prohens *et al.* (2005) and Tümbilen *et al.* (2011) with AFLP markers, and by Cericola et al. (2013) that considerable genetic diversity exists within each of the cultivar groups studied, which were mostly distinguished by the fruit shape. Also, Muñoz-Falcón *et al.* (2011) using SSR markers also found that one of the groups studied here (Listada de Gandía) was genetically diverse. The existence of intra-varietal group genetic diversity has important implications for selection and breeding as it indicates that important genetic advances can be obtained with intra-cultivar group selection, and also that hybrids heterotic for yield may be obtained when crossing genetically different accessions of the same cultivar group (Rodríguez-Burruezo *et al.*, 2008).

Here we have found that a certain degree of genetic differentiation exists among the four cultivar groups. This is in agreement with previous reports (Cericola *et al.*, 2013; Prohens *et al.*, 2005; Tümbilen et al., 2011), in which cultivar groups were found to present a moderate degree of differentiation. However, the Round group accessions were intermingled in the multivariate analyses with accessions of other groups indicating that this cultivar group is genetically highly variable. Wild relatives of eggplant have round, ovoid, or obovoid fruit shape (Knapp *et al.*, 2013), indicating that the fruit shape characteristic of the Round cultivar group (Hurtado *et al.*, 2013) is an ancestral trait probably present in the first domesticated eggplants. As occurred in tomato (Brewer *et al.*, 2007), artificial selection of mutations affecting fruit shape has led to other fruit shapes, like those characteristic of the Listada de Gandía, Semi-Long and Long types, which may have undergone genetic bottlenecks resulting in a lower diversity and higher degree of genetic differentiation. However, further studies should be undertaken to confirm this hypothesis.

In our study, the Long and Listada de Gandía groups are clearly differentiated at the genetic level. In this respect, Muñoz-Falcón *et al.* (2011) and Prohens et al. (2005) found that the Listada de Gandía cultivar group was genetically clearly differentiated from the rest of eggplant materials of other types. In our study, only one of the Listada de Gandía accessions used (VS11) seems to be genetically closer to one Round accession (VS9) that to other Listada de Gandía materials. This accession is characterized by an odd shape for the Listada de Gandía type, as it is ovoid instead of having the normal obovoid shape characteristic of the Listada de Gandía materials (Hurtado *et al.*, 2013) and may have been derived from introgression of the striped trait characteristic of Listada de Gandía into a different genetic background.

CONCLUSIONS

A reduced number of selected genomic SSR markers have allowed detecting considerable genetic variation in a collection of eggplants including different cultivar types. These markers have also been useful for studying relationships among four cultivar groups differentiated by fruit shape, showing that they are highly homozygous, have considerable intra-varietal group diversity, and present a certain degree of genetic differentiation. In particular the Listada de Gandía and Long varietal groups are clearly differentiated from each other. This information is of interest for the genetic improvement and conservation of genetic resources of eggplant.

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Phenomics of fruit shape in eggplant (*Solanum melongena* L.) using Tomato Analyzer software

Maria Hurtado, Santiago Vilanova, Mariola Plazas, Pietro Gramazio, F. Javier Herraiz, Isabel Andújar, Jaime Prohens*

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

*Corresponding author.

Tel.: +34 963879424; fax: +34 963879422

E-mail address: jprohens@btc.upv.es (J. Prohens)

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ABSTRACDetailed characterization of fruit shape in eggplant (*Solanum melongena*) is important for horticulturists and breeders. In fact, commercial varieties are classified according to their fruit shape. However, traditional morphological descriptors provide limited information on this complex attribute. Recently, a software tool (Tomato Analyzer) for the processing of scanned images of sections of tomato fruits has been developed. Tomato Analyzer is adequate for phenomics studies of fruit shape, as it provides quantitative and objective data for a large number of fruit morphology traits. We used Tomato Analyzer for evaluating fruit shape in a collection of 21 accessions of eggplant from four varietal groups (Round, Listada de Gandía, Semi-long, and Long). For each accession we evaluated 20 fruits, for which we measured fruit weight, length, and width (manually), and 23 fruit shape parameters using the Tomato Analyzer. Significant differences among accessions have been found for all traits, except for Shoulders Height. For many traits, high values for the coefficient of genotypic variation (CV_G) and broad-sense heritability (H^2) have been obtained, indicating that selection will be efficient. Significant differences have also been found among varietal groups for 20 out of the 26 traits, and for six of them each of the four varietal groups differed significantly from the others. Multivariate principal components analysis (PCA) shows that accessions of each of the four varietal groups plot together and in separate areas of the PCA graph, with the exception of some overlapping between Round and Listada de Gandía accessions. Discriminant analysis resulted in 57.14% of the individual fruits being correctly assigned to

their accession, and of those incorrectly classified, 21.67% were correctly classified to their varietal group. The results obtained show that the Tomato Analyzer image software tool is of great utility for phenomics studies of fruit shape in eggplant, which is of interest for characterization of germplasm and cultivars, and for selection and breeding.

INTRODUCTION

Fruit shape is one of the most relevant attributes in eggplant (*Solanum melongena* L.) characterization and breeding (Daunay, 2008). In fact, classification of eggplant cultivars is fundamentally performed on the basis of fruit shape (Nunome et al., 2001; Daunay, 2008; Marín, 2013). Commercially, eggplant cultivars are commonly classified in three major varietal groups: Round, Semi-long and Long (Nunome et al., 2001; Prohens et al., 2005; Tümbilen et al., 2011; Marín, 2013). In Spain, Listada de Gandía, which corresponds to striped eggplants very popular in the Mediterranean coast of Spain (Muñoz-Falcón et al., 2008), is also commonly considered as a differentiated varietal group (Prohens et al., 2005). Each of these groups encompasses a considerable genetic diversity, as assessed by molecular markers (Prohens et al., 2005; Muñoz-Falcón et al., 2011; Tümbilen et al., 2011).

As occurs with other Solanaceae crops domesticated for their fruits, like tomato (*Solanum lycopersicum* L.) or pepper (*Capsicum* spp.) (Ben Chaim et al., 2001; Paran and van der Knaap, 2007; Rodríguez et al., 2011), a wide diversity exists for fruit shape in eggplant (Nunome et al., 2001; Daunay, 2008; Wang et al., 2008), even within varietal group (Prohens et al., 2005; Muñoz-Falcón et al., 2008). Small differences in eggplant fruit shape are relevant for breeders and may be determinant for the success or failure of a commercial cultivar. Several studies performed to analyze the inheritance of fruit shape in eggplant have allowed the discovery of a number of QTLs for fruit shape in eggplant (Nunome et al., 2001; Doganlar et al., 2002). However, a large part of the genetic factors responsible for natural variation for fruit shape in eggplant remain to be discovered. In this respect, studies of the diversity and genetics of fruit shape in eggplant lags behind tomato and pepper, in which many studies have been made (Paran and van der Knaap, 2007; Gonzalo and van der Knaap, 2008; Rodríguez et al., 2011; Tsaballa et al., 2011). Further advances in this field in eggplant require phenomics tools that provide an accurate and detailed description of fruit shape.

Studies of fruit shape characterization in eggplant are generally based in simple traits measured manually, like fruit length or width (IBPGR, 1990; Nunome et al., 2001; Doganlar et al., 2002; Prohens et al., 2005). Although these traits provide relevant information and are easily measurable by non-specialized staff, they do not allow a precise characterization of fruit shape, which is necessary for different objectives, including the detailed description of germplasm accessions and cultivars, and selection and breeding (Costa et al., 2011). Although several tools based on machine vision systems have been developed for studying fruit shape of different fruits, including eggplant (Saito et al., 2003), they are mostly used for grading and detection of abnormal shapes in the industry (Ruiz-Altisent et al., 2010) and their implementation in horticulture and breeding laboratories generally is not feasible.

Recently, a free software for the analysis of fruit shape of tomato fruits (Tomato Analyzer) has been developed (Brewer et al., 2006; Gonzalo and van der Knaap, 2008; Rodríguez et al., 2010a, 2010b). Tomato Analyzer allows phenomics studies of fruit shape as it allows scoring a large number of fruit shape traits from scanned images of fruit sections. Although this software was initially devised for the morphological and morphometric analysis of tomato fruits, for which it has been proved of great value (Gonzalo and van der Knaap, 2008; Gonzalo et al., 2009; Mazzucato et al., 2010; Rodríguez et al., 2011), it has also been successfully used for studying the fruit shape of other fruits, like papaya (*Carica papaya* L.) (Blas et al., 2012). We have also used the Tomato Analyzer software to study fruit shape in interspecific families derived from the crossing between *S. melongena* and its relative *S. aethiopicum* L. (Prohens et al., 2012), and found considerable differences between parents and interspecific hybrids for many traits. However, up to now, no reports exist of the use of phenomic tools like Tomato Analyzer to study fruit shape variation in collections of eggplant materials.

In this study, we characterize and classify according to fruit shape (as evaluated by the software tool Tomato Analyzer), a collection of 21 eggplant accessions from different varietal groups. We have studied the differences among accessions and varietal groups and used multivariate principal components and discriminant analyses to group and classify accessions for fruit shape. This is the first fruit shape phenomics

study in a germplasm collection of eggplants and is aimed at providing relevant information for the characterization, classification, selection and breeding for fruit shape in eggplant.

MATERIAL AND METHODS

Plant material

Fruits from a total of 21 eggplant accessions, of which 19 correspond to local varieties from the provinces of Alicante and Valencia (Region of Valencia, Spain), one of a commercial variety of the Listada de Gandía type (Listada Clemente), and a breeding line from the Institut National de la Recherche Agronomique (LF3-24) were used for the present study. Both Listada Clemente and LF3-24 are commonly used as controls in eggplant germplasm characterizations (Prohens et al., 2005; Muñoz-Falcón et al., 2008). The collection of accessions used included materials corresponding to different shape types, as recognized in commercial classifications in Spain (Prohens et al., 2005; Marín, 2013): Round (4 accessions), Listada de Gandía (7 accessions), Semi-long (4 accessions), and Long (6 accessions) and represent the diversity for fruit shape in the "occidental" type of eggplants (Hurtado et al., 2012). Previous molecular studies (Prohens et al., 2005; Muñoz-Falcón et al., 2011), as well as our unpublished results, reveal that all the accessions used here are genetically distinct. Plants from which the fruits were harvested were grown in the open field during the summer season of 2011 at the Agricultural Experimental Farm of Carcaixent (Valencia, Spain) using the standard horticultural practices (Table 1.)

Table 1 Accessions used for the study of fruit shape variation in eggplant grouped according to the different varietal groups, and their geographical origins.

Accession	Code	Origin
		Round
B-31	B31	Carcaixent, Valencia, Spain
V-S-9	V9	La Aparecida (Orihuela), Alicante, Spain
V-S-10	V10	La Aparecida (Orihuela), Alicante, Spain
V-S-13	V13	San Fulgencio, Alicante, Spain
		Listada de Gandía
07-A25-01	07A	Vilareal, Castellón, Spain
IVIA-025	I025	Moncada, Valencia, Spain
IVIA-347	I347	Foios, Valencia, Spain
Listada Clemente	LC	Semillas Clemente, Spain (commercial cultivar)
V-S-1	V1	Alzira, Valencia, Spain
V-S-7	V7	Xeraco, Valencia, Spain
V-S-11	V11	Dolores, Alicante, Spain
		Semi-long
V-S-14	V14	Rafal, Alicante, Spain
V-S-16	V16	Novelda, Alicante, Spain
V-S-17	V17	Elx, Alicante, Spain
V-S-18	V18	Elda, Alicante, Spain
		Long
LF3-24	LF3	INRA, France (breeding line)
V-S-3	V3	Gandía, Valencia, Spain
V-S-4	V4	Gandía, Valencia, Spain
V-S-6	V6	Xeraco, Valencia, Spain
V-S-12	V12	Benijófar, Alicante, Spain
V-S-19	V19	Mutxamel, Alicante, Spain

Fruit shape characterization

For each accession, 20 fruits were harvested at the commercially ripe stage (evaluated by the size, and color and glossiness of the fruit skin). Fruits were brought to the laboratory and washed. Then, they were weighted, and the fruit length (curved length) and fruit width (maximum width) were measured with a ruler following the common protocols for manual characterization of eggplant (IBPGR, 1990; Doganlar et al., 2002). Subsequently, fruits were cut longitudinally, scanned with a HP Scanjet G4010 photo scanner (Hewlett-Packard, Palo Alto, CA, USA) at a resolution of 300 dpi (Figure 1) and subjected to morphometric analysis with Tomato Analyzer version 3 software (Rodríguez et al., 2010a).

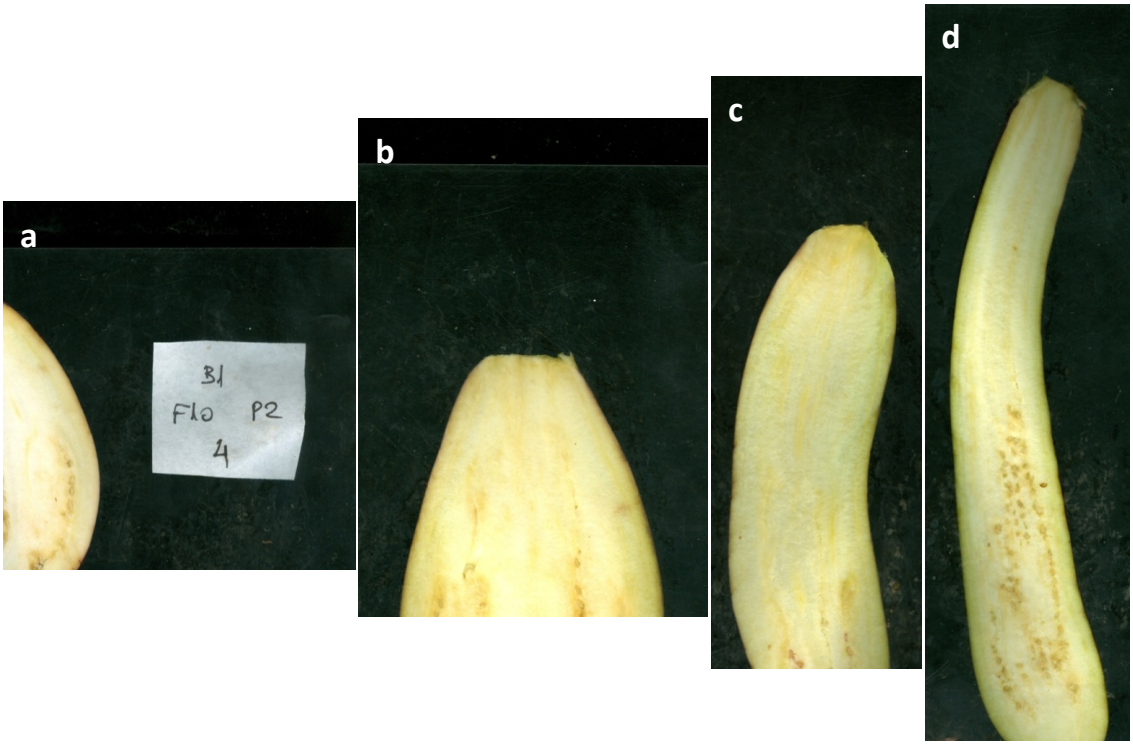

Fig. 1. Scanned longitudinal sections of eggplant fruits of representative fruits of the Round (a), Listada de Gandía (b), Semi-long (c), and Long (d) varietal groups. Scanned images like these ones were used for the measurement of fruit shape traits by the Tomato Analyzer software.

A total of 23 fruit shape traits, corresponding to basic measurements (6), fruit shape index (2), blockiness (3), homogeneity (3), proximal fruit end shape (1), asymmetry (2), internal eccentricity (5), and slenderness (1), were evaluated with Tomato Analyzer (Table 2). For blockiness traits, the default settings, given by the software (0.1 and 0.9 for the upper and lower positions, respectively) were used (Rodríguez et al., 2010a). Full details on the description of each of the traits can be found elsewhere (Brewer et al., 2006; Gonzalo and van der Knaap, 2008; Rodríguez et al., 2010a, 2010b), with the exception of the trait Slender, included within the slenderness category. Slender is the new denomination we use here for Lobedness Degree (Rodríguez et al., 2010a), which was originally devised for measuring the lobedness of the latitudinal section, and which we use as a measure of the slenderness of the longitudinal fruit section.

Table 2. Fruit traits studied and their description. All traits, except those under the category *Manual measurements*, were measured with Tomato Analyzer software. Further details for the measurement of fruit shape traits with Tomato Analyzer can be obtained from Brewer et al. (2006), Gonzalo and van der Knaap (2008), and Rodríguez et al. (2010a, 2010b).

Trait	Code	Units / Description
Manual measurements		
Weight	Weight	Fruit weight (g)
Fruit Width	Width	Fruit width (cm)
Fruit Length	Length	Fruit length (cm)
Basic measurements		
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 (cm)
Fruit shape index		
Fruit shape index external 1	Fruit_Shape1	The ratio of the Maximum Height to Maximum Width.
Fruit shape index external 2	Fruit_Shape2	The ratio of Height Mid-width to Width Mid-height
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		
Circular	Circular	Fitting precision (r^2) of the actual shape to a circle; larger values indicate that the fruit is more circular
Ellipsoid	Ellipsoid	Fitting precision (r^2) of the actual shape to an ellipse; larger values indicate that the fruit is more ellipsoid
Rectangular	Rectangular	The ratio of the rectangle bounding the fruit to the rectangle bounded by the fruit; larger values indicate that the fruit is more rectangular
Proximal fruit end shape		
Shoulder Height	Sh_Height	The ratio of the average height of the shoulder points above the proximal end point to Maximum Height.
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.
Internal Eccentricity		
Eccentricity	Eccentricity	The ratio of the height of the internal ellipse to the Maximum Height.
Proximal Eccentricity	P_Eccentricity	The ratio of the height of the internal ellipse to the distance between the bottom of the ellipse and the top of the fruit.
Distal	D_Eccentricity	The ratio of the height of the internal ellipse to the distance between the top of the ellipse and the bottom of the

Eccentricity		fruit.
Fruit Shape Index Internal	F_S_Index_I	The ratio of the internal ellipse's height to its width.
Eccentricity Area Index	Ecc_Area_Index	The ratio of the area of the fruit outside the ellipse to the total area of the fruit.
***Slenderness*[a]**		
Slender[a]	Slender	The standard deviation of distances from the center of weight to the perimeter, multiplied by 100

[a]*Slenderness* and Slender are, respectively, new denominations for the original *Latitudinal section* and Lobedness Degree, and which were developed for taking measures of the latitudinal section of the fruit (Brewer et al., 2006, Gonzalo and van der Knaap, 2008, and Rodríguez et al. 2010a, 2010b). Here we use them as a measure of the slenderness of the longitudinal fruit section.

