UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Application of conventional, biotechnological, and genomics approaches for eggplant (*Solanum melongena* L.) breeding with a focus on bioactive phenolics

PhD dissertation by

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Jai Bholenath Ji…..

*“**“Karmanye vadhikaraste Ma Phaleshu Kadachana, Ma Karma Phala Hetur Bhur Ma Te Sango Stv Akarmani”**.”*

Bhagvad Gita

Never doubted that it is only your blessings or encouragement or hope that I was able to succeed in completing my degree nothing else I think could work with me. Therefore, dedicated to you, as you have always unveiled in me a desire to thrive on the challenge of always striving to reach the highest mountain in everything I do.

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Resumen

Las hortalizas son una fuente importante de compuestos nutracéuticos y la berenjena (*Solanum melongena* L.) se encuentra entre las diez hortalizas con mayores contenidos en compuestos bioactivos. El compuesto más importante que proporciona capacidad nutracéutica a la fruta de berenjena es el ácido clorogénico, que es un ácido fenólico que deriva del ácido cinámico. Los compuestos fenólicos del fruto de la berenjena también están relacionados con el pardeamiento y los caracteres relacionados con el color de la pulpa de la fruta. La mejora del contenido en compuestos fenólicos del fruto de la berenjena se puede realizar a través de varios enfoques de mejora genética, como los convencionales, biotecnológicos y basados en la genómica. Antes del inicio de esta tesis, la información disponible sobre el uso de parientes silvestres de cultivos en el desarrollo de genotipos de berenjena ricos en compuestos fenólicos era limitada. La información sobre la genética de importantes características morfológicas y bioquímicas de la berenjena también fue limitada. Además, no había ningún protocolo estandarizado para realizar un estudio de agroinfiltración en frutos de berenjena. Por lo tanto, sobre la base de estos antecedentes, se plantearon varios objetivos para obtener información sobre estos temas en berenjena utilizando los parientes silvestres de cultivos y las variedades cultivadas tradicionales.

En el primer capítulo, se caracterizó una colección de seis accesiones de berenjenas, 21 accesiones de 12 especies silvestres (la única especies del germoplasma primario *S. insanum* y 11 especies del germoplasma secundario) y 45 híbridos interespecíficos de berenjena

con especies silvestres utilizando 27 descriptores morfológicos y 20 descriptores morfométricos de frutos basados en la herramienta fenómica Tomato Analyzer. Observamos diferencias significativas entre los tres grupos, con híbridos que muestran valores intermedios para la mayoría de los descriptores. Las especies silvestres mostraron una amplia diversidad para ampliar la base genética de la berenjena.

En el segundo capítulo, el mismo material se caracterizó para el contenido en compuestos fenólicos del fruto, el color de la pulpa de la fruta y los caracteres relacionados con el pardeamiento. Los parientes silvestres mostraron una mayor diversad para los contenidos en compuestos fenólicos del fruto que la berenjena cultivada. Mientras que el ácido fenólico predominante en la berenjena cultivada fue el ácido clorogénico (> 65.0%), en los parientes silvestres fue de menos del 50% del área del pico del cromatograma HPLC. Las variedades cultivadas presentaron un color de carne más claro y baja actividad de polifenol oxidasa (PPO). Los híbridos interespecíficos fueron intermedios para todos los caracteres estudiados. Curiosamente, no encontramos correlaciones significativas entre el contenido de compuestos fenólicos totales o ácido clorogénico y el pardeamiento de la pulpa de la fruta.

En el tercer capítulo, realizamos un estudio genético Linea por Probador (L × P) utilizando dos líneas cultivadas, una con citoplasma oriental y otra occidental con cuatro probadores que representan tres especies silvestres: *S. insanum*, *S.anguivi* y *S . lichtensteinii*. Además, evaluamos el material para 3 descriptores bioquímicos, 12 morfológicos y 8 de Tomato Analyzer. Encontramos diferencias

significativas para los 23 caracteres estudiados. Los valores más altos para la componente SCA se determinaron en comparación con la componente GCA. En general, los probadores del genepool secundarios fueron mejores para la mejora de los caracteres bioquímicos, mientras que las especies de genepool primarias fueron mejores para los caracteres morfológicos.

En el cuarto capítulo, para obtener información sobre la genética de caracteres importantes en la berenjena, realizamos el primer estudio genético detallado de la berenjena con 10 genotipos diversos de berenjena, entre ellos una accesión de *S. insanum*, mientras que el resto fueron variedades tradicionales con forma del fruto muy diversa. Cuando se cruzaron en un diseño de medio dialelo, los 10 padres produjeron un conjunto de 45 híbridos. Evaluamos padres e híbridos para 14 descriptores de características morfológicas y 14 características morfométricas del fruto. También determinamos las distancias genéticas utilizando 7,335 marcadores de SNP polimórficos. En el estudio de la herencia de los caracteres morfológicos importantes para el desarrollo de híbridos en berenjena, se encontró que la distancia genética entre los padres tiene un valor limitado para predecir el rendimiento de los híbridos en este cultivo.

Del mismo modo, en el quinto capítulo, estudiamos los compuestos fenólicos del fruto, el color de la pulpa del mismo y los caracteres relacionados con el pardeamiento en el mismo material. Se determinó que la accesión de *S. insanum* accession INS2 presenta valores altamente significativos para los compuestos fenólicos totales y el contenido de ácido clorogénico. Se encontraron efectos

significativos para la aptitud combinatoria específica y general para los caracteres bioquímicos estudiados. De manera similar a lo encontrado anteriormente, la distancia genética entre los padres no fue útil para predecir el comportamiento de los híbridos.

Finalmente, en el sexto capítulo, hemos desarrollado un protocolo de agroinfiltración para la expresión transitoria de un gen en el fruto de la berenjena utilizando GUS; Vector pCAMBIA1304. Posteriormente, para probar la utilidad del protocolo, utilizamos la hidroxicinamoil CoA-quinato transferasa (SmHQT) de berenjena, que es la enzima central estudiada para aumentar el contenido de ácido clorogénico, en la construcción del gen con el promotor específico en un vector de transformación de plantas (pBIN19). Además, en nuestro casete, también coexpresamos la proteína P19 del *Tomato bushy stunt virus* para sobreexpresar la proteína.

En esta tesis proporcionamos información sobre los parientes silvestres de berenjenas para caracteres morfológicos y bioquímicos. A partir de ellos, hemos proporcionado la base genética de la herencia de importantes caracteres cuantitativos en berenjena, en particular para alto contenido en compuesto fenólicos de interés nutracéutico. También hemos desarrollado una aplicación con un enfoque de ingeniería genética en berenjena. Esperamos que esta información sea útil para alcanzar un ideotipo de berenjena exitoso.

Summary

Vegetables are an important source of nutraceutical compounds and eggplant (*Solanum melongena* L.) is among the top ten vegetables rich in bioactive compounds. The most important compound that provides nutraceutical capacity to eggplant fruit is chlorogenic acid which is a kind phenolic acid that is derived from the cinnamic acid. The phenolics of eggplant fruit are also related to browning and fruit flesh colour related traits. The improvement of fruit phenolics of eggplant can be performed via various breeding approaches like the conventional, biotechnological and genomics based approaches. Before, the start of this thesis there was very limited information available regarding the use of crop wild relatives in the development of phenolic-rich and morphological comprehensive eggplant genotype. The information on the genetics of important morphological and biochemical traits of eggplant was also limited. Furthermore, there was not any standardised protocol to perform agroinfiltration studies on eggplant fruit. Therefore, based on this background several objectives were taken to find out the information on these lines for eggplant using the crop wild relatives and popular cultivated varieties.

In the first chapter, a collection of six eggplant accessions, 21 accessions of 12 wild species (the only primary genepool species *S. insanum* and 11 secondary genepool species) and 45 interspecific hybrids of eggplant with wild species were characterized using 27 morphological descriptors and 20 fruit morphometric descriptors based on the phenomics tool Tomato Analyzer. We observed significant differences among the three groups with hybrids showing intermediate

values for most of the descriptors. The wild species showed an extensive diversity for broadening the genetic base of eggplant.

In the second chapter, the same material was characterized for the fruit phenolics, fruit flesh colour and browning related traits. Wild relatives showed greater variation for the fruit phenolics than cultivated eggplant. While, the predominant phenolic acid in cultivated eggplant was chlorogenic acid (>65.0%), in the wild relatives it was less 50% of HPLC chromatogram peak area. Cultivated varieties had lighter flesh colour and low polyphenol oxidase (PPO) activity. The interspecific hybrids were found to be intermediate for the all the characters studied. Interestingly, we found no significant correlations between total phenolics or chlorogenic acid contents and fruit flesh browning.

In the third chapter, we performed a Line by Tester (L ×T) genetic study using two cultivated lines one with oriental and another with occidental cytoplasm along with four testers representing three wild species namely, *S. insanum*, *S.anguivi*, and *S. lichtensteinii*. Further, we evaluated the material for 3 biochemical, 12 morphological and 8 Tomato Analyzer based descriptors. We found a significant amount of variation for all the 23 traits studied. The higher values for the SCA component were determined as compared to the GCA component. Overall, the secondary genepool testers were better for the biochemical traits improvement whereas, the primary genepool species was better for the morphological traits.

In the fourth chapter, in order to gain information regarding the genetics of important traits in eggplant we performed the first detailed genetic study of eggplant with 10 diverse eggplant genotypes among

them an *S. insanum* accession, while the remaining nine were highly diverse shaped fruits of popular eggplant cultivars. When crossed in a half-diallel matting design 10 parents produced a set of 45 hybrids. We evaluated parents and hybrids for 14 morphological and 14 fruit morphometric traits descriptors. We also determined the genetic distances using 7,335 polymorphic SNP markers. In the study of the genetics of important morphological traits for hybrid development in eggplant, we found that genetic distance among parents had limited value to predict hybrid performance in this crop.

Likewise, in the fifth chapter, we studied the fruit phenolics, fruit flesh colour and browning related traits in the same material. We determined that *S. insanum* accession INS2 displayed values highly significant for the total phenolics and CGA content. Significant specific and general combining ability effects were found for the biochemical traits studied. Similarly, the genetic distance among parents was nonsignificant to predict the hybrids performance.

Finally, in the sixth chapter, we have developed an agroinfiltration protocol for the transient expression of a gene in the eggplant fruit using GUS bearing; pCAMBIA1304 vector. Thereafter, to prove the usefulness of the protocol, we have used the eggplant hydroxycinnamoyl CoA-quinate transferase (SmHQT), which is the central enzyme studied to increase the chlorogenic acid content, in a gene construct with the specific promoter in a plant transformation vector (pBIN19). Also, in our cassette, we also co-expressed the P19 protein of *Tomato bushy stunt virus* to overexpress the protein.

Overall, in this thesis, we have provided information regarding the wild relatives of eggplants from a morphological and biochemical prospective. Thereafter, we have provided the genetic basis of the inheritance of important quantitative traits in eggplants, in particular for high contents of phenolic compounds of nutraceutical interest. We have also developed an application of a genetic engineering approach in eggplant. We hope this information will be useful in reaching a successful eggplant ideotype.