Data analyses

For each trait, data of individual fruits were subjected to analyses of variance (ANOVA) in order to detect significant differences among accessions. The total sum of squares was partitioned into sums of squares for accession and residual effects and expressed in percentage over the total sum of squares. The coefficients of phenotypic variation (CV_P) and genotypic variation (CV_G) for each trait were estimated from the mean value and from phenotypic or genotypic variance estimates obtained from the ANOVAs, and expressed in percentage (Wricke and Weber, 1986). Broad-sense heritability (H^2) was estimated from phenotypic and genotypic variance estimates (Wricke and Weber, 1986). Mean values for each accession were used to perform additional ANOVAs in order to detect differences for the traits studied among varietal groups. The average number, standard deviation, and range for the number of significant differences (according to the Student-Newman-Keuls multiple range test) between pairs of accessions within and between varietal groups was calculated. Principal components analysis (PCA) was performed for standardized values of fruit shape traits using pairwise Euclidean distances among accession means. Discriminant analysis was used to study the percentage of individual fruits correctly classified to the actual accession. All statistics were conducted using specific software (Statgraphics Centurion XVI, StatPoint Technologies, Warrenton, VA, USA).

RESULTS

Differences between accessions

Highly significant differences (P<0.001) were found among the 21 eggplant accessions evaluated for 24 out of the 26 fruit shape traits studied (Table 3). For P_Eccentricity significant differences were also found, although at a lower level of significance (P<0.05). For Sh_Height no significant differences were detected. For 17 out of the 26 traits the sums of squares for accession were higher than for the residual, and for four traits (F_S_Index_I, Fruit_Shape2, Slender, and Circular) the sum of squares for accession was higher than 80% (Table 3).

Mean values for the manually measured traits Width and Length (6.71 cm and 15.6 cm, respectively) were greater than the Tomato Analyzer measured Max_Width and Max_Height (4.99 cm and 10.5 cm, respectively). For two traits (Sh_Height and Ovoid), the mean values were very low (0.005), resulting from the fact that fruits of many accessions presented values of 0. The coefficient of phenotypic variation (CV_P) ranged between 5.4% for P_Eccentricity and 495.6% for Ovoid (Table 3). The extremely high values of CV_P for Ovoid and Sh_Height result from the low mean values for these traits (Table 3). For the rest of traits, with the exception of the three internal eccentricity traits Eccentricity, P_Eccentricity, and D_Eccentricity, which presented values below 5.0%, the CV_P values were situated in the range between 12.1% (Circular) and 74.4% (Slender). Regarding the coefficient of genotypic variation (CV_G), the lowest and highest values again corresponded, respectively, to P_Eccentricity (1.1%) and Ovoid (362.2%). Here, too, Eccentricity, P_Eccentricity, and D_Eccentricity, presented low values (below 2.5%). When excluding these three latter traits and Ovoid, the CV_G values were comprised between 7.9% (Rectangular) and 67.8% (Lobedness). Broad-sense heritability (H^2) presented a wide range of variation, from 0.01 for Sh_Height to 0.85 for Fruit_Shape2 and F_S_Index_I. A total of 10 traits presented H^2 values above 0.7 (Length, Width_MH, Max_Height, Fruit_Shape1, Fruit_Shape2, D_Blockiness, Ellipsoid, Circular, F_S_Index_I, and Slender), while six traits had H^2 values below 0.3 (Weight, Area, Sh_Height, Eccentricity, P_Eccentricity, and D_Eccentricity).

Table 3 Percentage of the total sum of squares for the effects of accession and residual, and mean, coefficient of phenotypic variation (CV_P), coefficient of genotypic variation (CV_G), and broad-sense heritability (H^2) for the fruit traits evaluated in a collection of 21 eggplant accessions.

	Sum of squares (%)					
Trait	Accession[a]	Residual	Mean	CV_P (%)	CV_G (%)	H^2
Manual measurements						
Weight (g)	30.4***	69.6	232	40.2	21.2	0.28
Width (cm)	66.6***	33.4	6.71	28.0	22.7	0.66
Length (cm)	73.9***	26.1	15.6	37.0	31.7	0.73
Basic measurements						
Perimeter (cm)	59.4***	40.6	28.3	26.9	20.6	0.58
Area (cm^2)	31.6***	68.4	36.8	35.8	19.4	0.29
Width_MH (cm)	72.1***	27.9	4.30	32.9	27.8	0.72
Max_Width (cm)	45.6***	54.4	4.99	23.7	15.7	0.44
Height_MW (cm)	66.3***	33.7	10.1	37.5	30.4	0.66
Max_Height (cm)	70.8***	29.2	10.5	36.4	30.6	0.70
Fruit shape index						
Fruit_Shape1	77.9***	22.1	2.27	46.9	41.3	0.78
Fruit_Shape2	84.7***	15.3	2.75	59.8	55.0	0.85
Blockiness						
P_Blockiness	55.0***	45.0	0.66	21.1	15.5	0.54
D_Blockiness	74.4***	25.6	0.89	22.7	19.5	0.74
Triangle	36.6***	63.4	0.76	19.5	11.4	0.34
Homogeneity						
Circular	80.7***	19.3	0.62	55.4	49.7	0.81
Ellipsoid	63.8***	36.2	0.82	12.1	10.1	0.70
Rectangular	28.8***	71.2	0.47	15.4	7.9	0.26
Proximal fruit end shape						
Sh_Height	5.9[ns]	94.1	0.005	367.8	40.7	0.01
Asymmetry						
Obovoid	65.7***	34.3	0.30	43.9	35.4	0.65
Ovoid	54.5***	45.5	0.005	495.6	362.2	0.53
Internal Eccentricity						
Eccentricity	15.6***	84.4	0.77	5.8	2.1	0.13
P_Eccentricity	8.9*	91.2	0.90	5.4	1.1	0.04
D_Eccentricity	22.4***	77.6	0.90	5.5	2.4	0.19

F_S_Index_I	84.8***	15.2	2.76	59.9	55.1	0.85
Ecc_Area_Index	64.7***	35.3	0.44	13.4	10.8	0.64
Slenderness						
Slender	83.2***	16.8	24.6	74.4	67.8	0.83

[a] ***, **, *, [ns] indicate, respectively, significant at P<0.001, P<0.01, P<0.05, and non-significant.

3.2. Differences between varietal groups

Important differences in fruit shape traits were also found between the four varietal groups considered, with highly significant ($P<0.001$) differences for 17 traits, and significant ($P<0.01$ or $P<0.05$) differences for three other traits (). Only six out of the 26 traits studied (Weight, Triangle, Sh_Height, Ovoid, Eccentricity, and P_Eccentricity) did not present significant differences between varietal groups. For six other traits (Length, Perimeter, Height_MW, Max_Height, Fruit_Shape1, and Slender) each of the varietal groups differed significantly from all the others. For two of these traits (Length and Max_Height) the ranges of variation of the four groups did not overlap (Table 4).

For the 26 fruit shape traits measured, the average for the number of significant differences between pairs of accessions within a group ranged between 6.9 for Long accessions and 9.7 for Semi-Long accessions. However, in all of the four varietal groups there are some pairs of accessions for which no significant differences were found for any traits (V9 and V13 for the Round group; I025, I347, and V1 for the Listada de Gandía group; V14 and V17 for the Semi-long group; and, V4 and V6 for the Long group). When considering the average number of differences among groups, they range between 9.7 for Round vs. Listada de Gandía and 18.5 for Round vs. Long. The number of significant differences for the traits studied between accessions corresponding to different varietal groups ranges between 2 (V18 from the Semi-long group and V19 from the Long group) and 21 (three pairs involving Round group accessions on one hand and Long group accessions on the other, and three pairs involving Listada de Gandía group accessions on one hand and Long group accessions on the other).

Table 4. Means and range (in italics) for the fruit traits measured in the four eggplant varietal groups studied and *F*-ratio for significance of differences between varietal groups means.

Traits	Round (n=4)		Listada de Gandía (n=7)		Semi-long (n=4)		Long (n=6)		*F*-ratio[a]
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Manual measurements									
Weight (g)[b]	202 a	*168-233*	271 a	*182-346*	234 a	*168-290*	207 a	*164-252*	2.75^{ns}
Width (cm)	8.03 b	*6.72-9.42*	7.71 b	*6.02-9.24*	6.21 a	*5.08-7.73*	5.01 a	*4.71-5.21*	11.97***
Length (cm)	8.6 a	*6.8-10.2*	13.5 b	*11.6-17.4*	17.6 c	*17.5-17.9*	21.2 d	*18.9-25.3*	38.16***
Basic measurements									
Perimeter (cm)	20.5 a	*18.3-24.3*	25.6 b	*22.0-29.2*	30.6 c	*29.3-32.6*	35.0 d	*32.1-40.5*	29.26***
Area (cm^2)	26.4 a	*20.9-35.3*	38.2 b	*27.9-50.8*	39.8 b	*34.8-46.1*	40.3 b	*37.0-44.6*	5.35**
Width_MH (cm)	5.32 c	*4.59-6.05*	5.14 c	*3.71-6.54*	3.91 b	*3.04-4.76*	2.90 a	*2.64-3.12*	15.58***
Max_Width (cm)	5.49 b	*4.75-6.42*	5.39 b	*4.09-6.78*	4.77 ab	*4.42-5.79*	4.32 a	*4.12-4.68*	3.54*
Height_MW (cm)	5.8 a	*4.5-6.9*	8.8 b	*7.4-10.8*	11.3 c	*11.1-11.6*	13.6 d	*11.5-16.0*	38.63***
Max_Height (cm)	6.1 a	*4.8-7.3*	9.0 b	*7.8-11.1*	11.8 c	*11.6-12.0*	14.3 d	*12.7-17.0*	41.81***
Fruit shape index									
Fruit_Shape1	1.13 a	*0.88-1.53*	1.74 b	*1.31-2.48*	2.56 c	*2.13-2.80*	3.43 d	*2.84-4.08*	37.37***
Fruit_Shape2	1.12 a	*0.85-1.54*	1.79 a	*1.30-2.69*	3.07 b	*2.76-3.64*	4.74 c	*3.90-6.10*	47.39***
Blockiness									
P_Blockiness	0.56 a	*0.52-0.59*	0.58 a	*0.55-0.66*	0.69 b	*0.66-0.76*	0.79 c	*0.74-0.86*	38.01***
D_Blockiness	0.76 a	*0.70-0.83*	0.75 a	*0.63-0.82*	0.90 b	*0.77-1.05*	1.12 c	*1.06-1.22*	29.07***
Triangle	0.75 a	*0.65-0.85*	0.78 a	*0.71-1.06*	0.78 a	*0.71-0.87*	0.71 a	*0.66-0.81*	0.75^{ns}

Homogeneity									
Circular	0.89 b	*0.80-0.95*	0.87 b	*0.76-0.95*	0.50 a	*0.24-0.64*	0.22 a	*0.12-0.31*	58.23***
Ellipsoid	0.93 c	*0.89-0.97*	0.86 b	*0.79-0.93*	0.77 a	*0.72-0.84*	0.73 a	*0.71-0.74*	25.23***
Rectangular	0.50 b	*0.47-0.52*	0.48 ab	*0.46-0.51*	0.45 a	*0.40-0.47*	0.44 a	*0.37-0.47*	4.50*
Proximal fruit end shape									
Sh_Height	0.007 a	*0.002-0.012*	0.004 a	*0.000-0.008*	0.009 a	*0.002-0.018*	0.004 a	*0.000-0.014*	0.91ns
Asymmetry									
Obovoid	0.23 a	*0.15-0.30*	0.23 a	*0.05-0.30*	0.31 a	*0.24-0.36*	0.44 b	*0.38-0.49*	12.57***
Ovoid	0.003 a	*0.000-0.011*	0.014 a	*0.000-0.086*	0.000 a	*0.000-0.000*	0.000 a	*0.000-0.000*	0.72ns
Internal Eccentricity									
Eccentricity	0.76 a	*0.74-0.78*	0.78 a	*0.77-0.79*	0.76 a	*0.73-0.78*	0.76 a	*0.72-0.78*	1.44ns
P_Eccentricity	0.89 a	*0.89-0.89*	0.89 a	*0.89-0.89*	0.89 a	*0.89-0.90*	0.91 a	*0.88-0.93*	2.71ns
D_Eccentricity	0.89 a	*0.89-0.89*	0.89 a	*0.89-0.89*	0.91 a	*0.90-0.92*	0.93 b	*0.92-0.97*	14.68***
F_S_Index_ I	1.12 a	*0.85-1.54*	1.80 a	*1.30-2.69*	3.08 b	*2.77-3.65*	4.76 c	*3.91-6.13*	47.31***
Ecc_Area_ Index	0.41 a	*0.40-0.42*	0.40 a	*0.39-0.41*	0.45 b	*0.41-0.51*	0.50 c	*0.48-0.53*	27.11***
Slenderness									
Slender	5.7 a	*2.6-10.2*	14.1 b	*6.7-26.9*	30.3 c	*23.6-36.1*	45.7 d	*37.6-56.9*	45.61***

[a] ***, **, *, ns indicate, respectively, significant at P<0.001, P<0.01, P<0.05, and non-significant.

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

Table 5. Mean ± standard deviation, and range (between brackets, in italics) within and between each of the eggplant fruit shape varietal groups for the number of pairwise significant differences (according to the Student-Newman-Keuls multiple range test) between accessions for the 26 fruit traits studied in a collection of 21 eggplant accessions.

Varietal groups	Round	Listada de Gandía	Semi-long	Long
Round	7.0±4.5	9.7±4.4	16.0±1.9	18.5±1.4
	(*0-11*)	(*3-16*)	(*12-20*)	(*17-21*)
Listada de Gandía		8.2±4.5	14.0±4.2	18.0±1.8
		(*0-16*)	(*3-20*)	(*14-21*)
Semi-long			9.7±5.2	12.5±3.7
			(*0-14*)	(*2-19*)
Long				6.9±4.8
				(*0-13*)

Multivariate analyses

The first and second components of the PCA accounted, respectively, for 64.3% and 13.1% of the total variation among accession means. The first component was positively correlated with fruit width traits, like Width, Width_MH, and Max_Width, and round or blocky fruits, like Circular, Ellipsoid and Rectangular, and negatively correlated with elongated fruit traits, like Length, Perimeter, Height_MW, Max_Height, Fruit_Shape1, Fruit_Shape2, F_S_Index_I, and Slender, as well as to traits that are mostly expressed in long fruits (P_Blockiness, D_Blockiness, Obovoid, D_Eccentricity, Ecc_Area_Index) (Figure 2). The second component was positively correlated with fruit size traits (Weight, Width, Area, Max_Width), and negatively to ovoid fruits (Triangle, Ovoid) (Figure 2).

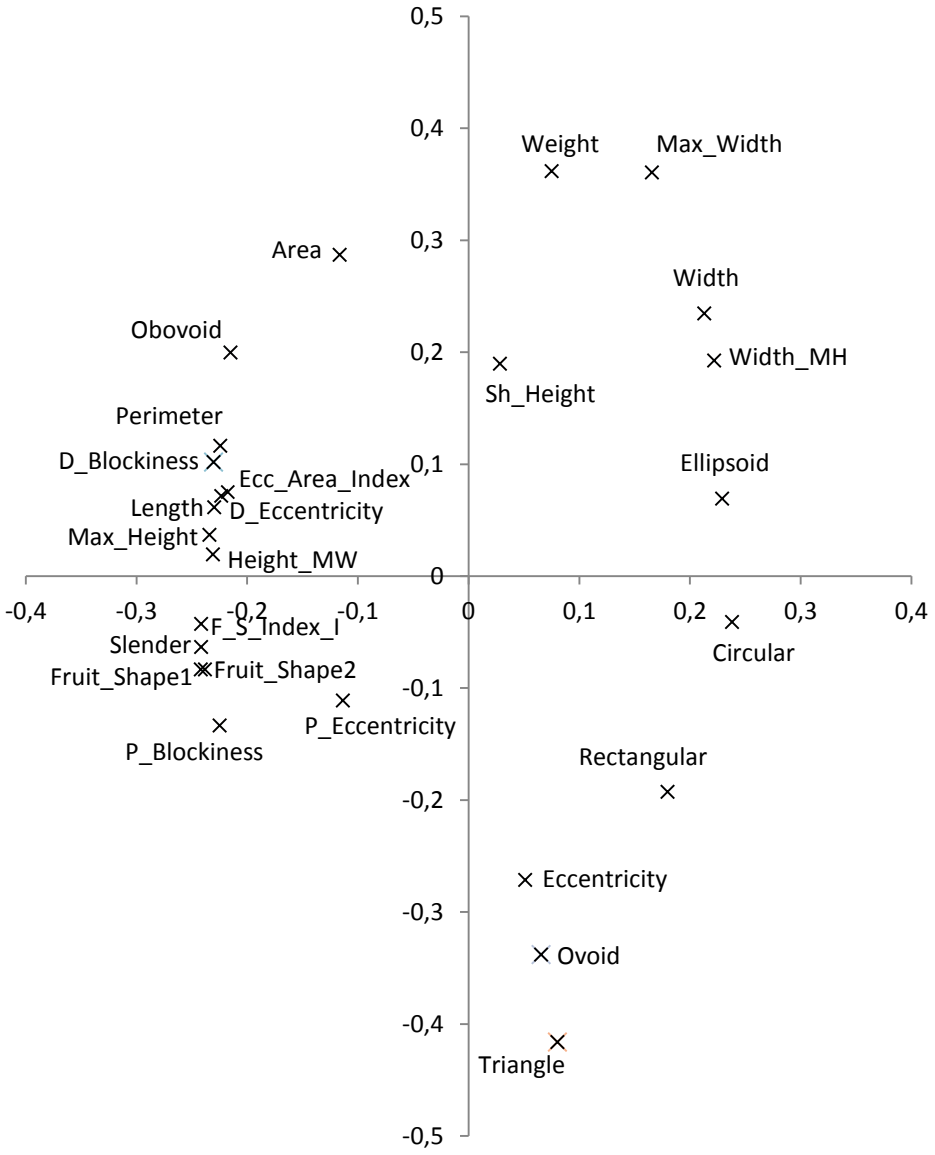

Fig. 2. Loading plot of the first and second principal components for the 26 fruit traits evaluated (see Table 2) for the 21 eggplant accessions studied. First (X-axis) and second (Y-axis) components of the PCA account for 64.3% and 13.1% of the total variation, respectively.

The projection of the accessions on a two-dimensional PCA plot shows that accessions of each varietal group plot together and are situated in a different part of the graph (Figure 3). Accessions of the Round and Listada de Gandía groups present positive values for the first component, while the Semi-Long and Long accessions present negative values for this first component. Accessions of the Semi-long and Long varietal groups do not overlap for the values of this first component, as Semi-long accessions present values between -4 and 0, while Long accessions have values

between -8 and -4. However, Listada de Gandía accessions and Round accessions present some overlap for this first component. In this respect, Listada de Gandía accession I347 presents values for this component higher than those of Round accessions B31 and V10, and Listada de Gandía accessions V7 and V11 present values higher than those of Round accession V10 (Figure 3). Regarding the second component each of the four groups includes accessions presenting positive or negative values. The Listada de Gandía is the varietal group presenting the widest range of variation, including the only accession presenting ovoid shape (V11) and the heaviest accession (V7), and which presented the lowest and highest values, respectively, for the second component (Figure 3).

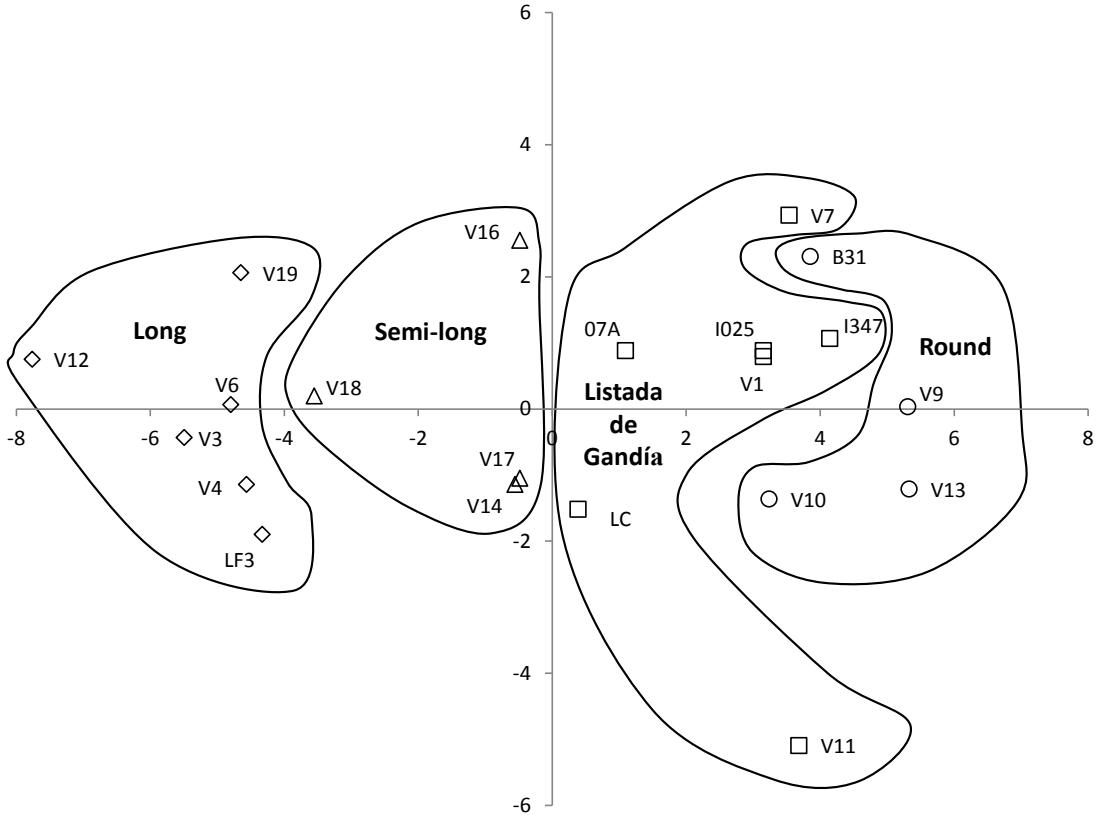

Fig. 3. Score plot of the first (X-axis) and second (Y-axis) principal components for the 21 eggplant accessions evaluated based on 26 fruit traits (see Table 2). First (X-axis) and second (Y-axis) components of the PCA account for 64.3% and 13.1% of the total variation, respectively. The different varietal groups are represented by different symbols: Round (circle), Listada de Gandía (square), Semi-long (triangle), Long (rhombus).

Discriminant analysis shows that with the 26 traits studied, individual fruits were correctly classified to their actual accession in 57.14% of the cases (Table 6). For the accessions of the extreme groups Round and Long, the percentages of correct classification were higher (73.75% and 66.67%, respectively) than for the intermediate groups Listada de Gandía (48.57%) and Semi-Long (41.25%). A total of 21.67% of individual fruits (between 12.50% for the Semi-Long group and 30.00% for the Long group) were incorrectly classified to their actual accession, although they were classified correctly to their actual varietal group, which makes a total of 78.81% of fruits correctly classified to their actual varietal group (between 53.75% for the Semi-Long group and 96.67% for the Long group). Finally, 21.19% of the individual fruits are misclassified to accessions from other varietal groups. This misclassification is lowest in the Long (3.33%; all of them classified as Semi-long) and Round (12.50%; all of them classified as Listada de Gandía) groups, and highest in the Semi-Long (46.25%; of which 8.75% classified as Round, 15.00% as Listada de Gandía, and 22.5% as Long) and Listada de Gandía (27.14%; of which 23.57% classified as Round and 3.57% as Semi-long) groups.

Table 6. Classification table for the fruits of each accession indicating the percentage of cases correctly classified to their actual accession, to other accessions of the same varietal group, and to accessions of other varietal groups, according to multivariate discriminant analysis.

		Incorrectly classified	
Accession	Correctly classified to actual accession (%)	To same varietal group (%)	To other varietal groups (%)
Round	73.75	13.75	12.50
Listada de Gandía	48.57	24.29	27.14
Semi-long	41.25	12.50	46.25
Long	66.67	30.00	3.33
Total	57.14	21.67	21.19

DISCUSSION

Varietal groups in eggplant are established according to the fruit shape (Nunome et al., 2001; Prohens et al., 2005; Daunay, 2008; Tümbilen, 2011; Marín, 2013). In consequence, classification of eggplant fruits and cultivars for fruit shape is an important objective for horticulturists and breeders. Despite the importance of fruit shape in this crop, its characterization is generally based on simple traits, like fruit length and width (IBPGR, 1990; Doganlar et al., 2002; Prohens et al., 2005). The use of modern image tools, like the Tomato Analyzer software (Brewer et al., 2006, Gonzalo and van der Knaap, 2008, Rodríguez et al. 2010a, 2010b), has allowed us to perform the first fruit shape phenomics study in a collection of eggplant germplasm.

We have measured 26 fruit shape traits (3 manually and 23 with Tomato Analyzer) in eggplant fruits. In all cases but one (Shoulder Height) significant differences have been found among accessions. This shows that the use of Tomato Analyzer software in eggplant represents an improvement over prior fruit shape manual characterizations, as it provides quantitative data on many fruit shape traits that are not dealt with when using manually measured morphological descriptors (IBPGR, 1990; Doganlar et al., 2002; Prohens et al., 2005, 2012). In fact, some of the

traits that may seem redundant when measured manually and with Tomato Analyzer, actually are not. In this way, the fact that the manually measured Width and Length traits present values higher than those of the Tomato Analyzer measured Max_Width and Max_Height reflects that they are not measuring the same fruit shape characteristic (Brewer et al., 2006, Gonzalo and van der Knaap, 2008, and Rodríguez et al. 2010a), and therefore are complementary. Width measures the maximum width of the fruit, while Max_Width measures the maximum width of the longitudinal section used for scanning, which is not necessarily made in the widest part of the fruit. Also, Length is an estimate of the curved length, while Max-Height is the maximum vertical distance of the fruit.

In all fruit trait categories, with the exception of proximal fruit shape (i.e., Shoulder Height), we found traits with high heritability values, which indicates that, as occurs in tomato (Brewer et al., 2006, 2007; Gonzalo and van der Knaap, 2008; Rodríguez et al., 2011), there is an important degree of genetic determination in the expression of fruit shape traits. This indicates that for those fruit shape traits with high heritability and CV_G values, important genetic advances can be achieved by means of selection (Wricke and Weber, 1986). Also, we have found significant differences among means for 20 fruit shape traits among the four varietal groups (Round, Listada de Gandía, Semi-Long, and Long) studied. For six of the fruit shape traits we found significant differences among all of the four varietal groups, and for two of them there was no overlap among groups in the ranges of variation. This shows that, as occurs in other crops (Venora et al., 2009; Mazzucato et al., 2010; Ercisli et al., 2012; Lootens et al., 2013), image analysis is useful for classification for cultivar types in eggplant and is much better than conventional morphological descriptors for discrimination among varieties.

Within each of the varietal groups, most of the accessions presented significant differences for several to many fruit shape traits, showing that a precise characterization, as well as within varietal group selection, can be performed using Tomato Analyzer traits. However, the fact that for some accessions no significant differences have been found for any of the multiple traits studied suggests that they

may present the same genetic constitution for fruit shape genes (Rodríguez et al., 2011). Amazingly, the number of significant differences between accessions of different varietal groups has, in some cases, been smaller than the differences between accessions of the same group, providing further evidence that fruit shape variation in eggplant is continuous. In this respect, establishment of varietal groups based on fruit shape or other specific morphological traits for which continuous variation exists is considered as arbitrary (Grandillo et al., 1999; Stepansky et al., 1999; Spooner et al., 2003).

The multivariate PCA analyses shows that, as occurs when using standarized morphological descriptors for plant, flower and fruit traits (Prohens et al., 2005), the different varietal groups are clearly distinguished, although the Round and Listada de Gandía groups present some degree of intersection. In this respect, Listada de Gandía presents an important genetic diversity and is basically characterized by the presence of stripes (Muñoz-Falcón et al., 2008, 2011) and depending on the shape of individual cultivars, they are frequently included in the Round or Semi-long groups (Marín, 2013). Also, the discriminant analysis shows that, despite the fact that some of the accessions tested were genetically very similar (Prohens et al., 2005; Muñoz-Falcón et al., 2008) and presented a very similar fruit shape, the percentages of correct classification of individual fruits are quite high. This shows that Tomato Analyzer provides a very precise characterization of fruit shape of eggplant fruits. The discriminant analysis results also indicate that several fruits are necessary for an adequate characterization of fruit shape of eggplant accessions or cultivars.

CONCLUSION

The detailed characterization of fruit shape is important in eggplant. Use of the Tomato Analyzer software has allowed the classification of eggplant fruits according to their fruit shape, the characterization of the large diversity that exists for multiple fruit shape traits within and between eggplant varietal groups, as well as to estimate genetic parameters of interest for breeding. This first phenomics study of fruit shape in eggplant opens the way to using image analysis software (like Tomato Analyzer) for the genetic dissection, through the identification of genes and QTLs, of this important attribute in this crop. The availability of phenomics fruit shape data sets represents an improvement over manual characterization, and will be of great utility for the characterization of germplasm resources and cultivars, as well as for selection and breeding programs of eggplant.

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PROGRAMAS MEJORA GENÉTICA

Development of Breeding Programmes in Eggplant with Different Objectives and Approaches: Three Examples of Use of Primary Genepool Diversity

M. Hurtado, S. Vilanova, M. Plazas, P. Gramazio and J. Prohens

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

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ABSTRACT

Diversity available in the eggplant (*Solanum melongena* L.) primary genepool can be used for developing new improved materials with better characteristics. We have initiated three breeding programmes in this crop aimed at different objectives: a) development of commercial hybrids for greenhouse cultivation (hybrids programme); b) improvement of the pickling 'Almagro' eggplant landrace (*Almagro* programme); and, c) introgression of traits of interest from the wild *S. incanum* into the genetic background of *S. melongena* (introgression lines programme). In the case of the hybrids programme we used as sources of variation a number of commercially successful hybrids of the black-type which presented a broad genetic diversity, as assessed with AFLP and SSR markers. These hybrids have been selfed for several generations and have been subjected to phenotypic selection for the traits of commercial interest. The lines obtained have a high degree of intra-line uniformity and we are obtaining experimental hybrids using as parents lines showing a good performance and a high genetic divergence. The *Almagro* programme is aimed at reducing the calyx prickliness of the original landrace. A selection programme has allowed selecting *Almagro* materials with lower prickliness. A backcross breeding programme using as donor parents a pickling eggplant with low prickliness and prickle-free fresh market eggplants has been initiated, in which selection using a participatory approach has been done for low prickliness and *Almagro* eggplant morphotype. At present, backcross materials up to the BC3 generation have been obtained and are being tested in commercial fields of farmers. Finally, the introgression lines programme is aimed at developing a set of introgression lines of *S. incanum* in the genetic background of *S. melongena* by means of backcrossing and marker assisted selection. In this case, a genetic map has been obtained and selection is done so that the whole genome of *S. incanum* is represented in overlapping segments present in the materials used in the successive backcross generations. At present the BC3 generation is being selected for further backcrossing. These three programmes are aimed at different objectives and are a typical example of different

approaches, conventional and modern tools, for exploiting the diversity present in the primary genepool for the genetic improvement of a vegetable crop.

INTRODUCTION

Breeding programmes in eggplant (*Solanum melongena* L.) are aimed at improving the yield, resistance or tolerance to biotic and abiotic stresses, improvement of the quality, and diversification of types (Daunay, 2008). In this respect, the use of the primary genepool of eggplant, which comprises the cultivated and weedy forms of eggplant as well as the wild ancestor *S. incanum* (Lester and Hasan, 1991; Daunay, 2008), is of interest for the genetic improvement and broadening of the genetic base of eggplant.

The approaches to be used in a breeding programme depend on the reproductive system, the objective to be achieved, the available sources of variation, and the time available to reach an acceptable breeding material or cultivar (Acquaah, 2007). In the case of cultivated eggplant, the reproduction is mostly autogamous (Pessarakli and Dris, 2004) and landraces usually have a high degree of fixation. Therefore, the breeding approaches to be used are the usual for autogamous crops (Acquaah, 2007; Daunay, 2008). Here we present the results of three ongoing programmes for the breeding of eggplant using the primary genepool of eggplant as source of variation, which consist in the development of: a) commercial hybrids of black eggplant (hybrids programme); b) non-prickly lines of the local *Almagro* pickling landrace (*Almagro* programme); c) introgression lines of *S. incanum* into the genetic background of the cultivated *S. melongena* (introgression programme).

The black eggplant is the economically most important cultivar group in this crop, at least in Europe and America (Muñoz-Falcón et al., 2009a). Although pure lines of black eggplant have been developed since long time ago, the market for commercial production of off-season (greenhouse cultivated) eggplant is dominated by hybrids (Marín, 2011). Developing new hybrids could benefit from the heterosis

derived from crossing selected lines situated at high genetic distances (Rodríguez-Burruezo et al., 2008). In this respect, commercial hybrids are élite cultivars for greenhouse cultivation (Muñoz-Falcón et al., 2008), and therefore may represent an important source for developing pure lines useful for obtaining new hybrids well adapted to commercial cultivation in a short time. Introduction of exotic materials (i.e., black landraces adapted to open field cultivation), while can be of interest for broadening the genetic base of eggplant (Muñoz-Falcón et al., 2009a) may also require pre-selection of materials for good performance in greenhouse (Muñoz-Falcón et al., 2008).

The *Almagro* eggplant is a local heirloom from the central part of Spain used for making pickles (Seseña and Palop, 2007). The pickled eggplants made with this heirloom have a protected geographical indication (PGI) status since 1994 (Castro, 2005). The plants of this landrace are characterized by producing small fruits covered by an acrescent calyx, and the final pickled produce elaborated with the fruits of Almagro eggplants has a high quality (Prohens et al., 2007). However, *Almagro* eggplant fruits present prickles in the calyx and this difficults harvesting and processing, as the prickles have to be manually removed before the fruits are processed. Other pickling materials, like the *Andalusian* eggplant, which has fewer prickles give a final produce with lower quality, as the processed fruit has a softer texture (Prohens et al., 2007). Therefore, breeding for low prickliness is a breeding objective of interest in this local landrace. Both the *Andalusian* eggplant and non-prickly fresh market varieties could be used as sources of variation for reaching this objective.