Resum

Les hortalisses són una font important de compostos nutracèutics i l'albergínia (*Solanum melongena* L.) es troba entre les deu hortalisses amb majors continguts en compostos bioactius. El compost més important que proporciona capacitat nutracèutica al fruit d'albergínia és l'àcid clorogènic, que és un àcid fenòlic que deriva de l'àcid cinàmic. Els compostos fenòlics del fruit de l'albergínia també estan relacionats amb el pardejament i els caràcters relacionats amb el color de la polpa de la fruita. La millora del contingut en compostos fenòlics del fruit de l'albergínia es pot realitzar a través de diversos enfocaments de millora genètica, com els convencionals, biotecnològics i basats en la genòmica. Abans de l'inici d'aquesta tesi, la informació disponible sobre l'ús de parents silvestres de cultius en el desenvolupament de genotips d'albergínia rics en compostos fenòlics era limitada. La informació sobre la genètica d'importants característiques morfològiques i bioquímiques de l'albergínia també va ser limitada. A més, no hi havia cap protocol estandarditzat per a realitzar un estudi d' agroinfiltració en fruits d'albergínia. Per tant, sobre la base d'aquests antecedents, es van plantejar diversos objectius per a obtindre informació sobre aquests temes en albergínia utilitzant els parents silvestres de cultius i les varietats cultivades tradicionals.

En el primer capítol, es va caracteritzar una col·lecció de sis accessions d'albergínies, 21 accessions de 12 espècies silvestres (l'única espècie del germoplasma primari *S. insanum* i 11 espècies del germoplasma secundari) i 45 híbrids interespecífics d'albergínia amb espècies silvestres utilitzant 27 descriptors morfològics i 20 descriptors

morfomètrics de fruits basats en l'eina fenómica Tomato Analyzer. Observarem diferències significatives entre els tres grups, amb híbrids que mostren valors intermedis per a la majoria dels descriptors. Les espècies silvestres van mostrar una àmplia diversitat per a ampliar la base genètica de l'albergínia.

En el segon capítol, el mateix material es va caracteritzar per al contingut en compostos fenòlics del fruit, el color de la polpa de la fruita i els caràcters relacionats amb el pardejament. Els parents silvestres van mostrar una major *diversad per als continguts en compostos fenòlics del fruit que l'albergínia cultivada. Mentre que l'àcid fenòlic predominant en l'albergínia cultivada va ser l'àcid clorogènic (> 65.0%), en els parents silvestres va ser de menys del 50% de l'àrea del pic del cromatograma HPLC. Les varietats cultivades van presentar un color de carn més clar i baixa activitat de polifenol oxidasa (PPO). Els híbrids interespecífics van ser intermedis per a tots els caràcters estudiats. Curiosament, no trobem correlacions significatives entre el contingut de compostos fenòlics totals o àcid clorogènic i el pardejament de la polpa de la fruita.

En el tercer capítol, realitzem un estudi genètic Línia per Emprovador (L × P) utilitzant dues línies cultivades, una amb citoplasma oriental i una altra occidental amb quatre emprovadors que representen tres espècies silvestres: *S. insanum*, *S. anguivi* i *S. lichtensteinii*. A més, avaluem el material per a 3 descriptors bioquímics, 12 morfològics i 8 de Tomato Analyzer. Trobem diferències significatives per als 23 caràcters estudiats. Els valors més alts per a la component SCA es van determinar en comparació amb la

component GCA. En general, els emprovadors del genepool secundari van ser millors per a la millora dels caràcters bioquímics, mentre que les espècies de genepool primari van ser millors per als caràcters morfològics.

En el quart capítol, per a obtindre informació sobre la genètica de caràcters importants en l'albergínia, realitzem el primer estudi genètic detallat de l'albergínia amb 10 genotips diversos d'albergínia, entre ells una accessió de *S. insanum*, mentre que la resta van ser varietats tradicionals amb forma del fruit molt diversa. Quan es van creuar en un disseny de mitjà dial·lel, els 10 pares van produir un conjunt de 45 híbrids. Avaluem pares i híbrids per a 14 descriptors de característiques morfològiques i 14 característiques morfomètriques del fruit. També determinem les distàncies genètiques utilitzant 7,335 marcadors de SNP polimòrfics. En l'estudi de l'herència dels caràcters morfològics importants per al desenvolupament d'híbrids en albergínia, es va trobar que la distància genètica entre els pares té un valor limitat per a predir el rendiment dels híbrids en aquest cultiu.

De la mateixa manera, en el cinqué capítol, estudiem els compostos fenòlics del fruit, el color de la polpa del mateix i els caràcters relacionats amb el pardejament en el mateix material. Es va determinar que l'accessió de *S. insanum* accessió INS2 presenta valors altament significatius per als compostos fenòlics totals i el contingut d'àcid clorogènic. Es van trobar efectes significatius per a l'aptitud combinatòria específica i general per als caràcters bioquímics estudiats. De manera similar a l'oposat anteriorment, la distància genètica entre els pares no va ser útil per a predir el comportament dels híbrids.

Finalment, en el sisé capítol, hem desenvolupat un protocol d'agroinfiltració per a l'expressió transitòria d'un gen en el fruit de l'albergínia utilitzant GUS; Vector pCAMBIA1304. Posteriorment, per a provar la utilitat del protocol, utilitzem la hidroxicinamoil CoA-quinat transferasa (SmHQT) d'albergínia, que és l'enzim central estudiat per a augmentar el contingut d'àcid clorogènic, en la construcció del gen amb el promotor específic en un vector de transformació de plantes (pBIN19). A més, en el nostre casset, també coexpresem la proteïna P19 del *Tomato bushy stunt virus* per a sobreexpresar la proteïna.

En aquesta tesi proporcionem informació sobre els parents silvestres d'albergínies per a caràcters morfològics i bioquímicos. A partir d'ells, hem proporcionat la base genètica de l'herència d'importants caràcters quantitatius en albergínia, en particular per a alt contingut en compost fenòlics d'interés nutracèutic. També hem desenvolupat una aplicació amb un enfocament d'enginyeria genètica en albergínia. Esperem que aquesta informació siga útil per a aconseguir un ideotip d'albergínia exitó

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INTRODUCTION

Breeding Vegetables with Increased Content in Bioactive Phenolic Acids

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Abstract

Vegetables represent a major source of phenolic acids, powerful antioxidants characterized by an organic carboxylic acid function and which present multiple properties beneficial for human health. In consequence, developing new varieties with enhanced content in phenolic acids is an increasingly important breeding objective. Major phenolic acids present in vegetables are derivatives of cinnamic acid and to a lesser extent of benzoic acid. A large diversity in phenolic acids content has been found among cultivars and wild relatives of many vegetable crops. Identification of sources of variation for phenolic acids content can be accomplished by screening germplasm collections, but also through morphological characteristics and origin, as well as by evaluating mutations in key genes. Gene action estimates together with relatively high values for heritability indicate that selection for enhanced phenolic acids content will be efficient. Modern genomics and biotechnological strategies, such as QTL detection, candidate genes approaches and genetic transformation, are powerful tools for identification of genomic regions and genes with a key role in accumulation of phenolic acids in vegetables. However, genetically increasing the content in phenolic acids may also affect other traits important for the success of a variety. We anticipate that the combination of conventional and modern strategies will facilitate the development of a new generation of vegetable varieties with enhanced content in phenolic acids.

Keywords: breeding; bioactive properties; genetic variation; molecular markers; phenolic acids; vegetable

1. Introduction

Plant breeding programs have mostly concentrated on yield improvement, resistance to diseases, tolerance to abiotic stresses, longer shelf life, early or late production, and varietal diversification. However, consumers are increasingly becoming aware of the potential benefits resulting from diets rich in fruits and vegetables for maintaining a good health and preventing diseases [1]. In this respect, the scientific literature provides a wealth of information that correlates a diet high in fruits and vegetables with better health and disease prevention [2,3]. This has stimulated a growing demand for vegetables with enhanced contents in bioactive compounds. Many bioactive molecules derived from vegetables are effective due to their antioxidant activity, which prevents the formation of reactive oxygen, nitrogen, hydroxyl and lipid species, by scavenging free radicals or by repairing or removing damaged molecules [4,5]. The most relevant antioxidant bioactive molecules found in fruits and vegetables generally include hydrosoluble vitamins, carotenoids, and phenolics [6–8]. Occasionally, other classes of molecules, like glucosinolates in the case of brassicas [9], have relevant bioactive properties that contribute to the functionality of fruits and vegetables.

Among the major groups of bioactive compounds of vegetables, phenolic acids (molecules containing a phenolic ring and an organic carboxylic acid function) are becoming the focus of attention of many researchers given their properties for human health and their relative abundance in vegetables (Table 1). Phenolic acids are one of the

diverse classes of the many different phenolic compounds synthesized by plants and are commonly found in plant-derived foods [10–12]. The bioactive properties of phenolic acids from vegetables are numerous (see below in the section “Properties of phenolic acids”). This has resulted in an increasing interest in breeding for enhanced content in phenolic acids content in vegetables [13,14].

Increasing the content in phenolic acids content of vegetables can be achieved by a variety of means, including development of improved cultivars, use of specific cultivation conditions, and application of postharvest treatments [15]. In this review, we will focus on breeding new cultivars with improved content in phenolic acids. This will require identifying the phenolic acid compounds most important and abundant in vegetables, the search for sources of variation (including crop wild relatives) with potential as breeding materials, and discussion of breeding strategies and biotechnological approaches appropriated for developing new vegetable varieties with enhanced content in phenolic acids.

Table 1. Average contents of total phenolic acids in different vegetables (mg/100 g of fresh weight) ranked according their average concentration (adapted from [12]).

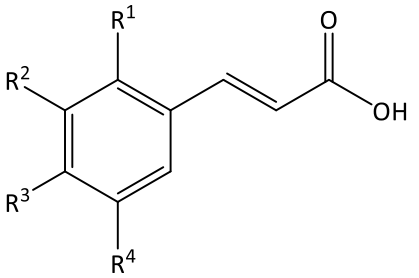
Vegetable	Total Phenolic Acids [mg/100 g fw]	Major Soluble Phenolic Acids
Eggplant (*Solanum melongena*)	32.0	chlorogenic
Carrot (*Daucus carota*)	29.5	chlorogenic, caffeic, protocatechuic
Red beet (*Beta vulgaris*)	27.0	ferulic
Basil (*Ocimum basilicum*)	22.0	chlorogenic
Broccoli (*Brassica oleracea var. italica*)	15.0	sinapic, caffeic
Radish (*Raphanus sativus var. sativus*)	12.0	*p*-coumaric, ferulic
Spinach (*Spinacia oleracea*)	11.0	chlorogenic, protocatechuic, gallic
Chinese cabbage (*Brassica pekinensis*)	7.7	sinapic, chlorogenic
Parsley (*Petroselinum crispum*)	6.2	protocatechuic
Parsnip (*Pastinaca sativa*)	5.7	chlorogenic
Lettuce (*Lactuca sativa* var. *capitata*)	5.1	chlorogenic
Pepper (*Capsicum annuum*)	4.7	chlorogenic, *p*- coumaric, ferulic, protocatechuic
Cauliflower (*Brassica oleracea var. botrytis*)	4.6	*p*-coumaric, sinapic, chlorogenic
Turnip (*Brassica rapa*)	4.6	sinapic, ferulic, chlorogenic

White cabbage (*Brassica oleracea var. capitata* f. *alba*)	3.8	sinapic, *p*-coumaric
Grean bean (*Phaseolus vulgaris*)	3.5	chlorogenic, protocatechuic
Tomato (*Solanum lycopersicum*)	3.5	chlorogenic
Pea (*Pisum sativum*)	1.3	sinapic
Onion (*Allium cepa*)	1.0	protocatechuic, *p*-coumaric
Zucchini (*Cucurbita pepo*)	0.9	*p*-coumaric, caffeic
Cucumber (*Cucumis sativus*)	0.1	*p*-coumaric, ferulic

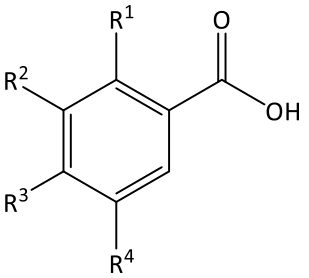
2. What are phenolic acids?