Solanum incanum L. is the wild ancestor of eggplant (Lester and Hasan, 1991; Meyer et al., 2012), and it gives fertile hybrids with cultivated eggplant (Lester and hasan, 1991; Daunay, 2008). *Solanum incanum* is naturally distributed in several parts of Africa and the Middle East, but not in Southeast Asia, where cultivated eggplant was domesticated (Lester and Hasan, 1991; Meyer et al., 2012). It is unknown how the *S. incanum* plants from which *S. melongena* was domesticated reached Southeast Asia, although several hypotheses have been established (Lester and Hasan, 1991; D'Arcy and Pickett, 1993). In any case, as occurred with the tomato (Bai and Lindhout, 2007), the domestication of eggplant outside the area of natural distribution of *S. incanum* means that eggplant underwent a genetic bottleneck during domestication (Daunay, 2008). The development of *S. melongena* lines with introgressions from *S. incanum* would be of interest for introducing traits present in materials of this wild species, as well as to study the evolution and domestication of the crop (Mennella et al., 2010). For example, *S. incanum* presents resistance to *Fusarium oxysporum* f.sp. *melongenae*, resistance to drought and low and high temperatures, as well as a high content in phenolics (Yamakawa and Mochizuki, 1979; Stommel and Whitaker, 2003; Daunay, 2008).

The aim of this paper is to present the approaches followed and results obtained in the three breeding programmes aforementioned, and which have different objectives. In all cases, we have used different levels of diversity available in the primary as sources of variation.

MATERIALS AND METHODS

Hybrids programme

Thirty commercial hybrids and lines with economic importance and belonging to the black group were used for the breeding program. These accessions were characterized using morphological descriptors using 15 traits, of which four correspond to plant traits, seven to fruit traits, and four to flower and inflorescence traits (Prohens et al., 2005). Commercial hybrids were selfed. Also, in order to generate more variation crosses between hybrids and between hybrids and pure lines were performed among materials that were situated at a high genetic distance, as assessed with AFLP and SSR markers.

Plants of the segregating generations have been selected according to a pedigree selection scheme. All selected plants for their interesting phenotype were selfed for several generations in order to develop pure lines. The next step of the programme is the use of the selected lines for obtaining improved F1 commercial hybrids. The cultivation of plants, characterization, and selection of materials have been carried out in greenhouses of the main producing area in Spain, situated in the province of Almería, using the commercial production practices.

Almagro programme

The materials initially used for this programme consisted of: 18 accessions of the local *Almagro* eggplant, 15 accessions of pickling eggplant from the *Andalusian* type, 1 accession of a landrace of black eggplant (CS16) without prickles, and 1 experimental hybrid of black eggplant (E1) without prickles. The morphological variation among the *Almagro* and *Andalusian* accessions was studied using 14 descriptors, as indicated in Muñoz-Falcón et al. (2009). The morphological diversity was tested using three combinations of AFLP primers and thirty-six SSR markers.

Plants of *Almagro* selected for low prickliness were selfed for developing pure lines and were also crossed with non-prickly Andalusian pickling eggplants and with

the CS16 and E1 black non-prickly eggplants. A backcross programme with selection for low prickliness and gross morphology according to the *Almagro* ideotype were selected using a participatory approach, in which farmers have participated in the selection (Prohens et al., 2007). All the evaluations of plants have been performed in farmers' fields in the *Almagro* eggplant producing areas using commercial production practices.

Introgression lines programme

An F1 hybrid, obtained by crossing the accession of *S. incanum* 'MM 577' and *S. melongena* accession 'AN-S-26' was backcrossed to the *S. melongena* parental accession. A total of 96 BC_1 plants were randomly selected to construct the genetic linkage map. All the markers were initially tested on parents and the F_1 hybrid. Those markers for which we detected polymorphisms were analyzed in the whole mapping population. A total of 12 AFLP primer combinations, generated by four EcoRI primers (E+ACG; E+ACT; E+AGC; E+ACA) combined with three MseI primers (M+CAC, M+CTA, M+CAA) were used. A total of 110 SSR markers from different sources (Nunome et al., 2003; Stàgel et al., 2008; Nunome et al. 2009) were tested in the segregating population including 57 new SSRs developed in our lab (Vilanova et al., 2012). In addition, 123 markers based on conserved orthologous sequences (COS) (Wu et al., 2009) were amplified and subsequently sequenced in parents in order to develop CAP markers. Linkage analysis was carried out using Joinmap 3.0 software (van Ooijen and Voorrips, 2001). Linkage groups were established at a LOD ≥3 and map order was determined using maximum recombination fraction θ=0.4. Kosambi mapping function was used to convert recombination units into genetic distances (cM).

Subsequent BC populations were selected using the polymorphic molecular markers obtained in order to conserve the whole genome of *S. incanum* scattered into different materials, while at the same time recovering the *S. melongena* genetic background. Important phenotypic traits like prickliness, flesh browning, polyphenol

content, colour of the fruit, shape of the fruit, etc. were characterized on the BC1 plants.

RESULTS

Hybrids programme

Considerable morphological and molecular (both AFLP and SSR) variation was found among the materials initially used and also in the subsequent generations. A total of 53 AFLP fragments and 61 SSR alleles were found to be polymorphic in the materials tested. Also, a high morphological variation was found when crossing hybrids and landraces situated at a high genetic distance. As expected, the intra-family variation decreased as the number of selfing generation increased. The selection programme has allowed the development of uniform pure lines with a good performance under greenhouse conditions, and some of them could even be competitive with F1 commercial hybrids, as assessed by their performance under commercial conditions. These selected lines display variation for a number of traits, like fruit size, shape, and skin colour, including intensity of colour and glosinness). The molecular characterization of the selected materials will allow assessing the genetic diversity maintained during the programme.

The selected pure lines are going to be intercrossed using complementary and transgressive taking into account the genetic distance among them and also their agronomic and phenotypic characteristics following to obtain the first experimental hybrids. These hybrids will be evaluated under commercial conditions and compared to the most important commercial F1 hybrids. We expect that some of these hybrids will be of commercial interest and at the same time will have increased the genetic diversity of the élite germplasm of black eggplant adapted to greenhouse cultivation conditions.

Almagro programme

The morphological characterization of eggplant showed that *Almagro* and *Andalusian* eggplants were very similar for most of the morphological traits evaluated, and that some diversity existed for relevant traits within each of these pickling eggplant landraces. However, compared to the *Andalusian* eggplants, the *Almagro* eggplants, had significantly smaller leaves (shorter leaf petiole length, blade lengths and blade width), were more prickly (both in the leaves and the calyx), had a shorter pedicel length and narrower pedicel at the proximal end, shorter calyx length, and smaller fruits. The study of the molecular diversity, allowed scoring 25 polymorphic AFLP fragments, and eight polymorphic SSR loci, with a total of 33 SSR alleles. Both markers showed that a considerable diversity existed within both the *Almagro* and *Andalusian* eggplants, and also that these two pickling varieties clustered together when compared to accessions of eggplants for fresh consumption. AFLP markers were unable to separate *Almagro* and *Andalusian* eggplants. However, SSRs allowed a clear distinction between both types. In this respect, two SSR alleles were found to be specific and universal to *Almagro* accessions.

Given the diversity found in *Almagro* accessions, including prickliness, thousands of individual plants were visually scored in the eggplant fields of several farmers associated to the *Almagro* PGI. A total of 27 plants were pruned, uprooted, and transplanted into a greenhouse in Valencia. Selected *Almagro* plants were self-pollinated, and the selfed generations were tested and selected for several years in the fields of several farmers for uniformity and also for prickliness, yield, and for the *Almagro* eggplant ideotype. Several hundred plants have been screened during this selection programme. As a result of the phenotypic selection, a pure line, named H15, which has a significantly lower prickliness and higher yield than the *Almagro* materials grown by the farmers was finally selected.

Selected *Almagro* materials, including the H15 line, were crossed with non-prickly *Andalusian* and CS16 and E1 fresh market eggplants. Hybrids showed heterosis for prickliness but not for hybrid vigour. Selected hybrids were crossed with *Almagro*

materials for obtaining the BC1. Several BC1 plants with no or low prickliness were detected among the different progenies; however, these plants did not fit completely to the *Almagro* ideotype, in particular those having CS16 and E1 as donor parents. However, the plants with no or very low prickliness were those having CS16 and E1 as donor parents. The selected BC1 individual plants were backcrossed again, this time only to H15 to obtain the BC2 generations, which were again subjected to selection. Some of the plants in this generation again had no or almost no prickliness, and, as expected, presented morphological characteristics much more similar to those of the *Almagro* eggplant. A new cycle of backcross to H15 was performed to obtain the BC3, which is being evaluated at the moment of writing the present paper (May 2012). The next steps of the programme will consist in a new cycle of selection in the BC3, and those plants that already conform to the *Almagro* ideotype and with no or very low prickliness, will be self-pollinated for developing pure lines selected for no prickliness. The evaluations will be made for several years in order to select new *Almagro* non-prickly materials. Molecular fingerprinting will be performed to confirm that the genetic background of the selected materials has been recovered.

Introgression lines programme

The BC1 map obtained includes a total of 116 AFLP, 53 SSRs and 35 COS markers. The SSR and COS markers mapped allowed us to establish synteny with other *Solanum* maps like 'EPL1 x WCGR112-8' intra-specific map obtained by Nunome et al. (2009), '*S. linnaeanum* (MM195) x *S.melongena* (MM738)' inter-specific map developed by Wu et al. (2009), and the '*S. lycopersicum* (LA925) x *S. pennellii* (LA716)' inter-specific tomato map obtained by Fulton et al. (2002).

The map is organized into 13 linkage groups covering 809 cM and includes 204 loci. The average distance between markers (cM/marker) is 3.96, and the largest gap (>20 cM) is located in G4. Loci order was compared with the previously constructed eggplant maps and was maintained in all groups. The moephological characterization of the BC1 plants allowed the identification of candidate QTLs for

some phenotypic traits like prickliness, flesh browning, phenolics content, fruit colour, or fruit shape.

Evenly distributed markers were chosen in order to perform the subsequent plant selection. Seventeen BC1 plants were selected and backcrossed to obtain the BC2. Ten plants derived from each BC2 were selected again to obtain the BC3. This process will be continued for several more generations and will be followed by selfing of the selected individuals in order to obtain the collection of *S. melongena* lines with introgressed fragments of the *S. incanum* genome.

DISCUSSION

Compared to tomato (Solanum lycopersicum L.) or pepper (Capsicum annuum L.), the two other major Solanaceae fruit crops, breeding efforts in eggplant have been limited. While for tomato, the use of the primary and secondary genepools has been extensively used for breeding new cultivars (Díez and Nuez, 2008), in the eggplant, the practical use of genetic resources in breeding programmes has been more restricted. In this respect, we have initiated three breeding programmes, each with a different objective, and in which we have used different levels of diversity contained in the primary genepool, in which there is no hybridization incompatibility and offsprings are completely fertile and with regular meiosis (Daunay, 2008).

The first breeding programme we present is the aimed at developing commercial hybrids of black eggplants for the competitive market of off-season cultivation in greenhouse, in which there is a high rate of replacement of cultivars (Marín, 2011). These hybrids must have a high yield under greenhouse conditions, intense black colour, earliness, and uniformity (Daunay, 2008; Muñoz-Falcón et al., 2009a). We have used élite well adapted F1 hybrids for developing pure lines well adapted to greenhouse cultivation and selected for the traits aforementioned. AFLP and SSR markers will be used to fingerprint the selected pure lines and hybrids will be obtained among those situated at high genetic distance, which we expect will result in

heterotic hybrids (Rodríguez-Burruezo et al., 2008) well adapted to greenhouse conditions. Also, the selected pure lines with acceptable performance under greenhouse from black-skinned local landraces and exotic Oriental cultivars, which have a genetic base broader than the modern black eggplant F1 hybrids (Muñoz-Falcón et al., 2009a) will be used to develop hybrids with the lines obtained from commercial F1 hybrids. The aim is to increase the genetic diversity of commercial F1 hybrids and to make a greater exploitation of heterosis.

The Almagro eggplant breeding programme is aimed at solving a specific problem (prickliness) of this local landrace (Muñoz-Falcón et al., 2009b). Given that the acreage devoted to this local landrace is limited and that the economic profit obtained by each individual plant is much lower than in the case of the greenhouse grown plants (Castro, 2005), no private breeding programmes are underway for this landrace. The selection within this diverse landrace has allowed the selection of a pure line with high yield and prickliness (Prohens et al., 2009), showing that individual selection within heterogeneous populations of homozygous plants of eggplant may be a highly efficient and fast way to obtain improved materials. The use of a conventional backcross programme (Rodríguez-Burruezo et al., 2010) is allowing the introgression of the non-prickly trait (Doganlar et al., 2002) into the genetic background of Almagro eggplant. At present the BC3 generation is being evaluated and the selected plants will be selfed to derive improved Almagro pure lines. In theory, in the BC3 the Almagro genetic backgroung has been recovered in a 93.75% (Rodríguez-Burruezo et al., 2010). However, given the selection made for the Almagro ideotype probably there has been a higher degree of recovery, at least for the genomic regions conferring the typical morphological characteristics of this landrace. Molecular SSR markers will be used to confirm that the materials selected present a high percentage of the Almagro background (Muñoz-Falcón et al., 2009b).

Developing a set of introgression lines, so that each of the lines has a single fragment of the genome of the donor parent, and that on the whole the set of lines cover the whole genome of the donor parent, is difficult (Zamir, 2001). This is because a genetic map covering the whole genome is required and marker assisted selection

needs to be done in each backcross generation in order to select the plants more having the desired donor genome fragments. Although lines of the eggplant with introgressions from the scarlet eggplant (S. aethiopicum L.) and S. sodomaeum L. have been obtained (Mennella et al., 2010), no set of introgression lines covering the whole genome of a donor have been obtained. In our case, given that S. incanum is the ancestor of eggplant (Lester and Hasan, 1991), development of such set of introgression lines is of major interest, as it will allow introgressing traits of interest from this fully compatible species into the genetic background of eggplant and will also be a major tool for understanding the genetics, evolution and domestication of this crop (Zamir, 2001). In order to achieve this objective we have developed a molecular map (Vilanova et al., 2010), which is being used for the selection of plants from the BC1 and BC2 generations. At present, the BC3 is being genotyped for the selection of individuals for obtaining the BC4. The backcross and selection process will continue until the set of desired materials have been obtained with introgressions in heterozygosis. Subsequently, the materials will be selfed in order to fix in homozygosis the fragments introgressed from the S. incanum donor parent. We expect that, as occurred with tomato (Lippman et al., 2007), some of these lines will be of direct use in eggplant breeding.

In summary, we have shown that the use of different breeding approaches and distinct levels of primary genepool diversity are appropriate for breeding programmes with specific objectives. The materials derived from these programmes will be useful for developing improved cultivars of eggplant and for broadening the genetic base of this crop.

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Enhancing conservation and use of local vegetable landraces: the *Almagro* eggplant (*Solanum melongena* L.) case study

Maria Hurtado · Santiago Vilanova · Mariola Plazas · Pietro Gramazio · Isabel Andújar · F. Javier Herraiz · Angel Castro · Jaime Prohens

M. Hurtado · S. Vilanova · M. Plazas · P. Gramazio · I. Andújar · F.J. Herraiz · J. Prohens (✉): Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

A. Castro: Asociación para la Promoción de la I.G.P. "Berenjena de Almagro", C/Ramón y Cajal 12, 13260 Bolaños de Calatrava, Spain

Corresponding author e-mail, telephone and fax:

e-mail: jprohens@btc.upv.es

Telephone: (+34) 963879424

Fax: (+34) 963879422

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ABSTRACT

We have used the *Almagro* pickling eggplant landrace as a model for the enhancement of a local vegetable landrace. The programme has included characterization, selection, and breeding activities. Considerable intra-landrace diversity has been found for morphological traits and molecular markers. Characteristic morphological traits have allowed its registration as conservation variety. Also, universal and specific simple sequence repeat (SSR) markers have been found for the *Almagro* landrace. The chemical characterization revealed that *Almagro* eggplant has a high content in bioactive phenolics. Organoleptic tests revealed that pickles produced with *Almagro* eggplant are superior to those of the related *Andalusian* landrace. The selection programme has led to the development of a pure line (H15) with increased yield and reduced fruit calyx prickliness. A participatory breeding programme, in which selection is made by farmers in their own field, has been initiated to introduce the no-prickles trait from three other varieties in the genetic background of the *Almagro* eggplant. The results of the programmes show that plants with the *Almagro* eggplant ideotype and with reduced prickliness can be selected in the backcross generations. As a result of the enhancement programme the acreage and total production of *Almagro* eggplant has tripled in the last decade.

Keywords Backcross breeding · Characterization · Diversity · Landrace · Markers · Phenolics · Selection · *Solanum melongena*

INTRODUCTION

The use and enhancement of local landraces may have an important role for the development of horticulture. During the last years there has been an increasing interest for the recovery of local landraces by consumers and markets (Spataro and Negri 2013). Landraces of vegetables are associated to better flavour ("flavour of the past"), local tradition, and environmentally friendly production (Trichopolou et al. 2007). Because of this, consumers accept paying higher prices for the local landraces. In addition, cultivation of local landraces contributes to on-farm conservation of genetic resources and to preserving the ethnobotanical knowledge associated to them (Hammer et al. 2003, Polegri and Negri 2010).

Contrarily to commercially important cultivars, local landraces of vegetable crops often have been neglected and poorly studied, and little scientific information exists on their characteristics and unique features (Piergiovanni and Laghetti 1999, Polegri and Negri 2010, Cianconelli et al. 2013, Torricelli et al. 2013). We hypothesize that added value of local landraces can be increased by limited investments in their scientific study, characterization, selection, and breeding, leading to an improvement in its production, quality, or both, and contribute to the enhancement and demand of local landraces (Trichopolou et al. 2007).