Phenolic acids are secondary metabolites characterized by the presence of an aromatic ring with an organic carboxylic acid functionality. Phenolic acids derive from benzoic and cinnamic acids; and although their basic structure remains the same, the number of the hydroxyl groups and their positions on the aromatic ring vary greatly resulting in different phenolic acids [16–18]. The most commonly found phenolic acids derived from benzoic acid in vegetables include gallic, *p*-hydroxybenzoic, syringic and vanillic acids, while those derived from cinnamic acid include caffeic, chlorogenic, ferulic, *p*-coumaric and sinapic acids [18] (Figure 1). Generally, the concentration of the derivatives of cinnamic acid in fruits and vegetables is higher than that of benzoic acid, except for certain red fruits and other plant products [19]. In this respect, chlorogenic acid, which is caffeic acid esterified with quinic acid (Figure 2), is pre-

eminent among phenolic acids in many vegetables [12]. Phenolic acids can be found in plant tissues either as in a free or, more frequently, in a bound form. The bound fraction is generally found as esters, glycosides or in complexes [20,21].



Cinnamic acid derivatives



Benzoic acid derivatives

Substitution	**Cinnamic Acid Derivatives**	**Benzoic Acid Derivatives**
R^3=OH	*p*- Coumaric acid	*p*- Hydroxybenzoic acid
R^3=R^4=OH	Caffeic acid	Protocatechuic acid
R^2=OCH_3, R=OH_3	Ferulic acid	Vanillic acid
R^2=R^3=R^4=OH		Gallic acid
R^2=R^4=OCH^3, R^3=OH	Sinapic acid	Syringic acid
R^2=R^3=OH [plus the carboxylic group being esterified with quinic acid]	5-*O*-caffeoylquinic acid	

Figure 1. Chemical structures of major cinnamic and benzoic acids derivatives found in vegetables.

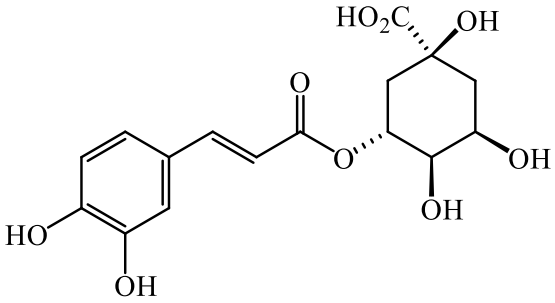


Figure 2. Structure of chlorogenic acid, a pre-eminent phenolic acid derivative present in many vegetables.

Apart from their interest for human health, phenolic acids are very important for the quality of plant-based foods: they are substrates for enzymatic browning, and may affect the overall flavour [22,23]. Furthermore, phenolic acids are the signaling molecules involved in plant-microbe interactions [24]. Knowledge of the biochemical pathway of phenolic acid is important for molecular breeding strategies. Phenolic acids are biosynthetically formed through the shikimic acid pathway from L-phenylalanine or, to a lesser extent, from L-tyrosine [25]. The core pathway for the biosynthesis of phenolic acids involves the synthesis of cinnamic acid from L-phenylalanine catalyzed by phenylalanine ammonia-lyase (PAL) [26]. Cinnamic acid is then further transformed, through the catalytic action of different enzymes (e.g., hydroxylases, methytransferases), into many varieties of phenolic acids, catalyzed by (Figure 3). Benzoic acid is synthesized from cinnamic acid via the β-oxidative pathway [27]. Regarding derivatives of benzoic acid, hydroxylation and methylation processes are similar to those

occurring for cinnamic acid derivatives, resulting in derived phenolic acids (Figure 3) [25,28].

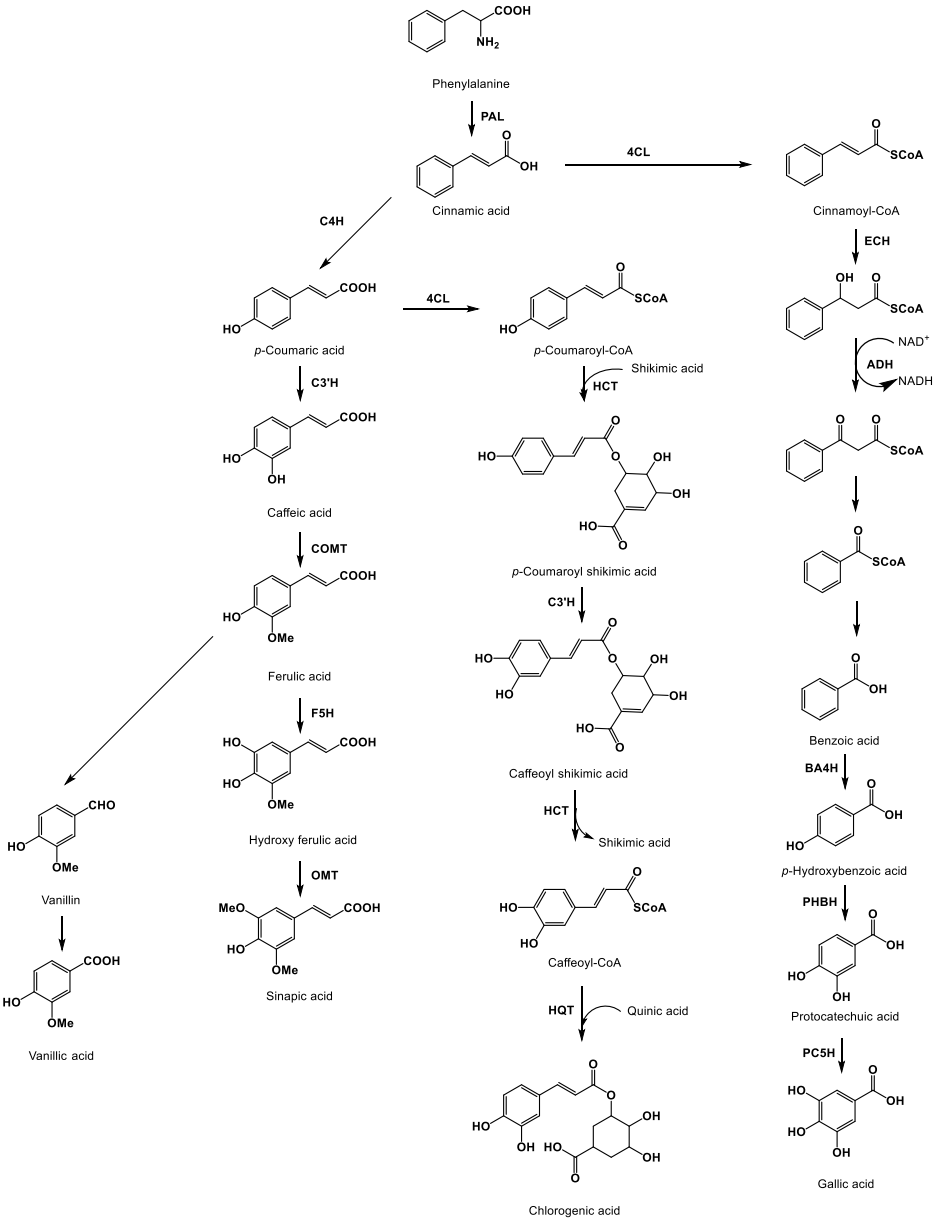


Figure 3. Schematic representation of some of the core biochemical pathways of major phenolic acids present in vegetables [25,29,30]. Enzymes involved in the pathways are indicated: PAL, phenylalanine ammonia lyase; C4H, cinnamate-4-hydroxylase; C3'H, p-coumarate 3'-hydroxylase; COMT, caffeic acid 3-*O*-methyltransferase; F5H, ferulate 5-hydroxylase; OMT, *O*-methyltransferase; 4CL 4-hydroxycinnamoyl-CoA-ligase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; HQT, hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase; ECH, enoyl-CoA hydratase; ADH, cinnamoyl alcohol dehydrogenase; BA4H, benzoic acid 4-hydroxylase; PHBH, p-hydroxybenzoic acid 3-hydroxylase; PC5H, protocatechuic acid 5-hydroxylase.

3. Bioactive Properties of Phenolic Acids

Phenolic acids are powerful antioxidants as they act by donating hydrogen or electrons, which can delay or inhibit the oxidation of bio-molecules (DNA, proteins, and lipids) [7]. The high correlation coefficient between phenolic acids content and antioxidant capacity in vegetables reveals that they play a main role in the bioactive properties of these plant products [31]. The antioxidant capacity of the phenolic acids depends on its structure, and it is higher in molecules with large number of hydroxyls [5]. In this respect, *in vitro* antioxidant activities of phenolic acids are even much higher than those of other major antioxidants present in vegetables, like vitamin C, E, and β-carotene [32]. There are many studies showing that phenolic acids are beneficial for human health and have a main role in preventing chronic diseases and therefore an adequate intake of phenolic acids should be part of a healthy and equilibrated diet [10,21,33,34]. Many epidemiological studies have revealed biological activities beneficial for human health of phenolic acids present in vegetables such as cardioprotective, anticarcinogenic, antimicrobial, hepatoprotective, antianxiety, antidiabetic and antiobesity properties [21,35–39].

4. Breeding for Increased Phenolic Acids Content

Conventional breeding techniques, based on selection and hybridization, have shown a high potential for enhancing the content of bioactive compounds in a wide range of plants [40,41]. Genetic improvement of phenolic acids content can be accomplished by different techniques, like simple mass selection or individual selection of plants with desirable characteristics for seed or vegetative propagation, or through the deliberate crossing of closely or distantly related individuals in order to produce new crop varieties or hybrids with increased contents (Figure 4). Genetic variation is necessary for efficient and successful selection and breeding for increased phenolic acid contents, and usually most of their variation is quantitative rather than qualitative [42,43]. Therefore, in general the conventional selection and breeding methods to be used for enhancing the content in phenolic acids in vegetables will be those of quantitative traits.

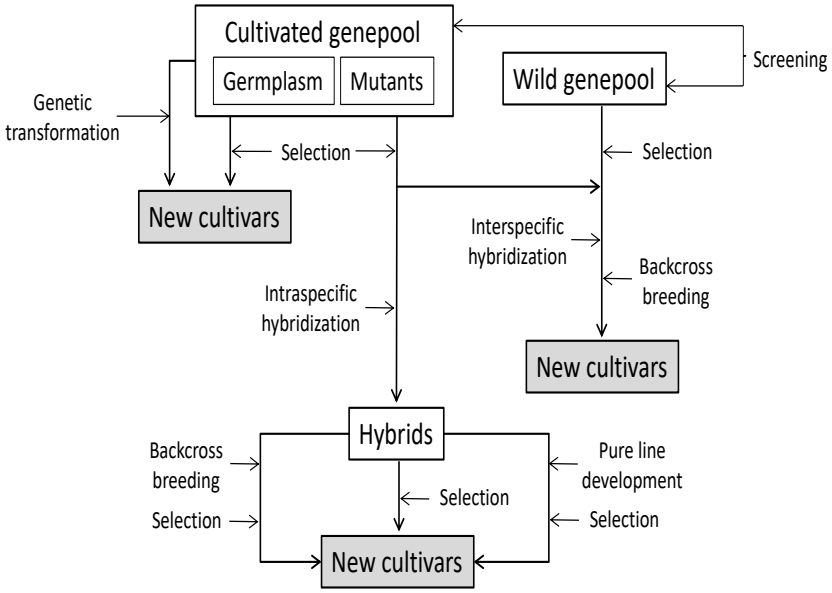


Figure 4. Summary of the main strategies for the development of new vegetable cultivars with increased content in phenolic acids. Screening and selection steps can be performed using phenotypic selection, marker assisted selection or both.

Identification of Sources of Variation

Large variation has been found for phenolics acid content among samples of cultivated species [43–52]. Table 2 presents the variation found in different vegetables for chlorogenic acid content, revealing that large differences may exist within a single species for a given phenolic acid. This variation, which can be of several fold differences among accessions of the same species, can be exploited to select

varieties with higher content in phenolic acids or to identify parental materials for breeding programmes (Figure 4).

Table 2. Intraspecific variation for chlorogenic acid [g·kg^{-1} dw] content in different vegetables.

Vegetable	Chlorogenic Acid [g·kg^{-1}]	References
Artichoke (*Cynara scolymus* L.)	0.4–7.3	[45]
Carrot (*Daucus carota*)	0.3–18.8	[46]
Chicory (*Cichorium intybus* L.)	0.1–0.9	[47]
Eggplant (*Solanum melongena*)	1.4–28.0	[48,49]
Lettuce (*Lactuca sativa* L.)	0.1–0.3	[50]
Pepper (*Capsicum annuum*)	0.7–0.9	[51]
Tomato (*Solanum lycopersicum*)	0.2–0.4	[52]

In some cases, morphological characteristics can provide an indication of the level of phenolic acids and therefore can be of interest for a preliminary selection of materials with potentially high content in phenolic acids. For example, Leja *et al.* [53] found that carrots with purple color roots possessed on average nine-fold higher phenolic acid content than carrots of other colors. Also, Vera-Guzmán *et al.* [54] reported that the color coordinates and chroma values presented a positive correlation with phenolic acid contents in *Capsicum* pepper. In the case of potato it was noticed that the pigmented cultivars like "Purple Majesty" and "Mountain Rose" contained considerably higher levels of chlorogenic acid isomers than the non-pigmented cultivars [55].

The origin may also be used on occasion for identification of sources of variation. For example, carrots of the Eastern (Asian) genepool often had higher content in phenolic acids than Western (European and American) genepool carrots [53]. Also, geographically-restricted Southeast Asian eggplants [*S. melongena* subsp. *ovigerum*] had a higher content in phenolic acids as well as greater diversity than eggplants from other regions [56].

Single mutations may represent an important source of variation for phenolic acid content (Figure 4). For example, mutants defective in light perception such as the high pigment [*hp*-1] mutant of tomato with increased fruit color result possess elevated chlorogenic acid content [57]. Also by utilizing somaclonal variation, a lettuce variety with high levels of chlorogenic acid was obtained [58].

Wild relatives are an important source of variability that can be used by plant breeders to develop vegetable varieties with increased contents in phenolic acids (Figure 4). For example, Meléndez-Martínez *et al.* [59] found that wild tomato species are a potential resource for increasing the phenolic acid content of tomato, as they presented higher concentrations than cultivated tomato. Also, in eggplant it has been found that artificial selection has resulted in a reduction in phenolic acids content and that wild relatives usually have higher contents in phenolic acids than cultivated eggplant [56]. In this crop, *S. incanum*, a wild relative of cultivated eggplant with high content in phenolic acids is being used in eggplant breeding programs as a source of variation for the introgression of this trait in the genetic background of eggplant by backcrossing [60,61]. Mennella *et al.* [62] studied the

content in chlorogenic acid in lines of eggplant containing introgressions from three related species that had been selected for resistance to *Fusarium* and agronomic traits and found that *S. sodomaeum* introgression lines were highest in chlorogenic acid compared to introgression lines derived from two other species [*S. integrifolium* and *S. aethiopicum*]. Nonetheless, despite the interest of wild species as sources of variation for high content in phenolic acids, there are also associated disadvantages for breeding programmes, as they present many undesirable traits from the agronomic and commercial point of view [60,63,64]. As a result, selection against these traits has to be performed in the backcross generations. When traits to be removed are monogenic and dominant, selection will be much easier to be done than when are polygenic and with recessive inheritance.

Gene Action and Heritability

Knowledge of gene action and heritability values is important for devising efficient breeding strategies. However, there are few examples of determining these parameters for phenolic acids in vegetable crops. In a recent study by Prohens *et al.* [60], using a backcross population between cultivated eggplant and *S. incanum*, it was found that a simple additive-dominance model, in which only the additive variance was significant, explained the genetic variance for phenolic acid conjugate constituents. This indicates that genes from the wild species favoring the accumulation of phenolic acids should be in

homozygosis in order to obtain higher contents in phenolic acids. Heritability studies for phenolic acid content of scarlet (*S. aethiopicum*) and gboma (*S. macrocarpon*) eggplants found moderate to high values of heritability for chlorogenic acid content and other phenolic acid contents and indicates that selection for these traits will be efficient in breeding programs [65].

The phenolic acid content is influenced by the growing environment and its interaction with the genotype [15,66,67]. For example, a recent study carried out by Stommel *et al.* [43] in order to evaluate the influence of the environment on fruit phenolics content in 12 different eggplant genotypes found a high genotype × environment interaction for phenolic acids content. However, these authors suggested that selection for stability could result in the selection of varieties with a reduced variability in phenolic acids content resulting from cultivation in different environments.

QTL and Candidate Genes for Phenolic Acids Conten

New developments in molecular biology, genomics and metabolomics have provided new relevant information on the synthesis of phenolic acids. Detection and mapping of quantitative trait loci (QTL) in segregating populations or germplasm collections provides information of high interest for marker assisted selection and breeding [68]. Therefore, mapping major QTL for phenolic acids content will facilitate incorporation of this trait into élite vegetable cultivars through marker assisted selection. Also, the candidate gene approach, which

may be linked to the detection of QTLs, shows promise given that the genes involved in the phenolic acid synthesis pathway are known [Figure 3]. These genes are candidates for having a role in the accumulation of phenolic acids. In this respect, the genes codifying for enzymes involved in the core chlorogenic acid synthesis pathway in eggplant [PAL, C4H, 4CL, HCT, C3′H, HQT] were mapped on the eggplant genetic map, and it was shown that all of them, except for 4CL and HCT, were not linked, which may facilitate pyramiding of favorable alleles in a single variety [61]. The role of genes involved in the pathway of synthesis of phenolic acids on the accumulation of these compounds has been confirmed in some studies. For example, in tomato, the overexpression of the HQT gene increased the content in chlorogenic acid [30], while in potato it was found that the suppression of the expression of the HQT gene resulted in a reduction in the chlorogenic acid content of over 90% [69]. Other genes are also of interest for increasing the content in phenolic acids in vegetables. For example, in the case of tomato a major candidate gene associated to higher phenolic acid content expressing in fruit was identified as ERF1, which is a key gene in orchestrating the genes for phenolic content production in tomato [70]. In addition, the availability in Arabidopsis and other model plants of a large number of mutants of genes from the various branches of the phenylpropanoid pathway [71] may facilitate the identification in vegetables of candidate genes for increasing the content in phenolic acids.

5. Genetic Transformation for Increasing Phenolic Acids Content

Many transgenic strategies are available to enhance the nutritional value of crops; these strategies offer a rapid way to introduce desirable traits into elite verities [72], including the development of new cultivars with increased contents in phenolics (Figure 4). However, only a few studies in vegetables have been reported to increase phenolic acids content by using genetic transformation approach. For example, chlorogenic acid was increased up to 1.8-fold in tomato via constitutive expression of the hydroxycinnamoyltransferase HQT gene [30]. In an another recent study by Amaya *et al.* [73], the ectopic expression of the D-galacturonate reductase (FaGalUR) gene from strawberry aimed at increasing the ascorbic acid content led to a moderate increase in this antioxidant, but it simultaneously resulted in an increase of more than two-fold in chlorogenic acid content of tomato fruit. Also, the MYB family transcription factor AtMYB11 from *Arabidopsis* was noticed to be involved in the regulation of caffeoylquinic acid synthesis in tomato, as after transformation the transgenic plants had a significant increase in chlorogenic acid (18.1-fold) content compared to the non-transformed wild-type; also the contents of dicaffeoylquinic acids and tricaffeoylquinic acids were 68.0-fold and 108.4-fold higher in transgenic plants as compared to the wild-type. In the case of potato, constitutively expressed anti-sense strawberry chalcone synthase gene (CHS) resulted in a dramatic reduction of anthocyanin, flavonol and proanthocyanidines levels, while the phenylpropanoid pathway was

upregulated leading to an increase in chlorogenic and caffeic acids contents [74].

Despite the potential of genetic transformation for increasing the content in phenolic acids in vegetables, the public acceptance of these genetically engineered crops is generally low [75]. In this respect, cisgenesis is a promising alternative to transgenesis for genetic engineering, with potentially less social rejection. Cisgenesis consists in the genetic transformation of a variety using only genetic material from the sexually compatible genepool [76]. In that case, it requires the identification of genes for phenolic acids from the sexually compatible genepool for introduction via genetic transformation.

6. Collateral Effects of Breeding for Phenolic Acids in Vegetables

Phenolic acids have relevant roles in plant life, including the response against biotic and abiotic stresses [77]. Apart from their bioactive properties for humans, phenolic acids have been associated with sensorial qualities of foods [78]. Additionally, the food industry has investigated the effects of phenolic acids on fruit maturation, enzymatic browning, and their roles as food preservatives [60,77]. In consequence, increasing phenolic acids content in vegetables may have an impact in other traits of interest, like tolerance to biotic and abiotic stress, browning, or flavor that should be taken into account in breeding new vegetable crops varieties.

Biotic and Abiotic Stresses

Phenolic acids are known to confer resistance to infection by a large number of pathogens, including fungi, bacteria, and viruses [79,80]. Increased synthesis of phenolic acids, which are incorporated to the cell wall of plants, takes place in response to biotic stress [81]. Phenolic acids are also known for their role in resistance to insect pests [82]. In this respect, resistance to thrips in chrysanthemum is attributed to higher chlorogenic and feruloyl quinic acid content [83]. Shivashankar *et al.* [84] found that resistance in chayote fruit against melon fly (*Bactrocera cucurbitae*) infestation was correlated with higher levels of *p*-coumaric acid. Nematoxic effects have also been reported for some acids like chlorogenic acid after nematode penetration [85]. It has also been demonstrated that phenolic acids may increase the tolerance to abiotic stresses. For example, salinity tolerance in lettuce is positively correlated with higher levels of chlorogenic acid [86]. In summary, the increase in the content in phenolic acids in vegetables may have a positive effect on resistance or tolerance to biotic and abiotic stresses. In this way, breeding for high content in bioactive phenolics in vegetables may lead to varieties more tolerant to stresses, which is an important objective in vegetable crops breeding.