We present the work done and new results of an enhancement programme directed to the *Almagro* pickling eggplant (*Solanum melongena* L., 2n = 2x = 24) landrace. We show how, with a limited effort it is possible to make an effective contribution to improving the commercial production, demand, and on-farm conservation of local landraces of vegetable crops.

The model landrace used: *Almagro* eggplant

The *Almagro* eggplant landrace is used for pickles and is native to the county of Campo de Calatrava, situated in the center of Spain (Muñoz-Falcón et al. 2009). The *Almagro* name of this landrace refers to the historic city of Almagro, which is the capital of the Campo de Calatrava county. This eggplant landrace has a small plant and fruit size (Fig. 1) and is very prolific, as it presents multiple inflorescences which often set several fruits. Like the rest of eggplants (Pessarakli and Dris 2004), *Almagro* eggplant is mostly autogamous. The fruits are obovoid-shaped and are harvested when they are 4 to 9 cm in length (Prohens et al. 2009). The berry is covered in a large proportion by an acrescent, usually prickly, calyx (Fig. 1). The berry skin presents a uniform green background colour and anthocyanins in the part exposed to the sun. The fruits of the *Almagro* eggplant are used by several local industries of the Campo de Calatrava county for elaborating *Almagro* eggplant pickles. The pickling process, which has been studied in detail (Seseña and Palop 2007), involves grading of fruits, blanching, lactic fermentation, stuffing or cutting into pieces (where appropriate) and canning in a brine solution (Fig. 1). Pickles made with *Almagro* eggplant have a long tradition in their region of origin, as well as in other parts of the Center and South of Spain, and in 1994 were recognized with a Protected Geographical Indication status (Muñoz-Falcón et al. 2009). In the last decades, other pickling eggplants from the neighbouring region of Andalucía (referred to as *Andalusian* landrace) have been introduced into the Campo de Calatrava county (Muñoz-Falcón et al. 2009). The introduced *Andalusian* eggplants present productive advantages over the *Almagro* landrace, as they have higher yields and lower prickliness. However, the pickles obtained from the *Andalusian* eggplant landrace have lower organoleptic quality (Prohens et al. 2007a).

The *Almagro* eggplant germplasm has traditionally been conserved by individual farmers. This strategy favours the maintenance of diversity of the landrace

(Gómez et al. 2005). Until we began our enhancement programme, no scientific breeding and selection had been applied for the improvement of *Almagro* eggplant.

Fig. 1 The *Almagro* eggplant landrace: commercial plantation in the Campo de Calatrava county (a), young plant (b), multiple infrutescences (c), typical fruit ready for harvest (d), highly prickly calyx (e), bag of harvested fruit ready to be transported (f), fruits ready for blanching (g), lactic fermentation (h), stuffing with pepper (in the case of stuffed eggplants) (i), and canning (j).

Enhancement through characterization

A detailed characterization is essential to assess the diversity and relationships of a landrace, to identify characteristics that make it distinct and unique, as well as to provide information on composition traits and properties that may be of interest to consumers (Hammer and Diederichsen 2009, Ciancaleoni et al. 2013, Torricelli et al. 2013). In the case of *Almagro* eggplant, we undertook the characterization from different points of view: morphological, molecular, chemical, and organoleptic.

Morphological characterization

Morphological characterization allows the description of a landrace as well as identifying the traits or combinations of traits which allow distinguishing it from other landraces or cultivars (Spataro and Negri 2013). The *Almagro* eggplant may be easily distinguished from eggplant varieties for regular use (cooking, frying) by a combination of multiple traits (e.g., small plant size, multiple inflorescences, fruit size, acrescent calyx, fruit colour). However, distinguishing it from other pickling landraces, like the *Andalusian* eggplant, may be more challenging as the gross morphology of the plant and the fruit is very similar (Prohens et al. 2007a; Muñoz-Falcón et al. 2009).

In order to obtain a morphological profile of the *Almagro* eggplant landrace, we characterized *Almagro* and *Andalusian* pickling eggplant accessions for morphological descriptors (Muñoz-Falcón et al. 2009). We found that the *Almagro* eggplant has smaller leaves, shorter fruit pedicels, smaller fruit area covered by the acrescent calyx, and higher prickliness than the *Andalusian* eggplant (Muñoz-Falcón et al. 2009). Therefore, measurement of a few morphological fruit traits can be of great utility for a rapid discrimination of *Almagro* eggplant fruits from the closely related *Andalusian* pickling eggplant. These morphological differences were essential for the registration of the *Almagro* eggplant as conservation variety in 2008, which may contribute efficiently to its conservation and enhancement (Spataro and Negri 2013).

However, environmental effects may difficult the distinction of authentic *Almagro* landrace materials from similar materials, especially if they have been cultivated under different environmental conditions. In this case, molecular characterization may be very useful for unambiguous identification.

Molecular characterization

The diversity and relationships of the *Almagro* eggplant with other regular use and pickling eggplant materials has been studied with AFLP and SSR markers (Muñoz-Falcón et al. 2009; Hurtado et al. 2012; Vilanova et al. 2012). A study of *Almagro* eggplant accessions with AFLP and SSR markers revealed that the *Almagro* eggplant materials presented considerable genetic intra-landrace diversity and were highly homozygous (Muñoz-Falcón et al. 2009), probably a consequence of autogamy (Pessarakli and Dris 2004). Genetic diversity is a typical characteristic of landraces (Hammer and Diederichsen 2009; Polegri and Negri 2010) and indicates that selection within the *Almagro* landrace may be effective.

Regarding the relationship of the *Almagro* landrace with other eggplant varieties, AFLP and SSR markers showed that *Almagro* eggplant clusters with other Occidental (i.e., from Europe, Middle East, Africa, America) eggplants (Hurtado et al. 2012, Vilanova et al. 2012). SSRs also clearly distinguished *Almagro* and *Andalusian* materials (Muñoz-Falcón et al., 2009), and two SSR alleles universal and specific to the *Almagro* eggplant were found. These markers can be used as a diagnostic tool to distinguish between *Almagro* and *Andalusian* pickling eggplant landraces and detect fake *Almagro* produce elaborated with the *Andalusian* landrace.

Chemical characterization

The chemical composition, in particular of bioactive compounds, of a vegetable landrace can give added value to the produce, as consumers are

increasingly valuing this type of information (Botonaki et al. 2006). Recently, San José et al. (2013) have studied the composition of one accession (H11) of *Almagro* eggplant and found it has higher values for protein content, vitamin C, glucose and total phenolics content than regular use varieties. Also, the high values of dry matter content, are probably the consequence of selection for good quality for pickling, as fruit with high moisture may result in pickles that do not present good firmness. Regarding bioactive constituents, Prohens et al. (2007b) found that *Almagro* eggplant ranked first out of 69 eggplant varieties for total phenolics content, a value that was up to four-fold higher than other eggplant varieties. The comparison of the content of chlorogenic acid content between *Almagro* eggplant, and the wild relative *S. incanum* L., which presents high content in chlorogenic acid (Prohens et al. 2013), has also revealed that *Almagro* eggplant presents chlorogenic acid content values higher than those of *S. incanum*. This may be of high relevance for the promotion of eggplant, as the healthy properties attributed to eggplant phenolics (Plazas et al. 2013), may contribute to increase demand substantially (Botonaki et al. 2006). In this respect, the reference to the high levels of phenolics of *Almagro* eggplant has been used in promotional advertisements of pickles elaborated with this landrace.

Organoleptic characterization

Good organoleptic properties are main drivers in the decision to purchase local varieties by consumers (Botonaki et al. 2006). The organoleptic properties of different accessions of *Almagro* and *Andalusian* pickling eggplants, as well as of hybrids between them, grown under the same conditions and subjected to the same pickling processing, was evaluated by a taste panel (Prohens et al. 2007a). The results from the panel showed that accessions of the *Almagro* eggplant presented higher scores for fruit colour, firmness, texture and global appreciation than the *Andalusian* landrace and inter-landrace hybrids. This is a clear indication that in order to obtain a final pickled produce with high quality it is necessary to use the local *Almagro* landrace instead of the introduced *Andalusian* landrace or hybrid materials. In

consequence, the certification that the elaborated prickles are produced with the local *Almagro* landrace provides an added value to the produce as it ensures a high standard of quality.

Enhancement through selection

Selection within a genetically heterogeneous landrace may result in an increase of favourable genetic combinations for the traits of interest. A participatory selection scheme was designed in order to select individuals of the *Almagro* eggplant landrace with high yield and low prickliness (Prohens et al. 2007a, 2009). Selected individuals could be used for bulk propagation, in order to maintain genetic diversity within the *Almagro* eggplant landrace or, alternatively, to obtain uniform *Almagro* eggplant pure lines. Fields of *Almagro* eggplant from farmers associated to the PGI were systematically screened by farmers, who looked in their own exploitation for plants that presented high yield and low prickliness. Selected plants were marked by farmers and a final selection of 27 plants from different farmers' fields was made by the team of breeders and farmers. The finally selected plants were pruned, uprooted, transplanted to pots into a greenhouse in Valencia and selfed during the winter season.

The increased seed of the selected plants was assessed for uniformity and tested in the fields of different farmers and evaluated for *Almagro* eggplant ideotype, including uniformity, yield, prickliness, and conformation with the typical characteristics of the *Almagro* eggplant. As a result of this process a pure line (H15), with higher yield and lower prickliness than the original *Almagro* landrace, was selected and registered as commercial variety (Prohens et al., 2009). This shows how individual selection within an autogamous landrace of a vegetable crop is an efficient and fast method to select pure improved pure lines with a limited effort.

Enhancement through breeding

Breeding programmes aimed at improving certain specific deficiencies of landraces while maintaining the genetic integrity of the landrace can result in the development of improved materials that maintain the characteristic traits of the landrace. In these cases, backcross breeding is an appropriate breeding method.

In the case of *Almagro* eggplant the presence of prickles in the calyx is the most detrimental characteristic. Therefore, we initiated a participatory backcross breeding programme in which we used the *Almagro* eggplant H15 selection as recurrent parent and three non-prickly eggplants as donors of the no-prickles trait. The three non-prickly donor parents were two black eggplants for regular use, of which one was a commercial F1 hybrid and the other a Spanish landrace, and a non-prickly *Andalusian* pickling eggplant accession. The latter accession, despite presenting some prickles under stress conditions was used as donor parent, as the genetic background is very similar to that of the *Almagro* eggplant (Muñoz-Falcón et al. 2009) and therefore, less backcross generations may be needed to recover the characteristics and genetic background of the *Almagro* eggplant.

Selection has been performed using a participatory approach in which segregating generations (500-1000 plants per selection cycle) are cultivated by farmers in the Campo de Calatrava county using the typical cultivation conditions (open field during summer season) for *Almagro* eggplant (Fig. 2). This allows an optimal exploitation of the genotype x environment interaction, as the selection is made under the specific conditions in which the finally selected materials will be grown. A pre-selection of individual plants most interesting on the basis of reduced prickliness and *Almagro* ideotype characteristics is made by farmers. A final selection of plants to be used for further backcrossing is performed by the breeders together with the farmers and the selected plants are uprooted and transplanted to greenhouses for making the backcrosses during the winter season so that seed from the next generation of backcrosses is available for the next summer season (Fig. 2).

We have found that plants of the F1 between the non-prickly parents and the H15 *Almagro* selection present a degree of prickliness intermediate between both parents. In the backcross generations, segregation has been observed being compatible with a model in which one major partially dominant gene and several minor genes control the prickliness trait (Doganlar et al. 2002). Therefore, it has been possible to select plants with lower prickliness in the backcross generations. Although segregation for fruit characteristics, like size, shape, and colour are observed in the early backcross generations (BC1, BC2), rapid recovery of the characteristics typical of the *Almagro* eggplant has been observed in the three backcross families. Once the *Almagro* eggplant genetic background has been recovered to a desired level (e.g., BC3: 93.75%; BC4: 96.88%; BC5: 98.44%), individual plants presenting the *Almagro* ideotype will be selfed or subjected to anther culture (Salas et al. 2012) in order to obtain pure lines in which the introgressed gene/s conferring low prickliness are in homozygous state. Preliminary results performed in the selfed generations from selected BC1 and BC2 plants show that there is segregation for prickliness and it is possible to select segregants with low or no prickliness in the selfed generations.

Development of F1 hybrids, which usually are heterotic for yield traits (Rodríguez-Burruezo et al. 2008), was also attempted to improve the *Almagro* eggplant. However, the *Almagro* hybrids were not more productive than the pure lines, probably because the genetic differences between accessions are too low to obtain heterosis for yield. Also, complementary crosses between *Almagro* and *Andalusian* eggplants were made. However, again, no heterosis was observed for yield and hybrids were intermediate for the morphological characteristics and prickliness (Prohens et al. 2007a; Muñoz-Falcón et al. 2009). In addition, the pickles elaborated with them did not reach the standards of quality of the *Almagro* eggplant landrace (Prohens et al. 2007a). Therefore, given that no productive advantage was evident for the F1 hybrids and that the production of F1 seed represents an additional cost, this breeding strategy was discarded.

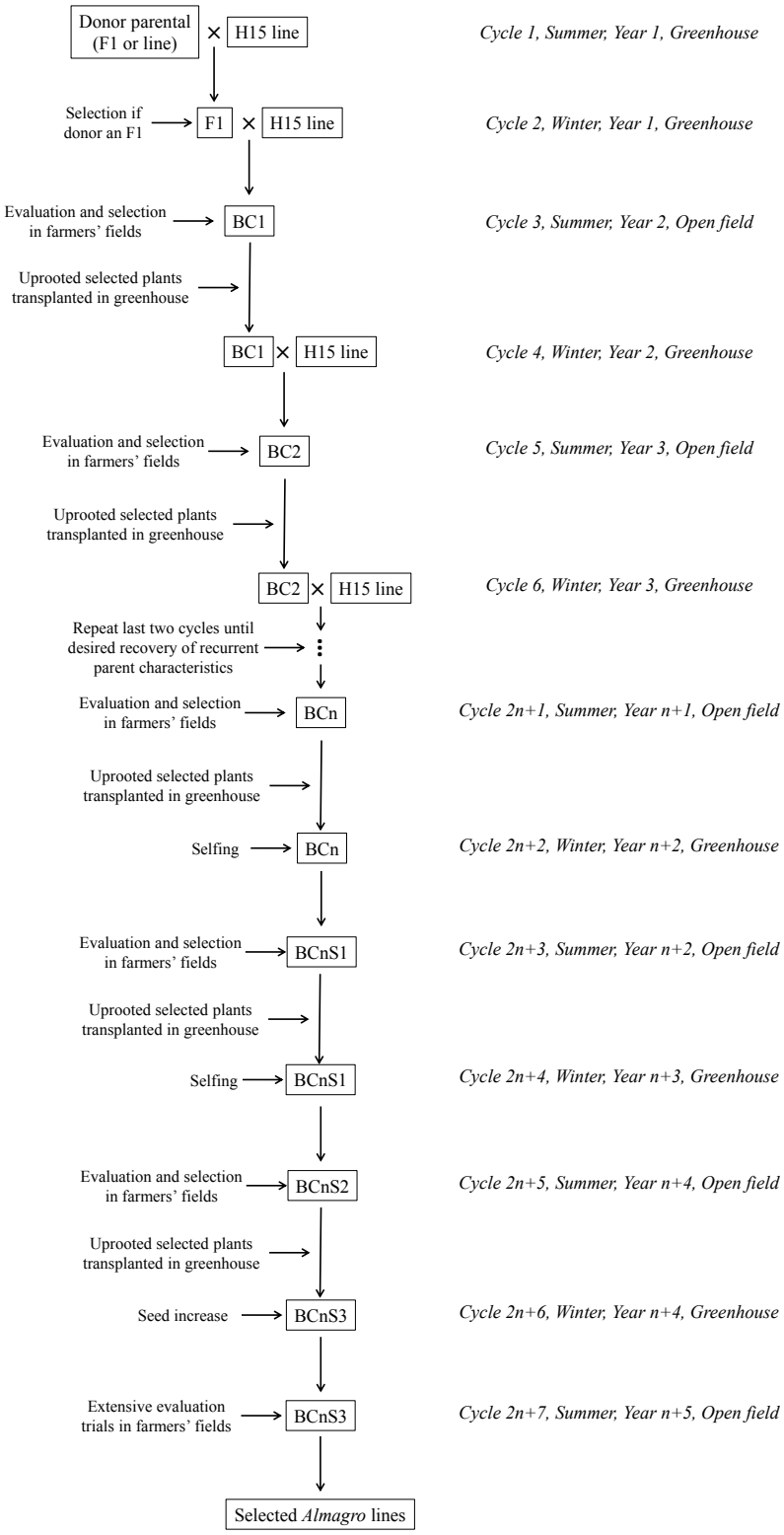

Fig. 2 Scheme of the participatory breeding programme for introgressing the no-prickles trait from three eggplant materials (a commercial F1 hybrid and a Spanish landrace for regular use, and a non-prickly *Andalusian* pickling eggplant accession). A single selection cycle is performed every year by

farmers in their own fields in the Campo de Calatrava county during the summer season, and crosses are made in greenhouse in Valencia during the winter season

CONCLUSIONS

Here we have shown that participatory enhancement programmes can make an effective contribution to recovering and providing added value to the *Almagro* eggplant landrace through different characterization, selection and breeding activities (Table 1). The use of a participatory approach, in which farmers have been involved in the characterization, selection, and breeding processes has facilitated the enhancement of the *Almagro* eggplant, including the registration as conservation variety and the selection of an improved line. In fact, since the programme was initiated, the cultivation of this landrace has increased steadily in the last 10 years from between 15-20 ha to 50-60 ha, while the total production has risen from 500-700 t to 1500-2000 t. Given the success with the *Almagro* eggplant landrace, we consider that the approach we have used can also be used as a model for the enhancement of other vegetable crop landraces (Table 1).