Browning

Raising the total phenolic acids content may cause a negative effect on apparent quality of the fruit. In the case of vegetables when the tissue of interest is cut, phenolic acids, mostly stored in vacuoles, are oxidized resulting in brown coloration, *i.e.*, enzymatic browning [22,87]. Enzymatic browning is mostly mediated by polyphenoloxidase enzymes. These oxidoreductases catalyze the hydroxylation of monophenols to diphenols. This reaction is comparatively slow and results in colorless products. Subsequently the same polyphenoloxidase enzymes catalyze the oxidation of diphenols to quinones, which is a fast reaction that yields brown colored products [88]. In consequence, a drawback of increasing the concentration of phenolic acids is that it may lead to a reduction in the apparent quality caused by the browning after exposure to the air [89]. However, it has been proposed that simultaneous selection for high content in phenolic acids combined with low activity PPO may result in a reduced or negligible impact on browning because of increased levels of phenolic acids in vegetables susceptible to enzymatic browning [61,90]. In this respect, it has been demonstrated, using transgenic approaches, that suppression of PPO activity results in a dramatic reduction of browning [91,92].

Flavour

Phenolic acids can contribute to the astringency and a have potential for causing bitterness in foods [93]. However, it has been found that phenolic acids, like chlorogenic acid, at the concentrations normally present in vegetables do not cause appreciable amount of bitterness [94], which is normally caused by other compounds like saponins, isocoumarins, glucosinolates and other compounds, like calcium, that may enhance bitterness [95–97]. In some cases, like in carrot, the content of isocoumarins increases with stress and can be responsible for the occasional bitter taste of carrots [96]. In the case, of phenolic acids, the cultivation environment may also have an important role in the phenolic acid levels [43], but it is unknown if this may have an effect on flavour of vegetables. Since literature is scarce on the effect of phenolic acids on flavour of vegetables further studies are needed to confirm the role of increased concentration of these acids on different flavour aspects of vegetables.

7. Future Prospects and Challenges

The development of vegetable crops with enhanced content in phenolic acids will benefit from the integration of conventional and modern techniques. In this respect, the germplasm collections of vegetable crops are largely unexplored regarding the content in phenolic acids and may allow the discovery of materials with high contents in phenolic acids [46,65,90]. Knowledge of candidate genes

involved in the synthesis of phenolic acids [29,30,61] may also lead to the detection of new alleles in germplasm collections using EcoTILLING or sequencing techniques [98]. Also, the sequencing of genomes and the use of synteny among related species may be of great interest for the very precise approach and better detection of the genes and QTLs involved in phenolic acids accumulation in vegetable crops with limited genomic information [99]. Genome editing is also creating new opportunities for designing new varieties with increased content in phenolics through a non-transgenic approach [100,101]. With all the information already available and new developments, breeders have the challenge to develop a new generation of vegetables with enhanced bioactive properties resulting from an increased content in phenolic acids. These new varieties will have to be adapted to market requirements in terms of yield, shape, and organoleptic properties, which requires an integral breeding approach.

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Conflicts of Interest

The authors declare no conflict of interest.

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Visiting Eggplant from a Biotechnological Prospective: A Review

Abstract

Eggplant is a member of family Solanaceae, and it is commonly cultivated in many parts of the world. Eggplant is susceptible to a number of biotic and abiotic stress, therefore, there is a continuous demand of varieties with insect pest and disease resistance, better nutraceutical capacity, and adapted to climate change. Biotechnological approaches/tools have helped in the expansion of eggplant ideotype. In this direction, tissue culture techniques for organogenesis and somatic embryogenesis are standardised in eggplant. Also, plant transformation techniques like agrobacterium mediated gene transfer has been established in eggplant. Even if, the information of eggplant from a biotechnology perspective is increasing yet there is a lot to explore. Techniques like gene editing have not been tried in eggplant, further, eggplant is still remains unexplored from the molecular farming prospective. In this review we compile the information regarding tissue culture, genetic engineering, and genome editing advancements so far accomplished in the eggplant

1. Introduction

Solanum melongena L. a member of Solanaceae is generously cultivated as fruit vegetable in sub- tropical and tropical regions of the world. It is commonly known as eggplant, aubergine, guinea squash or brinjal (especially in the Indian subcontinent) (Daunay, 2008). Eggplant, is majorly being grown in China, India, Egypt, Iran, and Turkey, etc. In 2018, 1.87 million ha were cultivated in the world for a total production of 51.28 million tonnes, of which 62% and 24% of the world production were covered by China and India respectively (FAO, 2016). There is an extensive diversity in cultivated eggplant in terms of its biochemical, phenotypic and physiological traits (Taher et al., 2017). Recently, breeders have focused on the wild relatives of eggplant with conventional, biotechnological, and genomics-based approaches (Gramazio, 2018).

Eggplant have a capacity to regenerate from tissues (explant), this regeneration capacity of plants has revolutionised the use of plants for genetic engineering approaches (Lynch, 2014; Smith, 2013; Vasil and Thorpe, 2013). In case of vegetable crops breeding, techniques involving gene transfer through sexual and vegetative propagation are well established (Bisognin, 2011; Lei, 2010). The general aim is to introduce genetic diversity into existing plant populations, further, to select and develop superior plants that carrying genes for desired explicit traits this is majorly done by the conventional breeding techniques (Dennis et al., 2008). The application of these conventional or classical techniques has resulted in significant accomplishments like

achieving the yield targets for vegetable crops (Borlaug, 1983). With the rapid development and advancement in genetic engineering techniques/methods, based on the knowledge of gene structure and function, the scope of vegetable improvement has been dramatically broadened (Dalal et al., 2006).

Genetic transformation technologies offer direct access to useful genes not previously accessible to plant breeders (Lusser et al., 2012). During the earlier days , techniques were available to transfer a single gene into the plant genome at a time, but now with the advancement, improved genetic engineering techniques, can simultaneously transfer several genes as a single event (Puchta and Fauser, 2013). Genetic engineering offers the possibility of integrating a desirable gene from closely related plants (cisgenic) without associated deleterious genes (linkage drag) or from related species, which do not readily cross with the crop of interest or from unrelated species (transgenic) even in other taxonomic phyla (Jacobsen and Schouten, 2007; Moradpour and Abdullah, 2017). Eggplant is susceptible to a wide range of biotic and abiotic stresses; therefore, insertion of resistance genes, along with yield and fruit quality improvement, is one of the main concerns of conventional breeding and biotechnology (Daunay, 2008; Daunay and Hazra, 2012).

Although, in many species, the development of rapid, highly efficient transformation, regeneration systems are still in progress and thus represents a bottleneck in the development of stable high yielding transgenic plants. Sustainable and economical use of genetic

engineering in crop improvement requires successful development and deployment of transgenic plants (Mba et al., 2012). Because of the advances in genetic transformation and gene expression technologies, a rapid progress has been observed during the last decade for the use of genetic engineering for crop improvement in terms of different biotic, abiotic stresses and male-sterility systems (Chase, 2006; Marco et al., 2015; Sree and Rajam, 2015). In case of eggplant some reviews are published on biotechnology and genetic engineering lines, but, they are either old or they lack all the important information at one place (Collonnier et al., 2001; Kashyap et al., 2003; Magioli and Mansur, 2005). Therefore, here, in this review article the use of biotechnology in eggplant improvement, in terms of plant regeneration, genetic transformation, and genome editing methods with their application to the eggplant as a milestone approaches for crop improvement has been presented.

2. Conventional breeding in eggplant

Although, eggplant wild relatives can be easily crossed with the cultivated eggplant (Plazas et al., 2016). But, interspecific crosses between *S. melongena* and the other related *Solanum* species bearing desirable agronomical traits, have sometimes been limited by sexual barriers (Behera and Singh, 2002). As eggplant, is susceptible to a wide range of biotic and abiotic stresses; therefore, insertion of resistance genes is, with yield and fruit quality improvement, one of the main concerns of conventional breeding and biotechnology. Till date, more

than 25 wild species of genus *Solanum* were used for crossing with cultivated eggplant with a limited success rate (Rotino et al., 2014). Interspecific hybrids in eggplants act as rootstocks in order to protect plants from fungal and bacterial infestations (Ali et al., 1990; Rotino et al., 1997). Also, it was shown that *S. incanum* can easily cross with cultivated eggplant and the resultant interspecific hybrids are fertile (Ranil et al., 2017). Importantly the use of wild species also results in transferring genes or genomic region with undesirable traits. These undesirable traits can be avoided with several generations of backcrossing. Traits like fruit size can be easily recovered with some generations of backcrossing (Kouassi et al., 2016).

Even though various techniques can get successful fertile hybrids, but still, wild relatives need to be used with caution because they may transfer unfavourable traits (such as high susceptibility to *Colletotrichum gloeosporioides* and the bitter taste, because of a high level of steroid saponin, found in *S. torvum* and *S. linneanum* respectively) to hybrids in addition to traits potentially useful for eggplant breeding (Kashyap et al., 2003). Overall, the use of conventional breeding for the improvement of brinjal is limited by several barriers such as crossing issues (pre and post fertilization) linkage drag etc. Several wild species have been reported to have resistance against economically important diseases and pests, but at a low level. For overcoming the above-mentioned limitations of conventional breeding, transgenic technology can be used as a

revolutionary approach in the genetic improvement of eggplant (Figure 1).

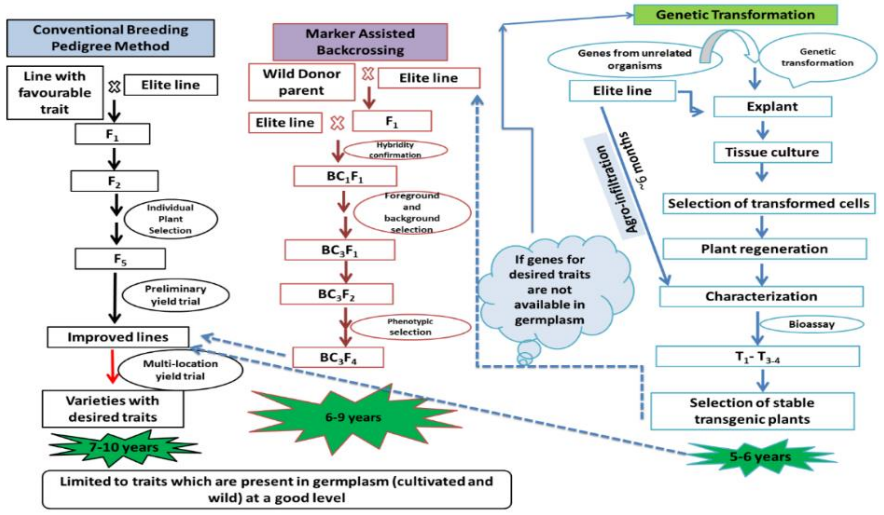

Figure1: A schematic representation of conventional breeding, marker-assisted backcrossing (MABC) and genetic transformation methods in eggplant improvement. Improved lines derived through genetic transformation can be either released as varieties or used as a donor parent in the MABC programs.

3. Plant Regeneration

Eggplant responds well to cell, tissue and organ culture (Kantharajah and Golegaonkar, 2004). Plants can be regenerated from the tissues of eggplant either through embryogenesis (Ammirato, 1983) or organogenesis (Litz, 1993). This ability of regeneration has allowed

the use of biotechnology, particularly the genetic transformation, exploitation of somaclonal variation, haploidy, and somatic hybridization (Collonnier et al., 2001).

Organogenesis

Numerous meristematic zones are formed from longitudinal sections of leaf explant that subsequently converted into shoot buds (Mukherjee et al 1991). The direct regeneration potential differs with the explant type used on a well-defined MS medium. Different explants have been found to show a differential response to regeneration on culture media having different combinations of cytokinins and auxins (Sidhu et al., 2014b). Hypocotyl, cotyledon, root, leaf explant had the different morphogenetic potential for numbers of adventitious shoots on different combinations of plant growth regulators (PGRs) (Mir et al., 2008; Sharma and Rajam, 1995; Zhang et al., 2014). Regeneration is also affected by 'age' of the explant as younger leaves showed better organogenesis than mature ones (Franklin et al., 2004; Prakash et al., 2015). Optimum combinations and concentrations of auxins and cytokinin have been determined for producing a maximum number of regenerated shoots in different cultivars of eggplant in many studies (Farhad et al., 2015; Foo et al., 2018; Yesmin et al., 2018) (Table 1). The dual role of sugar as carbon and the osmotic source is well known in shoot regeneration, and enhanced shoot regeneration efficiency was reported when low sugar concentrations were used (Mukherjee et al.,

1991). Genetic stability studies using Random amplified polymorphic DNA analysis also have been carried to reveal the monomorphic nature and true-to-type clones (Mallaya and Ravishankar, 2013).

Somatic embryogenesis

An artificial process in which an embryo is derived from a single somatic cell is called somatic embryogenesis (SE). There are two routes for SE (Sharp et al., 1980), first is direct embryogenesis in which the embryos are directly developed from the explant tissue in the absence of callus proliferation and second is indirect embryogenesis in which calluses are formed first and then embryos developed from callus itself (Horstman et al., 2017). For the first time, (Yamada et al., 1967) reported SE in eggplant from immature seed embryos cultured on MS medium supplemented with IAA. Effects of genotype, explant type and its age, and media composition on plant regeneration have been reported in various studies. Out of all these factors, the type of explant has been a most important factor for induction of somatic embryos in eggplant (Kantharajah and Golegaonkar, 2004). Different explant types such as immature seed embryo, hypocotyl, cotyledon, leaf, root have shown different potential for SE (Table 1). Effects of genotype and genotype-explant interaction on both organogenesis and somatic embryogenesis have been discussed by various studies presented in Table 1. Recently, various modified protocols were suggested for SE (Habib et al., 2016) and for organogenesis

(Muthusamy et al., 2014; Robinson and Saranya, 2013; Yesmin et al., 2018) for the efficient regeneration of plantlets in different cultivars. Several studies concerning with callus induction and somatic embryogenesis on a different medium supplemented with different combinations of auxins and cytokinins are listed in Table 1.

Table 1. Plant regeneration in eggplant varieties**.**

Explant	cultivar	Growth regulators and their effects	Remarks	Re
Somatic embryogenesis				
Immature embryo cultures		IAA-Somatic embryogenesis (SE)	First time reported somatic embryogenesis in eggplant	(Yamada et al., 1967)
Leaf	Imperial Black Beauty	10mg/L NAA-Maximal embryo yields 2: 1 (NO3-:NH4+)- essential for embryogenesis	Cultivars showed significant quantitative differences in their capacities to form embryos	(Gleddie et al., 1983)
Leaf	F1 hybrid "Suphal"	8.0 mg/L NAA and 0.1 mg/L Kn- High freq. SE	The frequency of plantlet regeneration varied from 27.0-49.7% in vitro and 2.0-4.5% in vivo.	(Rao and Singh, 1991)
Hypocotyl, epicotyl, leaf, cotyledon	F 100	54µM NAA - induction of somatic embryos	The effects of position and orientation of the tissue on the culture medium were also studied	(Magioli et al., 1998)
Cotyledon	Agroceres F-100 variety)	54 µM NAA-induction of somatic embryogenesis	Proembryo formation was observed after the second day of culture	(Tarré et al., 2004)
Anthe	10 F1 hybrids	1.0 mg/L 2,4-D + 1.0 mg/L KT- embryoids induction	The effects of genotypes background, plant hormones and pretreatments on embryogenesis studied	(Du-chen et al., 2008)
Hypocotyl, cotyledon and root	Punjab Barsati, Punjab Sadabahar, Jamuni Gola, PBSR-11 and BB-93C	0.5mg/L BAP + 1.0 to 2.0mg/L NAA- Highest SE (hypocotyl,cotyledon,root) 0.25 mg/L BAP- regeneration into whole plants	Effects of genotype, explant and culture medium effects on SE were reported	(Mir et al., 2008)
Hypocotyl	BARI Begun-1, BARI Begun-4, BARI Begun-5, BARI Begun-6 and Islampuri	0.5 mg/L 2,4-D- development of embryogenic calli 1.0 mg/L 2,4-D- highest number of embryo (BARI Begun-4)	Optimized somatic embryogenesis protocol for locally adapted varieties in Bangladesh	(Habib et al., 2016)

Organogenesis				
Excised hypocotyl segments	Cultivar and F1 hybrid	IAA 5.7 µM -90% bud regeneration Combinations of IAA and BA or IAA and Z or IAA and SD8339- 100% regeneration	Revealed that shoot differentiation is not essentially a function of cytokinin activity	(Kamat and Rao, 1978)
Hypocotyl	Fourteen commercial cultivars	0.8 mg /L NAA - only callus 0.016 mg/L NAA-adventitious roots 6BA-enhanced shoot production	Genotypic differences in response were observed	(Matsuoka and Hinata, 1979)
Stem pith	Florida Market	5.7 µM IAA and 4.5 µM each of 2,4- D and Kn - large aggregates	Suggested that some degradation product of ascorbic acid might be responsible for inducing leafy shoots.	(Fassuliotis et al., 1981)
Hypocotyls, cotyledons and leaves	Six cultivars	2mg/L IAA- callus and roots	Suggested that regenerative potential is under genetic control	(Alicchio et al., 1982)
Leaf	Pusa Kranti	44 mM Glucose or fructose - induction of shoot regeneration	Discussed the dual role of sugar as carbon and osmotic source in shoot regeneration	(Mukherjee et al., 1991)
Hypocotyl, epicotyl, node, leaf and cotyledon	Brazilian eggplant variety (F-100)	0.2 µM TDZ-optimal shoot bud induction rates (75–100 buds/explant) 0.6 µM IAA-half strength-root induction	First report on the use of TDZ for in vitro regeneration of eggplant	(Magioli et al., 1998)
Hypocotyl	Embu	0.25mg/L IAA+0.5 mg/L BAP-adventitious roots	Also evaluated influence of antibiotics (cefotaxime,timentin, kanamycin and hygromycin) on organogenesis	(Picoli et al., 2002)
Isolated microspores	hybrid DSa	KM + 250 mg/L PEG , 0. 2 mg/L 2, 4-D, 0. 5 mg/L ZT, 1 mg/L NAA-microspore culture	Showed that microspore dedifferentiation ability can be markedly improved by using the treatment of anther under the high temperature conditions	(LianYong et al., 2004)

Intact root explants	MEBH 11, MEBH 9, Kalpatru, and Rohini	0.45µM TDZ and 13.3 µM 6-BA- efficient regeneration	Observed negative correlation between regeneration and explant age	(Franklin et al., 2004)
Cotyledons and true leaves	two accessions of each gboma and scarlet	0.1 or 0.2 µM TDZ- eficient regenration (70–100% explants with shoots)	Concluded that TDZ shows a high capacity for inducing organogenesis in African eggplants	(Gisbert et al., 2006)
cotyledon and midrib	Loda	2.0 mg/LNAA + 0.05 mg/L BAP- best callusing (83-85%)	Information from this study could be used as an alternative path to induce genetic and epigenetic changes in regenerated plants	(Rahman et al., 2006)
Cotyledonary leaf, hypocotyl, shoot tip and root	Singhnath and Kazla	1.0 mg/L BAP and 1.0 mg/L Kn- high-frequency direct organo-genesis of shoots	Demonstrated the efficiency of regeneration system by obtaining viable seeds from the regenerated plants	(Sarker et al., 2006)
Cotyledons and under hypocotyls		3 mg/L 6BA-adventitious bud differentiation	Analyzed influence of auxin, genotype and explants on differentiation	(Guang-yuan and Sha-sha, 2007)
Microspore		0.2 mg/L 2,4-D, 0.5 mg/L Z + 1.0 mg/L NAA- callus induction	Observed significant genotypic differences for callus induction rate among the genotypes	(SONG et al., 2007)
Cotyledons and hypocotyls	Larga Negra and Black Beauty	2.0 mg/L NAA + 0.5 mg/L 6-BAP-callus induction	The highest per cent of callus induction was obtained from cotyledons	(Zayova et al., 2012)
Shoot tip, hypocotyl and midrib	Jhumky and Islampuri	2mg/L NAA- best for callus proliferation	Showed that it is possible to develop shoot and fruit borer tolerance brinjal genotypes through SE	(Ferdausi et al., 2009)
Microspore	Senryou 2 gou, Chikuyou and Hakata naganasu	0.4mg/L 2.4-D + 0.2mg/L BA - incubation of microspore induced calli	This improved method proved to increase the rate of plant regeneration from calli up to 70%	(Saiki et al., 2009)
Cotyledon	Meizi, Xianfeng I, Heijuren and Jiuye	2.0 mg/L Zeatin + 0.1mg/L IAA-bud induction	Analyzed genetic stability of the regenerated plants flow cytometry, RAPD and SSR molecular markers	(Xing et al., 2010)
Cotyledon	Larga Negra	1.0 mg/L BAP - plant regeneration	Observed somaclonal variations among regenerated plants	(Zayova et al., 2012)

Stem, leaf and root	Jhumki	2.0 mg/L BAP and 0.5 mg/L NAA-highest callus induction	Suggested that the given protocol might be useful for the production of disease-free and healthy plant materials	(Ray et al., 2010)
Cotyledonary leaf	Pusa Purple Long and Black Beauty	3 mg/L NAA + 0.5mg/L Kn-max. callus induction	The plantlets showed high survival rate (80%) in the soil	(Shivaraj and Rao, 2010)
Shoot tip and nodal explants	PKM-1	2.0 mg/L BAP + 1.0 mg/L 2iP-shoot proliferation	Efficient protocol was presented for micropropagation	(Kanna and Jayabalan, 2010)
Leaf	PLR	2mg/L BAP + 1.0 mg/L 2iP-organogenic calli induction	Studied indirect organogenesis from leaf explants	(Kanna and Jayabalan, 2010)
Cotyledons, hypocotyls and leaf	Punjab Barsati, BSR 229 and KS 331	2.5 mg/L BAP- regeneration	Studied the effect of genotype and explants on in vitro plant regeneration	(Charaya et al., 2011)
Hypocotyl, cotyledon and leaf	BL 5, BR 14, BSR 23	1.5mg/L IBA + 1.0mg/ L BAP-100% callusing and SE (cotyledon)	Cotyledonory callus of BSR 23 exhibited somatic embryos with highest regenerative potential	(Kaur et al., 2011)
Hypocotyl, cotyledon and root	Punjab Barsati, Punjab Sadabahar, Jamuni Gola, PBSR-11 and BB-93C	2.5 mg/L IAA + 0.5 mg/L BAP- adventitious shoot induction	Genotype, explant and genotype x explant interaction showed highly significant effects on organogenesis	(Mir et al., 2011)
Cotyledonary leave, nodal segment and shoot tip	Manjari Gota	2 mg/L 6-BAP + 1 mg/L Kn-best regeneration	Established In vitro rooted plantlets in polycarbonated polyhouse with 100 % survival rate	(Bhat et al., 2013)
Hypocotyls and cotyledon	Shisui	4.4 µM 6-BA + 0.2 µM TDZ-culturing of hypocotyl and cotyledon	Indicated that intact roots are important for explant shoot regeneration and development	(Tanaka et al., 2013)
Tender shoot tip, hypocotyls, leaf and stem	Valuthalai	0.6mg/l 2, 4-D- max. proliferation	Presented an improved method for the in vitro propagation	(Robinson and Saranya, 2013)
Leaf, cotyledon and hypocotyl	Mattu Gulla and Perampalli Gulla	2.0 mg/L BAP + 0.5 mg/L IAA-shoot initiation	Developed an efficient and reproducible in vitro regeneration method	(Muthusamy et al., 2014)