Table 1 Contribution of different characterization, selection, and breeding activities used for the enhancement of the *Almagro* eggplant landrace with have potential application to other landraces of vegetable crops

Activity	Contribution to landrace enhancement
Morphological characterization	Identification of traits that allow the fast and easy distinction from other similar landraces Information of relevance for the registration as conservation variety and for registration of improved selections as commercial varieties
Molecular characterization	Demonstration of the uniqueness of the landrace Specific and unique genetic fingerprint that ensures that the produce is elaborated with the landrace and protects from fakes
Chemical characterization	Identification of relevant nutritional and bioactive traits for which the landrace presents outstanding values
Organoleptic characterization	Demonstration of the superior value of the landrace over fakes and other similar materials
Selection	Development of materials by means of mass or individual selection, with improved performance while maintaining the characteristics of the landrace.
Breeding	Backcross breeding for the introgression of specific traits that improve the agronomic performance of the landrace while maintaining the characteristics of the landrace

Landraces and their elaborated products may be recognized by the European Union with a protected denomination, indication, or status (Trichopolou et al. 2007), which guarantees the quality of the produce and provides protection against imitation. In this respect, the morphological, molecular, chemical, and organoleptic characterization of landraces of vegetable crops, can make a relevant contribution to achieving and maintaining this recognition as they may support the claims that the landrace and/or its elaborated products are traditional, unique and have a high quality (Polegri and Negri 2010, Spataro and Negri 2013, Torricelli et al. 2013). Also, the information on the properties and characteristics of *Almagro* eggplant, in particular its high content in phenolics, could be relevant for increasing its demand and even for opening new markets (Botonaki et al. 2006).

The acreage devoted to landraces of vegetable crops is often limited, as the market is frequently local (Hammer and Diederichsen 2009; Spataro and Negri 2013). Therefore, seed companies usually do not establish selection and breeding programmes aimed at this type of materials. However, we have shown that a limited investment could result in a considerable enhancement of a vegetable landrace, which can result in an economically interesting alternative for farmers and that at the same time favours on-farm conservation of agricultural diversity (Piergiovanni and Laghetti 1999, Hammer et al. 2003, Polegri and Negri 2010).

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Increasing the Genetic Base of Modern Cultivars of Eggplant of the Semi-Long Black Type

Maria HURTADO[1], Santiago VILANOVA[2], Pietro GRAMAZIO[2], Mariola PLAZAS[2], Isabel ANDÚJAR[2], Francisco Javier HERRAIZ[2], Jaime PROHENS[2*]

[1]Meridiem Seeds S.L., Paraje Lo Soler 2, 30700 Torre Pacheco, Spain

[2]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

corresponding author: jprohens@btc.upv.es

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ABSTRACT

The eggplant (*Solanum melongena*) semi-long black varietal type is the most important in most European markets. Although open pollinated, pure lines and F1 hybrid cultivars exist within this varietal group, the latter predominate in the commercial production of eggplants, especially under greenhouse conditions. However, molecular markers studies have found that modern F1 hybrids present a reduced genetic base. This work is aimed at using a wide diversity of black eggplants for developing pure lines for obtaining hybrids heterotic for yield and increasing the genetic base of the black eggplants genepool. Thirty hybrid and non-hybrids varieties of black eggplants were used. Materials were characterized for morphological and agronomic traits of interest under greenhouse conditions. Molecular characterization was also performed using 16 SSR markers. A pedigree breeding programme was performed based on morphoagronomic traits until the F8 or F9 generations. Molecular analysis revealed that original materials could be separated in three main clusters groups, one made up by varieties from large companies that include the most successful varieties and the two other by an admixture of materials including non-hybrid varieties and F1 hybrids with moderate or low economic importance. The pedigree selection made allowed the final selection of 15 lines, which according to its origin, should have an increased genetic diversity compared to modern F1 hybrids. These lines, which present a very good performance under greenhouse conditions have been crossed in order to obtain hybrids heterotic for yield. These selected lines will be used to obtain a new generation of eggplant F1 hybrids with increased genetic base.

Keywords: *genepool, hybrids, pedigree breeding, Solanum melongena, SSR markers*

INTRODUCTION

Eggplant (*Solanum melongena* L.) is very variable for fruit colour, size and shape. These differences are commonly used to establish the different cultivar groups (Daunay, 2008; Muñoz-Falcón *et al.*, 2009). Fruit colour in eggplant is caused by the presence, distribution and amount of pigments in the epidermis, which are under the control of a few genes and QTLs and result in a wide diversity of colours (Daunay *et al.*, 2004; Daunay, 2008; Cericola *et al.*, 2014). The genetic control of fruit size and shape has also been studied and it has been found that a few major genes and QTL have a main role in determining fruit size and shape (Nunome *et al.*, 2001; Portis *et al.*, 2014). Also, an important fruit characteristic is calyx prickliness (Daunay, 2008). It has been found that a QTL in linkage group 6 plays a major role in prickliness of the different parts of the plant and fruit of eggplant (Frary *et al.*, 2014; Gramazio *et al.*, 2014)

In the European and Northamerican markets the most important varietal type is the black semi-long eggplant (Daunay, 2008; Muñoz-Falcón *et al.*, 2009). This type is characterized by presenting fruits of medium or large size (200-400 g), slightly obovate shape, intense black skin, and small calyx without prickles (Muñoz-Falcón *et al.*, 2009; Marín, 2015; Hurtado *et al.*, 2013). Most commonly used cultivars of this type cultivated under highly productive conditions of Spain and other countries corresponds to F1 hybrids (Marín, 2015). Although local varieties and non-hybrid cultivars of this type are still grown in several areas of Spain, they are increasingly being replaced by F1 hybrids, which have been generally available since several decades ago (Marín 2007, Daunay, 2008). Commercial F1 hybrids have almost exclusively been developed by private breeding programmes, which allow exploiting heterosis for yield (Sambandam, 1964; Rodríguez-Burruezo et al. 2008) and also do not breed true, therefore contributing to the protection of the variety from illegal reproduction. In the case of the black semi-long type, the development of F1 hybrids of the has been the result of selection of parental pure lines from a reduced genepool, which includes non-hybrid black varities like 'Black Beauty', 'Florida Market, and others (Savin, 1996; Daunay, 2008; Muñoz-Falcón *et al.*, 2009).

Most commercial breeding programmes rely on developing pure lines from already existing commercial F1 hybrids. Although reliable protocols exist for obtaining doubled haploids in eggplant through androgenesis (Dumas de Vaulx and Chambonnet, 1982; Salas *et al.*, 2012), developing pure lines directly from F1 hybrids is rarely used to obtain pure lines to be tested as potential parents for obtaining hybrids. The reason is that the frequency of pure lines with the desired characteristics or genotype is smaller than using a conventional method based on selfing and selection in each generation (Acquaah, 2012). In consequence, in comercial breeders normally develop pure lines in order to obtain hybrids using conventional methods based on selfing and selection. Androgenesis is mostly used to finally fix the selected materials in order to obtain completely homozygous materials that produce genetically uniform hybrids.

As a consequence of this common approach, the genetic diversity of the black semi-long F1 hybrids is becoming narrower, as it has been demonstrated with morphological and molecular markers (Muñoz-Falcón *et al.*, 2009) which difficults the development of significantly improved new cultivars. On the contrary obsolete and non-hybrid varieties of the black type present a much higher genetic diversity (Muñoz-Falcón *et al.*, 2009). Furthermore, a narrow genepool in the F1 hybrid genepool commonly used by breeders restricts the opportunities to exploit heterosis for yield resulting from obtaining hybrids from parents situated at a high genetic distance (Rodriguez-Burruezo *et al.*, 2008). In this respect, in many other crops it has been demonstrated that broadening the genetic diversity in the genepool available to breeders has allowed considerable advances in the development of new improved cultivars (Cooper *et al.*, 2001).

Our work was aimed at developing a breeding programme for the semi-long black type of eggplant in which new sources of diversity were included in order to broaden the genetic base of the genepool of this varietal type. The breeding programme has followed a pedigree scheme with phenotypic selection. Molecular markers have also been used to evaluate the diversity of the materials used for the breeding programme.

MATERIALS AND METHODS

Plant material. The starting plant material used consisted of thirty black eggplant varieties, of which 25 correspond to commercial F1 hybrids from different companies (Marín, 2015), including the most successful cultivars in the greenhouse vegetables production area of Almería (Southeast Spain), which is the most important in Spain (Torrellas et al., 2012), and 5 to non-hybrid varieties (Table 1). Some of the F1 hybrids varieties represent an élite genetic background, while others (those from the heirloom company Reimer) and the non-hybrid varieties were introduced in the programme in order to broaden the genetic diversity of the genepool and of the parental lines for obtaining commercial F1 hybrids. For the non-hybrid variaties crosses between them were used in order to develop F1 hybrids on which to start the selection programme. The varieties were transplanted to a greenhouse in Almería using a randomized block design, with five replicates (each with three plants) per variety.

Tab. 1. Varieties included as starting materials for the breeding programme, including the code used in the present work,

Code	Variety	Seed company	Type of material
102	Sultana	Batlle	F1 hybrid
103	Calanda	Ramiro Arnedo	F1 hybrid
104	Mulata	Ramiro Arnedo	F1 hybrid
105	AR04040	Ramiro Arnedo	F1 hybrid
106	Fantastic	Rijk Zwaan	F1 hybrid
107	Monarca	Rijk Zwaan	F1 hybrid
108	PX02326549	Petoseed	F1 hybrid
109	Petra	Fitó	F1 hybrid
110	Cristal	Fitó	F1 hybrid
111	Bellezza Nera	Vilmorin	Non-hybrid
112	De Barbentane	Vilmorin	Non-hybrid
114	Redonda Negra Lisa	F. Garden	Non-hybrid
115	Larga Negra	Ramiro Arnedo	Non-hybrid
116	LF3-24	INRA	Non-hybrid
117	Black Bell	Petoseed	F1 hybrid
118	Birga	Petoseed	F1 hybrid
119	Black Moon	Asgrow	F1 hybrid
120	Tasca	Vilmorin	F1 hybrid
121	Rendia	De Ruiter	F1 hybrid
122	Risorsa	Vilmorin	F1 hybrid
123	Brigitte	Rijk Zwaan	F1 hybrid
124	Longo	Rijk Zwaan	F1 hybrid
125	Money Maker	Reimer	F1 hybrid
126	Edna	Reimer	F1 hybrid
127	Millionaire	Reimer	F1 hybrid
128	Vittoria	Reimer	F1 hybrid
129	Nadia	Reimer	F1 hybrid
130	Night Shadow	Reimer	F1 hybrid
131	Dusky	Reimer	F1 hybrid
132	Epic	Reimer	F1 hybrid

For the rest of generations used in the breeding programme, a variable number of plants was used, ranging from 1200 to 1500 for each selection cycle. In these generations, in order to facilitate selection of all plants of a certain family, they were grown together in the same plot. Controls, consisting of F1 starting materials dominant in the market were included for comparisons in the generations in which selection was performed.

Morphological characterization.

A morphological characterization was performed based on agronomic and fruit traits of importance for the market in this varietal type: yield, fruit set under greenhouse conditions, fruit set recovery, fruit size and its uniformity, fruit shape and its uniformity, calyx size, calyx prickliness, skin colour and brightness. The ideotype was defined as a plant with high yield, good fruit set under greenhouse conditions, good fruit set recovery, intermediate fruit size with high uniformity, semi-long slightly obovate shape with highly uniform fruits, small calyx size, no calyx prickliness, intense black colour that does not fade, and high skin brightness.

Molecular characterization.

Genomic DNA was extracted from young leaves using the CTAB method (Doyle and Doyle, 1987) with some modifications. DNA quantitation was performed with a Nanodrop ND-1000 (Nanodrop Technologies, Wilminton, DE, USA) spectrophotometer, which allowed determining the DNA concentration and the absorbance ratios 260/280 nm and 260/230 nm, which provide an indication of DNA quality (Heptinstall and Rapley, 2000). In order to analyze DNA integrity we performed an electrophoresis in agarose gel at 1% and staining with ethidium bromide (0.8 mg/ml) and the bands were visualized with ultraviolet light in a transilluminator.

For the molecular analysis we used 16 genomic SSRs (Vilanova et al., 2012), which weew amplified by PCR using a PCR System 2700 Gene Amp (Applied Biosystems, Foster City, CA, USA) thermal cycler. Once the PCR reactions had been performed, amplifications were verified in an agarose (2%) gel using 2 ml of the PCR sample, 2 ml of bromophenol blue and 6 ml of milliQ H_2O. The analysis of the PCR products was performed using an ABI PRISM 3100-Avant (Applied Biosystems) automatic fragment analyzer. An UPGMA phenogram was obtained using Dice genetic distances.

Selection methodology. In order to develop pure lines for obtaining hybrids, we used the pedigree method (Fig. 1), based in selecting the best individuals in the F2

and then the best progenies and best individuals within each progeny (Acquaah, 2012). Seed obtained after selfing of the best individuals was used in order to obtain subsequent generations, up to the F8 generation. Traits used for selection were those indicated in the morphological characterization subsection. Molecular data were also considered in order to conserve a high diversity among the selected materials.

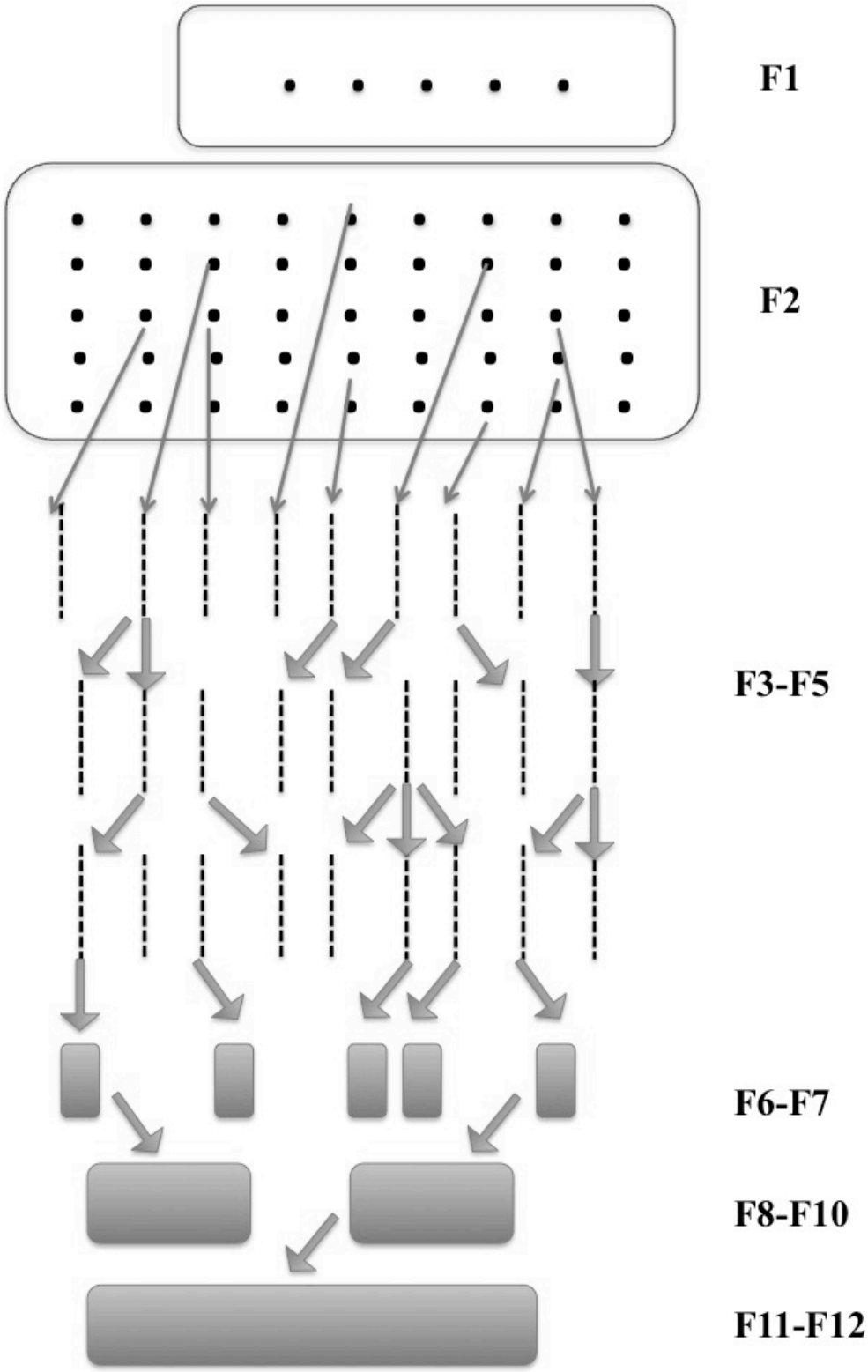

Fig. 1. Scheme of the pedigree method used for the selection of pure lines to be used as parents for developing new eggplant hybrids of the black semi-long type. Each generation corresponds to the selfing of selected individuals of the preceeding generation.

RESULTS AND DISCUSSION

Many differences were found among the original varieties in their performance for the traits studied. In general F1 hybrids performed better than the non-hybrid varieties, with the former varieties generally having higher yield and fruit set tan non-hybrid varieties. This result was expected, as most of the F1 hybrid varieties had been selected for greenhouse conditions, while the non-hybrid varieties include several varieties for open field cultivation (Marín, 2015). Given the important differences among greenhouse and open field cultivation in eggplant (Stommel *et al.*, 2015) F1 hybrids represent an élite germplasm with specific adaptation to the greenhouse conditions that include cultivation under reduced luminosity and cooler temperatures when compared to the typical cultivation conditions during the summer in the open field (Baixauli, 2001; Muñoz-Falcón *et al.*, 2009).