Leaf	Protab, Green Ball and Ghemma Begun	3 mg/L 2,4-D + 0.05 mg/L BAP- max. callusing	Studied the effect of genotypes and growth regulators on callus induction	(Alim et al.)
Hypocotyls and cotyledon	four cultivars	2.0 mg/L Z+1.0 mg/L 6-BA+0.2 mg/L IAA- best callus induction	Showed that, the regeneration capacity of hypocotyls was significantly higher than that of cotyledons	(ZHANG et al., 2014)
Leaf	Protab, Green Ball and Ghemma Begun	1mg/L NAA+ 0.1 mg/LBAP- max. regeneration	The variety Protab appeared as the best for the shoot and root formation	(Alim et al., 2015)
Microspore	F1 hybrid Bandera	0.2 mg/L IAA + 4 mg/L zeatin- shoot production	Regenerated doubled haploids from microspore-derived calli through organogenesis	(Rivas-Sendra et al., 2015)
Hypocotyl	Manjarigota	2μM BAP + 0.05μM NAA- shoot regeneration	Assessed the effect of size, age and position of the explant, pre-culture and high cytokinin concentration in the pre-culture medium on shoot regeneration	(Prakash et al., 2015)
Hypocotyl, cotyledon and leaf	BL-5, BR-14 and BSR-23	3.0 mg/L BAP- regeneration from cotyledon	Studied the effect of genotype, explant and culture media on direct plant regeneration	(Kaur et al., 2015)
Cotyledon and leaf		2 mg/L TDZ+ 0.5 mg/L BAP+ 0.5 mg/L NAA- shoot regeneration	Determined an efficient phytohormone concentration for development of organogenesis from explants	(Taghipour et al., 2015)
Leaf	PLR1	2.0 mg/L 2iP- max. shoot proliferation	Suggested to use this protocol for mass multiplication and also for regeneration of genetically transformed tissue	(Kanna and Jayabalan, 2015)
Hypocotyl	Nayantara, Kazla, Islampuri, ISD-006 and Uttara	2.0 mg/L ZR + 0.1 mg/L IAA- shoot regeneration	Established an efficient regenerating protocol for cultivated eggplant varieties	(Muktadir et al., 2016)
Cotyledon	BARI begun-4 and BARI begun-6	1 mg/L BAP+0.2 mg/L IAA- multiple shoot regeneration	The in vitro grown plantlets were acclimatized in soil, grew up to maturity, flowered, fruited and produced seeds as normal healthy plant like the control	(Yesmin et al., 2018)
Cotyledon	Bulat Putih	2.0 mg/L Kn- shoot regeneration	Indicated that kinetin alone is sufficient to induce shoots without the presence of BAP	(Foo et al., 2018)

Both somatic embryogenesis and organogenesis				
Cotyledons	Imperial Black Beauty	0.1 -0.5 mg/L NAA-rhizogenesis	Suggested a role for polyamines in eggplant somatic embryogenesis	(Fobert and Webb, 1988)
Cotyledons	Nakate shinkuro	50/µM 2,4-D-embryogenic calli induction	Discussed the effects of an aseptic ventilative filter on somatic embryogenesis	(Saito and Nishimura, 1994)
Hypocotyl, cotyledon and leaf explants	Pusa Purple Long, Long White Cluster, Pusa Kranti, and Pusa Purple Cluster	11.1µM BA + 2.9µM IAA - optimum for shoot regeneration	Genotype, explant and genotype-explant interaction had highly significant effects on both organogenesis and somatic embryogenesis	(Sharma and Rajam, 1995)
Cotyledon	Loda and China	0.05mg/L BAP+ 2.0 mg/L-best callus formation and SE	Developed an efficient and reliable method of indirect regeneration through SE	(Huda et al., 2007)
Embryo from mature seeds, cotyledon and shoot explantsNAA (10.6 mg/l)	Thengaithittu	10.6 mg/L NAA-callus production and SE (cotyledon)	Effects of various concentrations and combinations of NAA, Kinetin, 2, 4-D, TDZ and BAP on cultures were studied	(Swamynathan et al., 2010)
Hypocotyl, cotyledon and leaf	BSR-27, BR-16 and BL-7	1.5 mg/L IBA + 1.0 mg/L 6-BAP- highest SE	Studied the effect of media composition and explant type on the regeneration	(Kaur et al., 2013b)

4. Overview of gene transfer methods

The main purpose of genetic transformation is to manipulate the genome of important crop plant species by inserting one or few genes at a time. Using this technique we can transfer the DNA segment from any organism which has our trait of interest into the target crop plant. Initially, the genetic transformation has been used in tobacco for transferring different genes (insect resistance, disease resistance, herbicide tolerance, stress tolerance) (Binns, 1983; Potrykus et al., 1985). At the present time, several methods are available for the genetic manipulation of plant cells. These methods/ techniques can be grouped into two main categories: 1. vector-mediated gene transfer which includes exploitation of the natural gene transfer system of Agrobacterium 2. vector less or direct gene transfer including physical procedures of DNA transfer such as electroporation of protoplasts and tissues, microinjection and silicon carbide fibre-mediated transformation, microprojectile bombardment; and chemical procedure of DNA transfer by using polyethylene glycol (PEG). These methods are routine and reliable techniques for the production of transgenic plants and being used in different laboratories. The principle of every method used for transforming the cells of solanaceous crops and enlist the different studies conducted for the transformation of eggplant for different traits using various methods. The technique of particle bombardment, also known as microprojectile bombardment, biolistics and particle acceleration, has been proved to be a very effective and versatile way for integrating genes into plant genomes.

The DNA containing tungsten or gold particles, referred to as microprojectiles are carried by a microprojectile and are accelerated into living plant cells (Christou, 1992). Using this method requires careful consideration of three major parameters, first, physical parameters including nature and properties of the metal particles used to carry the foreign DNA, binding of DNA on to the particles and target tissue type; second, environmental parameters including environmental conditions of donor plants and bombarded tissues; third biological parameters including choice and nature of explant, and pre and post-bombardment culturing conditions (Twyman and Christou, 2004). In eggplant, different explants have been targeted for transformation using the biolistic approach, but the use of cotyledon as an explant was found advantageous over others in terms of regeneration efficiency and several buds per explant (Sidhu et al., 2014a). Electroporation is the method where electrical impulses of high field strength are used to reversibly permeabilize cell membranes to facilitate the uptake of DNA (Luft and Ketteler, 2015). This method has been used for a long time for transient and integrative transformation of protoplasts (Suginiura et al., 1999). Whereas, microinjection is the direct mechanical introduction of DNA under microscopical control into a cytoplasm or nucleus using a glass micro capillary-injection pipette (Chow et al., 2016). Although, there are several plant transformation methods but they are yet to be explored for the eggplant a schematic representation for steps involved in genetic transformation by different methods to generate transgenic plants is provided in Figure 2.

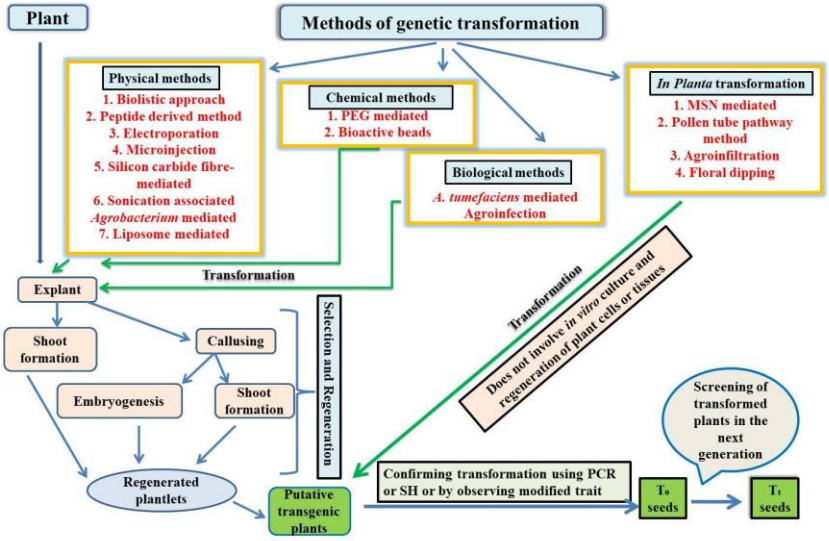

Figure 2: Schematic representation for steps involved in genetic transformation by different methods to generate transgenic plants.

Agrobacterium tumefaciens mediated gene transfer

Agrobacterium system was historically the first successful plant transformation system which revolutionised the field of plant genetic engineering. From then, *Agrobacterium tumefaciens* has been extensively used to introduce the gene into plant cells (Gelvin, 2003; Nester, 2015). The family Solanaceae of eggplant is well documented for its transformation efficiency (Van Eck, 02 2018). The first report of transformation in eggplant was in the late 1980s (Guri and Sink, 1988b). Routinely, agrobacterium strains with binary vectors are

employed to produce transgenic eggplants with kanamycin resistance (npt II) as the selective agent (Nester, 2015). The important aim of transformation in eggplant is to develop transgenic eggplant lines for resistance to Colorado potato beetle. In case, of eggplant different authors have developed regeneration and transformation procedures to transform eggplant (*Solanum melongena* L.) using seedling explants like hypocotyl, epicotyl, and node segments and cotyledon segments (Magioli and Mansur, 2005), leaf disks (Singh Yadav and Venkat Rajam, 1998), or roots (Franklin and Lakshmi Sita, 2003).

When an Agrobacterium-mediated transformation protocol was used with cotyledons, hypocotyls and leaves from two eggplant genotypes high transformation efficiencies were reported (Kole et al., 2010). Influence of several factors such as type of explants, Agrobacterium cell density, an infection time, pre-culture duration, co-cultivation duration, overgrowth control antibiotics on transformation efficiency also has been evaluated by various workers (Jayabalan, 2018; Prakash et al., 2016). For example, (Habde et al., 2017) found maximum transformation efficiency when they used a two-week old cotyledon and three-week old hypocotyls explants precultured for 2 days and followed by infection with Agrobacterium suspension (OD600= 0.2 to 0.4) for 1 min. Combination of cefotaxime and carbenicillin at 250 mg/l concentration was also found better for the effective control of Agrobacterium overgrowth. (Jadhav et al., 2015) transformed four eggplant cultivars with *cry1F* Gene and produced transgenic plants having resistance against shoot and fruit borer.