The molecular analysis of the thirty varieties used as starting material revealed that the materials were genetically diverse and differentiated into three major clusters (Fig. 2). One of the major clusters (cluster I) includes varieties from the major seed companies in eggplant seed marketing in southeastern Spain, like Asgrow, De Ruiter, Fitó, Petoseeds, Ramiro Arnedo, and Rijk Zwaan. These materials are the most extensively grown and have the greatest cost per seed (Marín, 2015). This is in agreement with the results obtained by Muñoz-Falcón *et al*. (2009), who using molecular markers, found that hybrids from major seed companies clustered together. No materials of the local varieties or from the small seed company Reimer are included in this cluster (Fig. 2), suggesting that modern greenhouse hybrids have an élite and differentiated genetic background. The results obtained from the morphological characterization also indicate that the F1 hybrids from cluster I are differentiated from the rest of varieties for having a larger and less elongated fruit than the rest of modern hybrids.

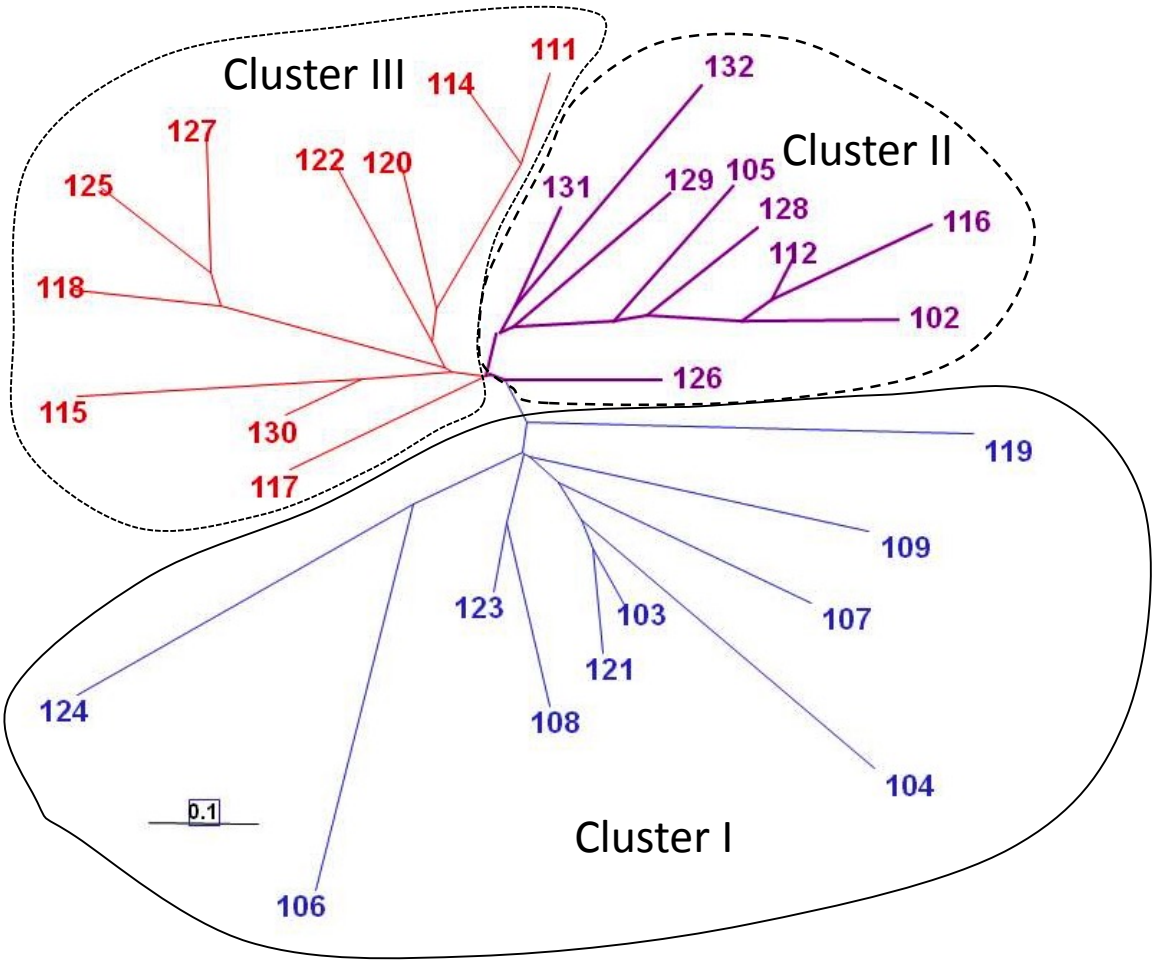

Fig. 2. UPGMA cluster analysis based and Dice genetic distance of genotyping data of 16 SSR markers. The three major clusters (I, II and III) have been indicated by lines of different typologies: cluster I (continuous line), cluster II (dashed line), cluster III (pointed line).

The two other major clusters (II and III) also contain a few modern hybrids not included in the cluster I, and which generally are not among the predominant cultivars, all the non-hybrid cultivars, and all the hybrids from Reimer company (Fig. 2). The hybrids of this latter company are generally recommended for open field cultivation. Amazingly, the two hybrids and the two non-hybrid varieties of Vilmorin are included in these two clusters. The eight hybrid varieties of Reimer seeds are very variable and are distributed in different branches in the clusters II and III. This is probably due to the fact that these hybrids include materials from the Occidental and Oriental genepools of eggplant (Vilanova et al., 2012). When compared with morphological traits, varieties 118 (Birga, from Petoseeds), 125 (Money Maker, from Reimer Seeds)

and 127 (Millionaire, from Reimer Seeds), which are situated in the same branch of cluster III, are the only ones which present anthocyanins in the calyx. The presence of anthocyanins in the calyx is controlled by four QTL, of which three are in linkage group 10 (Cericola *et al.*, 2014). This suggests that their common genetic background may arise from having taken this characteristic from the same source.

Overall, the results of characterization of the parents indicates that there is ample genetic diversity in the genepool of the black semi-long type of eggplant, and this could be used to increase the genetic diversity of modern cultivars of this type. This may contribute to increase the heterosis of F1 hybrids by crossing genetically distant parents (Rodríguez-Burruezo *et al.*, 2008), as well as to increase the morphological diversity (e.g., calyx with anthocyanins, smaller fruits, etc.) of modern F1 hybrids.

From the selfed progenies of each F1 hybrid, genealogical selection was performed using the target traits and selecting only those plants with better characteristics using a conventional pedigree selection scheme (Acquaah, 2015). In each generation, the selection was performed taking into account that a considerable genetic diversity, considering their provenance, was maintained (Fig. 3).

Fig. 3. One example of selected line, with a morphotype similar to the desired ideotype, and the way of tagging the plant.

This selection method resulted in that the morphotype of the selected materials in each generation was becoming more similar to the desired ideotype, as defined in Material and Methods, and more uniform, as the heterozygosis was reduced by one half in each generation of selfing. After subsequent selfing generations a total of 15 lines have been selected in the F8-F9 generations. These materials originate from different materials out of the thirty originally used, including varieties from each of the three clusters. Therefore, a large diversity is expected in these set of selected lines. Each of these lines is highly uniform and they are going to be genotyped and hybridized using a crossing-block scheme (Acquaah, 2015) in order to obtain the first hybrids, which will be used to select the most interesting for the market. Also, this fisrt set of hybrids will be used to evaluet the general combining ability of the different parental lines and the relationship between genetic distance and heterosis in these materials.

CONCLUSION

The methodology used, based in the use of molecular markers to select parents that represent an extended diversity compared to modern varieties combined with pedigree breeding has allowed developing phenotypically excellent pure lines of the black semi-long type of eggplant. These lines will be used to obtain a new generation of eggplant F1 hybrids with increased genetic base, effectively contributing to broadening the genepool of modern commercial F1 hybrids of eggplant.

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DISCUSIÓN

1. DIVERSIDAD GENÉTICA Y HERRAMIENTAS

1.1 Evaluación de la diversidad genética

Uno de los problemas con los que se encuentran la mayor parte de los mejoradores de berenjena en la empresa privada es la poca variabilidad genética que se encuentra en el material utilizado dentro de los programas de mejora, de forma que s variedades comerciales (y en particular los híbridos F_1) presentan una estrecha base genética (Muñoz-Falcón et al., 2009). Una de las razones es que la domesticación de la berenjena se realizó a partir de un acervo genético limitado, lo cual pudo originar un cuello de botella que contribuiría a que las variedades de berenjena cultivada presenten una baja diversidad genética (Isshiki et al., 1994, Karihaloo y Gottlieb, 1995; Meyer et al., 2012; Vorontsova et al., 2013). Por otra parte, durante la migración desde su zona de origen se produciría un nuevo cuello de botella genético, así como una diferenciación genética. A este respecto, en nuestro trabajo de marcadores moleculares en berenjenas de distintos orígenes hemos observado que la diversidad genética es moderada y que la diferenciación entre los materiales de China o Sri Lanka es menor que la existente con el material español. Esto es debido a que son dos países que se encuentran más cerca geográficamente y por tanto durante la domesticación y evolución del cultivo ha sido más fácil el intercambio o migración de materiales (Meyer et al., 2012: Cericola et al., 2013). Si a esta limitada diversidad del cultivo además le añadimos que dentro de los programas de mejora en empresa privada no se utiliza, en la mayoría de las ocasiones, recursos fitogenéticos de entidades públicas como Bancos de Germoplasma, sino que se usa material de la competencia para desarrollar nuevas líneas que se utilizarían como parentales para la obtención de nuevos híbridos, nos encontramos con un cuello de botella cada vez más acentuado.

Es por ello que se crea la necesidad de aumentar variabilidad, estudiando diferentes materiales como puede ser el de otros centros de origen, y compararlos con el nuestro para permitir conocer la diversidad existente en materiales exóticos. Teniendo en cuenta que la diversidad dentro de cultivares avanzados de *S.*

melongena no sólo se limita a caracteres de fruto como forma, tamaño y color, sino que también existen muchos otros caracteres en los cuales se puede encontrar una amplia variación como altura de la planta, habito de crecimiento, floración, pigmentación antociánica de algunas partes de la planta, presencia de espinas, lobulado de la hoja, etc. (Daunay and Hazra, 2012), esta diversidad puede ser útil para nuevos programas de mejora.

Al realizar estudios de comparación con otros centros de diversidad es cuando se observa que existen diferencias morfológicas entre orígenes, que seguramente será debido a diferentes criterios de selección durante la domesticación de la berenjena debido al uso o practicidad que se le dé a ésta en cada origen, y al efecto de deriva genética. En nuestro trabajo (Hurtado et al., 2012) se observó para la mayoría de los caracteres morfológicos diferencias significativas entre los tres orígenes demostrando que se podrían diferenciar entre ellos morfológicamente. Es más, con la utilización de un rango de 5-6 caracteres se podría asignar correctamente cualquier accesión a su centro de origen, lo cual indica un alto grado de diferenciación morfológica.

Dentro del material español, el análisis molecular da como resultado que las variedades utilizadas tipo negras tienen pocas diferencias genéticas. Como se ha dicho anteriormente era lo esperado (Vilanova et al., 2012), debido entre otras cosas a que es una planta con un alto grado de autogamia (Abak et al., 1998), y por tanto existe la fijación de alelos en homocigosis en aquellas poblaciones que no hay control artificial de la polinización.

Como se ha comentado anteriormente, la utilización de material de otros centros de diversidad es de gran importancia para los programas de mejora. Las diferencias encontradas entre orígenes indica la existencia de complementariedad para fuentes de variación en distintos caracteres morfológicos que no se encuentran dentro de tu centro de origen, pudiéndolo encontrar en otros centros de diversidad. A este respecto, Cericola et al. (2013) encontraron un resultado similar al utilizar marcadores moleculares. Por ejemplo, si como objetivo se busca la obtención de

material más grande y redondo se podría utilizar material de China, o si en cambio se busca material que nos dé un fruto con mayor brillo en la piel se podría utilizar material de Sri Lanka.

Además, a nivel genético y de interés comercial, utilizar material (como el estudiado) que presenta grandes diferencias entre orígenes, tiene gran interés para las empresas de semillas debido a que se puede utilizar como parentales dando híbridos muy heteróticos (Rodríguez-Burruezo et al., 2008). Además en este trabajo se ha observado que no existe correlación entre la caracterización morfológica y molecular ya que hay diferentes niveles de diversidad en los dos casos. Esto nos indica que, en los materiales que hemos usado, los dos métodos de caracterización son complementarios.

1.2 Utilización de marcadores moleculares.

El uso de técnicas moleculares puede ayudar a mejorar la eficiencia de los programas de mejora en varios aspectos. El análisis molecular de caracteres cuantitativos complejos permite determinar qué loci controlan aspectos fenotípicos de mayor interés, al igual que identificar genes con menores efectos fenotípicos, siendo también útil en programas de mejora de resistencia a plagas y enfermedades (Spooner et al., 2005). Mediante este tipo de técnicas es posible estudiar la diversidad y las relaciones existentes entre las distintas especies relacionadas de *Solanum*, lo que facilitaría que un acervo genético más diverso estuviese a disposición de mejoradores e investigadores para la mejora de la berenjena.

Por otro lado, las variedades tradicionales son el resultado de un proceso continuo de evolución basado en la selección, adaptación e interacción entre la planta, el agricultor y el medioambiente a lo largo de cientos de años. En este sentido, estas variedades tienen un valor intrínseco como patrimonio etnobotánico de un país, y como tal deberían ser conservadas (Zeven, 2002). No obstante como recurso fitogenético constituyen un excelente material de trabajo para el mejorador.

La utilización de un número reducido marcadores moleculares SSRs genómicos

ha permitido detectar una considerable variabilidad genética en una colección de variedades tradicionales de diferentes tipos morfológicos (Larga, Semi-larga, Redonda y Listada de Gandía). Este estudio vuelve a confirmar que España es un centro de origen secundario, igual que ya se propuso en otros trabajos anteriores (Prohens et al., 2005; Cericola et al, 2013). Y es que, al igual que se ha encontrado en otras variedades de berenjena (Muñoz-Falcón et al., 2011), los SSRs genómicos son más polimórficos que los EST-SSRs. Nuestro trabajo también confirma que los SSR genómicos desarrollados por Vilanova et al. (2012) a partir de una biblioteca genómica enriquecida, son de gran utilidad para el estudio de relaciones entre colecciones de germoplasma. Así, la mayoría de los loci SSRs estudiados son altamente informativos ya que presentan un número elevado de alelos (Na) y tienen valores bajos para la frecuencia del alelo predominante (fp) (valores cerca de mínimo teórico fp=1/Na), (Manzur 2009, Nunome et al., 2003, 2009). Por tanto, el trabajo realizado confirma que puede obtenerse información muy relevante sobre la diversidad y las relaciones de las berenjenas con un número bajo de marcadores SSRs genómicos.

Además dentro del material estudiado en esta tesis se han visto claramente diferencias genéticas entre y dentro de cuatro grupos de materiales locales valencianos de morfología diferente (Larga, Semi-larga, Redonda y Listada de Gandía). Por tanto, esto nos indica que los marcadores moleculares de tipo SSR, al igual que los AFLPs (Prohens et al., 2005) son útiles para estudiar grupos diferenciados por la forma del fruto, y confirmando que este tipo de variedades locales mostrando que son altamente homocigotos (debido a que son variedades tradicionales y autógamas) (Muñoz-Falcón et al., 2011). Esta información es de interés para la mejora genética, así como para la conservación de recursos genéticos de berenjena.

1.3 Herramientas para una caracterización morfológica precisa.

La caracterización de la forma del fruto de la berenjena es importante tanto para el mejorador, el agricultor, la empresa comercializadora, como para los bancos de germoplasma. Así, los grupos varietales en berenjena se establecen según la forma del fruto (Prohens et al, 2005; Daunay, 2008; Tümbilen, 2011; Marín, 2015). Hasta ahora, la caracterización fenotípica se realizaba con la utilización de descriptores morfológicos (Lester, 1990; Doganlar et al, 2002; Prohens et al, 2005, 2012), donde muchos de los caracteres que se podrían analizar en la práctica se descartaban debido a que eran demasiado cualitativos, pasando a caracterizarse sobre todo aquellos caracteres cuantitativos como longitud, anchura o peso del fruto.

El uso de nuevas herramientas de imagen, como el software Tomato Analyzer (Brewer et al, 2006; Gonzalo y van der Knaap, 2008; Rodríguez et al, 2010a, 2010b), ha permitido realizar el primer estudio de fenómica para la forma del fruto en una colección de germoplasma de berenjena. La utilización de dicho software demuestra que existe una mejora para la caracterización con respecto al modo manual o clásico, ya que se han podido analizar 23 caracteres cuantitativos y se han podido realizar comparaciones de forma directa para encontrar diferencias significativas entre materiales tanto dentro como entre grupos varietales. Con posterioridad en otros trabajos en berenjena se ha utilizado esta herramienta fenómica (Plazas et al., 2014). Por tanto, este primer estudio de fenómica para la forma del fruto de la berenjena abre el camino al uso de nuevas herramientas como software para análisis de imágenes (Tomato Analyzer en este caso) para la disección genética, a través de identificación de genes y QTLs que sean de interés para este cultivo. Además este tipo de herramientas presenta una mejora sobre la caracterización manual o clásica (tanto por comodidad y rapidez, como por precisión) y será de gran utilidad para la caracterización de recursos de germoplasma y de cultivos, así como para la selección y mejora de programas de berenjena.

2. APLICACIÓN DE LAS HERRAMIENTAS EN PROGRAMAS DE MEJORA GENÉTICA

En comparación con el tomate (*Solanum lycopersicum L.*) o pimiento (*Capscium annuum L.),* los dos otros principales cultivos dentro de las Solanaceae, los programas de mejora en berenjena han sido más limitados en cuanto al uso de material exótico en la mejora (Rotino et al., 2014). Mientras que en tomate por ejemplo se ha hecho uso de una amplia gama de diversidad genética para mejorar el cultivo (Díez y Nuez, 2008) , en berenjena como se ha comentado anteriormente el uso de recursos genéticos ha sido más restringido (Daunay y Hazra, 2012).

La intención de este trabajo en todo momento ha sido poder aumentar dicha diversidad, y sobre todo ayudar a los mejoradores e investigadores para que los programas de mejora de berenjena puedan llegar a ser tan genéticamente diversos como lo son los de otros cultivos, así como realizar selección de materiales con mejores características. Por ello se decide trabajar en tres programas de introgresión y mejora con objetivos diferentes, en los cuales se han utilizado diferentes niveles de diversidad genética y herramientas para la caracterización y selección.

2.1.Programa de Líneas de Introgresión (ILs)

Como se ha comentado al inicio de este trabajo, uno de los objetivos actuales en el mercado es obtener frutos con una alta calidad nutracéutica (Jenks y Bebeli, 2011). En este caso, se sabe que la berenjena tiene un alto poder antioxidante fundamentalmente debido a su contenido en polifenoles (Cao et al., 1996; Stommel y Whitaker, 2003) .

Sin embargo, al mismo tiempo uno de los caracteres que más se ha seleccionado en cuanto a la calidad aparente de la berenjena es el bajo pardeamiento de la carne (Plazas et al., 2013). El pardeamiento se produce al cortar el fruto y exponerlo al contacto del aire, produciendo una oxidación enzimática de los polifenoles mediada por 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, estudios como el de 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.

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). 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 es más fácil que utilizando especies más alejadas filogenéticamente (Lester y Hasan, 1991; Daunay, 2012).

Así que se considera de interés poder realizar un programa de Líneas de introgresión (ILs) con *S. incanum x S. melongena*. Las líneas de introgresión son un recurso genético valioso para la identificación, caracterización y confirmación de la presencia de QTL de importancia agronómica (Torjek et al. 2008; Falke et al. 2009; Di Matteo et al. 2010). De esta forma, el desarrollo de ILs proporciona ventajas para la mejora comercial al permitir estimar efectos de los QTLs en un fondo genético adecuado (normalmente una variedad élite) además, se pueden realizar estudios de interacciones QTL-ambiente, también, y al tener un bajo porcentaje de genoma silvestre se facilita la transferencia de un rasgo interesante a una variedad elite (Frary et al., 2003; Portis et al. 2014, 2015).

Las ILs presentan además ventajas sobre otras poblaciones segregantes, como la facilidad de piramidar caracteres deseables. Otras de las ventajas de las poblaciones de ILs es la posibilidad de visualizar un QTL como un factor mendeliano; esto es, transformar la genética cuantitativa en genética mendeliana (Liu et al. 2006;

Torjek et al. 2008; Ali et al. 2010). En nuestro caso, dado que *S. incanum* es parte del germoplasma primario de la berenjena (Knapp et al., 2013), el desarrollo de ILs es de gran importancia para la mejora, ya que permitirá que caracteres de interés de esta especie sean totalmente compatibles con el fondo genético de la berenjena. Y además será una herramienta de gran utilidad para el estudio de la evolución y domesticación de este cultivo (Zamir 2001).

Este trabajo se va seguir adelante, ya que en la actualidad se encuentra con el genotipado de la BC5, continuando hasta la obtención de un conjunto de líneas de introgresión deseado. Esperamos que, como ocurrió con el tomate (Lippman et al., 2007), algunas de estas ILs se utilicen directamente en programas de mejora de berenjena.

2.2 Programa de retrocruzamiento para la mejora de berenjena de Almagro.

Desde 1992, en la Unión Europea, los productos agrícolas que presenten características específicas asociadas a una alta calidad y que a su vez sean originarias de una cierta región, pueden ser protegidas de imitaciones por una Denominación de Origen Protegida (DOP) o una Indicación Geográfica Protegida (IGP). La principal ventaja de la protección mediante DOP o la IGP es que los productos que poseen esta categoría usualmente adquieren un valor añadido en los mercados (Gracia y Albisu, 2001; Babcock y Clemens, 2004).

En cuanto al cultivo de la berenjena a nivel europeo la única variedad protegida por una de las categorías antes mencionadas es la llamada "Berenjena de Almagro" la cual cuenta con una IGP, otorgada en 1994 (Castro, 2005). El programa de mejora de dicha berenjena está dirigido a resolver un problema específico, y es la presencia de espinas en el cáliz del fruto (Muñoz-Falcón et al., 2009b). Dado que la superficie dedicada al cultivo de esta variedad local es limitada y que el rendimiento (kg/ha) obtenido no da unos altos beneficios económicos en comparación con la

berenjena comercial (tipo negra) bajo invernadero (Castro, 2005), no existen programas de mejora como tal para dicha variedad.

Teniendo en cuenta que la protección IGP requiere que la producción o elaboración del producto final se realice dentro de una zona geográfica determinada, se consideró que sería de gran interés que este trabajo tuviera una interacción investigador-agricultor directa y estrecha. Es por ello que todo el proceso de selección y caracterización morfológica se realizó en campos de agricultores de Almagro. De ese modo, al realizar la selección en las condiciones específicas en que se cultivan los materiales seleccionados, permitió realizar una explotación óptima de la interacción *genotipo x ambiente*.

Al encontrarnos con una variedad local genéticamente heterogénea, era necesario basarse en un programa de selección individual (Prohens et al., 2007a, 2009). El material fue seleccionado tanto por su menor presencia de espinas como por su alto rendimiento en campo. Ello permitió obtener una línea pura (H15), con mayor rendimiento y menor espinosidad que se llegó a registrar como variedad comercial (Prohens et al, 2009). Por tanto, este tipo de selección individual dentro de un cultivo autógamo resulta un método eficaz y rápido para la obtención de líneas puras de interés.

En el trabajo para mejorar el carácter de espinosidad en la berenjena de Almagro como objetivo principal, hemos realizado un programa de retrocruzamiento con la utilización de la línea pura comercial H15 como parental recurrente y la utilización de berenjena (tipo negra y otra andaluza que morfológicamente se parece a de Almagro) con ausencia de espinas como parental donante. En las generaciones de retrocruzamiento, al ver la segregación para dicho carácter se ha observado que dicha segregación puede ser compatible con la idea que es un gen mayor parcialmente dominante y varios genes menores los que controlan dicho carácter (Doganlar et al., 2002, Frary y Doganlar, 2003, Prohens et al., 2009). Por tanto, ha sido posible la selección de individuos con menor presencia de espinas en las generaciones de retrocruzamiento. Y además aunque la morfología de los frutos de

los dos parentales presentaba grandes diferencias, se ha observado que existe una recuperación rápida (pocas generaciones BC2,BC3) de las características típicas de Almagro, siendo de gran interés para los programas de mejora y mejoradores de empresa privada, ya que interesa que dichos programas sean breves y obtener material comercial en el menor tiempo posible.

Es importante destacar que el haber realizado un programa de mejora donde la investigación pública ha estado en todo momento en contacto con el agricultor contribuye eficazmente a recuperar y aportar un valor añadido al cultivo a mejorar, en este caso a la berenjena de Almagro, tanto para la caracterización, selección como para el manejo del cultivo.

Además, sabiendo que la berenjena de Almagro tiene un alto contenido en polifenoles (Prohens et al, 2007), y habiendo observado que existe una recuperación rápida después de pocas generaciones de retrocruces hacia la forma del parental recurrente, resulta de interés poder utilizar este tipo de materiales en mejora comercial y así obtener líneas puras de berenjena tipo negra con mayor cantidad de polifenoles. Por otro lado, se ha demostrado con este trabajo que una inversión económica no muy elevada es posible mejorar este tipo de variedades locales, y que además el material obtenido puede resultar una alternativa económica para la empresa privada y al mismo tiempo favorecer la conservación de la diversidad agrícola (Pergiovanni y Laghetti 1999; Polegri y Negri 2010).

2.3 *Utilización de marcadores moleculares dentro de un programa de mejora en empresa privada.*

La mayor parte de variedades de berenjena se han obtenido utilizando la variación intraespecífica, siendo la variación interespecífica apenas utilizada para obtener líneas o materiales de premejora (Chadha, 1993, Plazas et al., 2014; Rotino et al., 2014). Si a eso la añadimos los cuellos de botella de la domesticación y del material vegetal utilizado dentro de programas de mejora genética en empresas privadas, nos encontramos con una diversidad cada vez más reducida. Y más si nos

basamos en la berenjena tipo semi-larga y negra, que es la que tiene mayor importancia económica (Marín, 2015).

Al empezar un programa de mejora en la empresa privada, interesa poder contar con una alta diversidad genética, ya que ello permite obtener combinaciones genéticas nuevas y explotar la heterosis (Rodríguez-Burruezo et al., 2008). Teniendo en cuenta el caso de la berenjena, se crea la necesidad de estudiar mediante la utilización de marcadores moleculares la variabilidad que existe a día de hoy en el mercado. Y poder de ese modo trabajar con material que presente grandes distancias genéticas para aumentar la variabilidad del material con el que vamos a trabajar.

Como se ha podido comprobar en el trabajo realizado (Hurtado et al, 2015), después de analizar con SSRs 30 variedades comerciales se pudieron separar 3 grandes grupos en los materiales de berenjena negra. Justamente, las variedades con mayor importancia económica (Marín, 2015) y que pertenecen a las casas de semillas más importantes (como Rijk Zwaan, Fitó o Seminis) se encuentran dentro de un mismo grupo, hecho que confirmar los resultados obtenidos por Muñoz-Falcón et al. (2009) con un menor número de variedades.

En general, los resultados de la caracterización molecular junto con la morfológica, indican que hay una amplia diversidad genética para determinar la forma del fruto de la berenjena tipo semi-larga y negra, sin recurrir a la utilización del material típicamente usado por los mejoradores, el cual tiene un fondo génetico común al haber sido derivado de las mismas fuentes. Por tanto se puede aumentar la heterosis de los híbridos F1 al cruzar parentales lo más distantes genéticamente (Rodríguez-Burruezo et al., 2008), así como para aumentar la diversidad morfológica y abrir mercados en otros lugares de interés económico. Por ejemplo, el mercado de berenjena en Italia busca frutos de tamaño mucho más globoso y variabilidad en el color de la piel, pasando de blanco a morado, tocando también tipo Listada de Gandía.

Mediante un programa de selección genealógica del material inicial, se fue seleccionando material con las mejores características mediante un esquema de selección de pedigree convencional (Acquaah, 2015). Después de generaciones de autofecundación se obtuvieron 15 líneas en generación F8-F9, que actualmente se encuentran en una finca de investigación comercial en El Ejido (Almería) con los cuales obtener los primeros híbridos comerciales mediante un esquema de cruces en bloque (Acquaah, 2015).

Es por ello que se considera, después del trabajo realizado, que la selección de parentales mediante el uso de marcadores moleculares ha permitido el desarrollo de líneas puras con un fenotipo excelente (dentro de los objetivos comerciales), y así poder obtener híbridos F1 con una base genética más amplia, y ampliar de ese modo el material comercial de la berenjena. Volviendo a confirmar que la utilización de nuevas herramientas en los programas de mejora es cada vez más necesario.

A lo largo de los años los programas de mejora en empresa privada se han basado en la selección por fenotipo como fuente de información de la variabilidad existente. Hoy en día la utilización de los marcadores moleculares que ayuden a estudiar las distancias genéticas, o que estén asociados a loci implicados tanto características cualitativas como cuantitativas (QTLs), permiten plantearse la selección asistida por marcadores basada en combinar la variabilidad fenotípica y genotípica como fuente de información de la variabilidad existente.

3. PRINCIPALES APORTACIONES DE ESTA TESIS

La relación entre la investigación pública y la empresa privada en nuestro país no ha sido fácil durante muchos años. En gran medida ello se ha debido a que los objetivos de cada uno de los dos sectores era distinto, de forma que en la investigación pública nos encontrábamos con objetivos para obtener conocimientos (ya sean más básicos o de innovación) de excelencia científica, mientras que en la empresa privada nos encontrábamos con estudios para obtener conocimientos y

tecnología que les permitiesen obtenerr productos a los clientes de forma más eficiente y competitiva.

Ahora mismo existe la necesidad de que haya además de excelencia científica, exista un objetivo con impacto económico y la mejor manera de conseguirlo es que quién dirija la investigación sepa dónde están las necesidades de la sociedad y que establezca objetivos de beneficio. Es por ello que és de gran interés la interacción "investigación pública-privada", en la que en todo momento exista una relación directa mientras se realice los proyectos conjuntamente.

En esta tesis se ha trabajado en todo momento conjugando los objetivos y necesidades de la empresa privada, generando así conocimientos o herramientas con una aplicación práctica a corto plazo en la sociedad, con la producción de conocimento de interés científico. Un ejemplo es la utilización de software de editores de imagen como el Tomato Analyzer, que presenta una mejora sobre la caracterización manual o clásica y será de gran utilidad para la caracterización de germoplasma y de cultivos, así como para la selección y mejora de programas de berenjena.

O como el caso del estudio de la diversidad genética de la berenjena, que como se ha comentado anteriormente resulta de gran interés para la empresa privada debido al cuello de botella existente para este cultivo. A nivel genético y de interés comercial, utilizar material (como el estudiado) que presenta grandes diferencias entre orígenes, tiene gran interés para las empresas de semillas debido a que se puede utilizar como parentales dando híbridos muy heteróticos. Al mismo tiempo permite obtener información de gran interés para entender la evolución y estructura genética del cultivo.

Es por ello que este trabajo además de demostrar que es factible y necesaria la interacción "investigación pública-privada", muestra que el estudio de la diversidad genética y el desarrollo y usos de herramientas para la caracterización morfológica, además de la obtención de material vegetal obtenido mediante un programa de

mejora, es de gran utilidad para el desarrollo de nuevas variedades de berenjena, así como para obtener información científico-técnica de interés para otros investigadores y mejoradores.

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CONCLUSIONES

CONCLUSIONES

1. La caracterización de accesiones de berenjena de tres centros secundarios de diversidad distanciados geográficamente (China, España y Sri Lanka), ha demostrado que existe una amplia diversidad y diferenciación tanto molecular como morfológica. A nivel morfológico existen evidencias de que a lo largo de la evolución del cultivo se han aplicado diferentes criterios de selección en cada centro secundario.

2. La falta de correlación entre la diversidad morfológica y molecular muestra que ambos caracteres proporcionan información complementaria entre ellos, y que ambos deben ser tomados en cuenta para el manejo de germoplasma. Esta información es de gran importancia para la mejora de la berenjena y el aumento de la diversidad, y sugiere que mediante el cruzamiento de materiales de diferentes orígenes se podrían obtener híbridos F_1 heteróticos para caracteres de rendimiento.

3. Dentro de los cuatro grupos de variedades locales valencianas establecidos por forma de fruto (Redonda, Listada de Gandía, Semi-Larga y Larga), se ha visto que molecularmente existe un alto grado de homocigosis, además de poder observarse que hay una cierta diferenciación entre los grupos. Estos resultados permitirán optimizar el uso de estos recursos en la mejora genética y conservación de germoplasma de variedades locales españolas.

4. La utilización de herramientas como el software Tomato Analyzer para la caracterización morfológica del fruto ha permitido una caracterización precisa de la forma del fruto de berenjena, de gran interés para el estudio de la diversidad de este carácter entre variedades de berenjena.

5. Mediante este primer trabajo de fenómica para la forma del fruto de la berenjena, hemos podido detectar diferencias entre variedades para la forma del fruto que no pueden ser detectados mediante caracterización con descriptores

convencionales. Así, la disponibilidad de datos fenómicos para la forma del fruto, representa una mejora para la caracterización manual y será de gran utilidad para la caracterización de recursos de germoplasma, así como para el trabajo en programas de mejora de berenjena.

6. Hemos iniciado una estrategia basada en distintos programas de mejora para la selección, mejora genética y ampliación de la base genética de la berenjena. A este respecto, el inicio del desarrollo de un conjunto de líneas de berenjena con introgresiones de la especie silvestre relacionada *S. incanum* es un primer paso para la obtención de un conjunto de materiales (líneas de introgresión) de gran interés para los mejoradores.

7. La realización de un programa de mejora participativo para la reducción de la espinosidad del cáliz de la Berenjena de Almagro basado en un esquema de retrocruzamientos está contribuyendo a la mejora de esta variedad local para un carácter muy importante a nivel productivo e industrial. Además toda la información generada sobre su diferenciación a nivel morfológico y molecular, obtención de una huella genética y determinación de su contenido en compuestos bioactivos está contribuyendo a la valorización de la misma.

8. El programa participativo de Berenjena de Almagro muestra que la inversión en programas de selección y mejora para variedades locales como la berenjena de Almagro, puede resultar una alternativa económica muy interesante para el sector productivo, además de favorecer la conservación in situ del germoplasma de variedades locales.

9. La utilización de marcadores moleculares para seleccionar materiales de berenjena con alta diversidad genética, ha permitido el desarrollo de líneas puras fenotípicamente excelentes para el tipo de berenjena negra semi-larga que presentan caracteres de interés comercial. Estos materiales serán de utilidad para la obtención de híbridos F_1 con una base genética mayor aumentado así la variabilidad dentro del material que existe en el mercado.

10. Se ha demostrado con este trabajo que la interacción "investigación pública-empresa privada" en mejora genética es sinérgica para el estudio de la diversidad genética y el desarrollo y uso de herramientas para la caracterización morfológica. De esta forma, se han producido avances de gran interés poder desarrollar nuevas variedades de berenjena y se ha obtenido información científico-técnica de interés para otros investigadores y mejoradores.