Gene transfer by Agroinfiltration

Grimsley et al., (1986) presented a method called, "Agroinfection" for the introduction of infectious viral DNA that uses the ability of Agrobacterium to transfer DNA from bacterial cells to plants. This combined use of the Ti plasmid and viral DNA has opened the way to molecular biological approaches that were not possible with either system alone. Recently, agroinfection by the croton yellow vein mosaic virus (CYVMV) of genus Begomovirus family Geminiviridae that has been achieved in tobacco and a replicon vector for expression of a foreign gene in plant was developed which can be used for studying the functional genomics, vaccine expression and gene silencing in plant (Jailani et al., 2016). This method is used for inducing the transient expression of genes in a plant organ, or isolated leaves, or even in cultures of plant cells (Leuzinger et al., 2013). In this method, firstly, a suspension of A. tumefaciens is introduced into a plant leaf either by direct injection or by vacuum infiltration or brought into association with plant cells immobilised on a support and then the bacteria transfer T-DNA into the plant cells (Han et al., 2015; Lee and Yang, 2006; Yang et al.) . Also, this might be quite useful to monitor the movement of elements like phosphorus (P) (Lin et al., 2016).

An improved protocol to enhance transformation efficiency of syringe agroinfiltration by combinative use of 5-azacytidine (AzaC), ascorbate acid (ASC) and Tween-20 was also developed using *N.*

benthamiana. Further, suggested the optimal concentrations of AzaC (20 µM), ASC (0.56 mM) and Tween-20 (0.03%v/v) that significantly improved the transformation efficiency of agroinfiltration resulting into the increase in the expression of the transgene by over 6-fold (Zhao et al., 2017). This agroinfiltration process was optimized for the incubation period, proper infiltration medium and the optical density of the Agrobacterium suspension and evaluated in the brinjal leaves through transient expression of a reporter gene driven by a constitutive promoter (Kumar et al., 2017). Further, the efficiency of agroinfiltration-based transient gene expression was increased in N. benthamiana. A dual vector delivery system was developed by combining the most effective features (chemical additives, heat shock and the co-expression of genes) which provided approximately 3.5-fold higher levels of absolute GUS protein compared to the pEAQ-HT platform (Norkunas et al., 2018).

For the first time, Guri and Sink (1988) successfully transformed the eggplant using agrobacterium-mediated genetic transformation with the cointegrate vector- pMON 200 harbouring *nptII gene*. This successful genetic transformation was then followed by several successful attempts to improve the transformation protocol and to produce the transgenics having desirable traits, using gene constructs with *nptII* as a selection marker and several reporter genes like gus (β-glucouronidase), cat (chloramphenicol acetyl transferase) and luciferase (see Table 2). Transgenic plants resistance to colarado potato beetle (Arpaia et al., 1997; Jelenkovic et al., 1998), root-knot

nematode (Frijters et al., 2000;Papolu et al., 2016), shoot and fruit borer (Kumar et al., 1998; Pal et al., 2009), tomato chlorotic spot virus (Picoli et al., 2006), cucumber mosaic virus (Pratap et al., 2011), Alternaria solani (Darwish et al., 2014) and fungal wilts (Singh et al., 2015) using different genes from various sources, have been produced successfully (Table 2). Likewise, transgenic plants bearing tolerance to abiotic stresses were also produced (Prabhavathi et al 2002; Prabhavathi and Rajam, 2007; Sagare and Mohanty, 2012; Kumar et al 2014) (Table 2). Useful agronomical traits have also been transferred into eggplant using transgene technology like parthenocarpic fruit development and a reduction in the enzymatic browning (Rotino et al., 1997; Cao et al., 2010; Toppino et al., 2011; Padma et al., 2012) (Table 2).

Table 2. Examples of agrobacterium mediated genetic engineering in eggplant for biotic, and abiotic stress tolerance along with other useful traits.

Explant (Tissue/Genotype)	Gene	Selectable marker gene	Trait	Remarks	Reference
Biotic stress tolerance					
Leaf sections/ Black Beauty		*NOS-NPT*		First successful genetic transformation	(Guri and Sink, 1988a)
Callus cultures from leaves		*NPT + LUC*		The efficiency of transformation of *S. melongena* was lower than *other Solanaceae species*	(Komari, 1989)
Cotyledonary and young leaves		*NptII*		The highest proportion of transformed explants were obtained from intact cotyledonary leaf pieces	(Filippone and Lurquin, 1989)
Leaf /Picentia		*NptII*		Development of a protocol for transformation using disarmed binary vector	(Rotino and Gleddie, 1990)
	Bt	*NptII*	Resistance against Coleopterans	Low expression of toxin	(Rotino et al., 1992)
Picentia		*NptII*		Developed a vivo method for screening of transgenic plants and observed Mendelian inheritance of Npt II	(Sunseri et al., 1993)
Cotyledons/ Kecskemeti		*NptII*		Developed a plant regeneration and genetic transformation protocol	(Fári et al., 1995)
Hypocotyl/ Black Jack	*Bt* (*CryIIIB*)	*NptII*	Coleopteran insect resistance	No significant resistance against the larvae of the Colorado potato beetle	(Chen et al., 1995))

Leaf, cotyledon and hypocotyl/female parent of the hybrid 'Rimina'	*Bt* (*CryIIIB*)	*NptII*	Colorado Potato Beetle resistance	Observed a significant insecticidal activity	(Arpaia et al., 1997)
Shoot tip/Hibush	*Bt* (*CryIIIB*)	*NptII*	Coleopteran insect resistance	Efficient protocol to increase the rate of transformation and regeneration	(Billings et al., 1997)
Harris special	*Bt* (modified *CryIIIA*)	*NptII*	Colorado Potato Beetle resistance	Transgenic lines against insect populations in the field at levels not significantly different from the insecticidal control	(Hamilton et al., 1997)
Leaf segments/ stock plants	*Bt* (synthetic *CryIIIA*)	*NptII*	Colorado Potato Beetle resistance	Resistant to neonate larvae and adult colorado potato beetle was observed	(Jelenkovic et al., 1998)
Cotyledonary explants/ Pusa Purple Long)	*Bt* (synthetic *cry1Ab*)	*NptII*	Fruit Borer (*Leucinodes orbonalis*) resistance	Proved that the synthetic gene based on monocot codon can be expressed in dicotyledonous plants for insect control	(Kumar et al., 1998)
	Mi-1		Resistance against *Meloidogyne incognita*	First report of transgenic nematode-resistant eggplant	(Frijters et al., 2000)
leaves/ Hibush	*luc*	*NptII*		Gene expression changes during a one-year period	(Hanyu et al., 1999)
Cotyledon		*NptII*		Optimization of factors for transformation efficiency	(Magioli et al.)
Root explants/MEBH 11, MEBH 9, Kalpatru and Rohini		*NptII*		Variety-independent method for producing transgenic eggplant	(Franklin and Sita, 2003)
Leaf explants/Haritha		*NptII*		Optimization of transformation protocol	(Kannapiran, 2003)

Cotyledon, hypocotyl and leaf explants /Pusa Purple Long		*Hpt*		Higher eggplant transformation efficiency using acetosyringone	(Kumar and Rajam, 2005)
Leaf explants/ Haritha		*Hpt*		Improvement in the protocol	(Soniya et al., 2005)
Cotyledons/ 12 different cultivars	*Bt* (codon-optimized *Cry2Aa*)	*NptII*	Resistance against brinjal shoot and fruit borer	Codon-optimized cry2Aa gene was used to obtain fully resistant plants	(Gupta et al., 2006)
HP83 and Moneymaker	*Mi-1.2*	*NptII*	Resistance against root-knot nematodes (Meloidogyne spp.)	Used Mi-1.2 gene from tomato for resistance against nematodes but no affect on aphids	(Goggin et al., 2006)
Hypocotyl segments/ Embú		*NptII*	Resistance against tomato chlorotic spot virus	Suggested that resistant phenotype depends on transgene copies	(Picoli et al., 2006)
Cotyledons/ proprietary brinjal line	*Bt (Cry2Ab)*	*NptII*	Resistance against fruit and shoot borer (*Leucinodes orbonalis*)	Simultaneously used T-DNA with cry2Ab gene and another T-DNA with nptII and GUS	(Narendran, 2006)
Hypocotyls/ line IVBL-9	*Bt (Cry1Ac)*	*NptII*	Resistance against shoot and fruit borer	Transformation frequency of 17.3% and 2.9 shoots per hypocotyl	(Pal et al., 2009)
Green stem segments		*aadA*		Method for plastid transformation in eggplant	(Singh et al., 2010)
Cotyledonary leaves/ Co2	*Bt* (*CryIAb*)	*NptII*	Resistance against *Meloidogyne incognita*	Regeneration and transformation efficiencies were compared	(Phap et al., 2010)

Cotyledonary leaves/ Pusa Purple Long	*CMV-CP*	*NptII*	Resistance against Cucumber Mosaic Virus (CMV)	Resistance against cucumber mosaic virus	(Pratap et al., 2011)
Hypocotyl/ Kashi Taru	synthetic *Cry1Aa3*	*NptII*	Resistance against shoot and fruit borer (*Leucinodes orbonalis* Guenée)	Used a gene with full codon-modification	(Rai et al., 2013)
Cotyledonary node/Jayant	*Bt (Cry1Ac)*	*NptII*	Resistance against lepidopteron insects	Efficient in vitro regeneration protocol was developed	(Kaur et al., 2013a)
Cotyledons/ Ichiban	*pEKH-WD*	*Ipt*	Resistance against *Alternaria solani*	R/RS site-specific recombination system	(Darwish et al., 2014)
Cotyledon and hypocotyl	*Bt (Cry1Ac)*	*Hpt*		Successfully transformed cotyledons with Cry 1AC	(Sidhu et al., 2014a)
Cotyledon and leaf/ Pusa Purple Long	*Chi*	*Hpt*	Resistance against fungal wilts	Higher chitinase activity	(Singh et al., 2015)
Hypocotyl/ Manjarigota		*NptII*		Standardization of shoot transformation efficiency	(Prakash et al., 2015)
Shoot tip/ Manjarigota, Ruchira, Poona selection, and Krishna Kathi	*Bt (Cry1F)*	*NptII*	Resistance against shoot and fruit borer (*Leucinodes orbonalis* Guenée)	Standardisation for transformation efficiency	(Jadhav et al., 2015)

leaf disks / Pusa Purple Long	*OC-IΔD86*	*NptII*	Resistance against root-knot nematodes (RKN)	Above 78% inhibition of root-knot nematode	(Papolu et al., 2016)
Hypocotyl and Cotyledonary leaves/ Manjarigota		*NptII*		Effects of growth regulators and explant-type on transformation method were studied	(Prakash et al., 2016b)
Hypocotyl/ Manjarigota		*NptII*		Effect of antibiotics and gelling agents was studied	(Prakash et al., 2016a)
Hypocotyl/ Arka Keshav and Manjarigota		*NptII*		Optimized the transformation system	(Hanur et al., 2016)
Cotyledon and hypocotyls/ RHRB-35 and Manjarigota	*Bt (Cry1Aabc)*	*NptII*	Resistance against shoot and fruit borer (*Leucinodes orbonalis* Guenée)	Factors influencing transformation efficiency were studied	(Habde et al., 2017)
Fully expanded leaves / Pant Ritura		*NptII*		Transient expression in leaves	(Kumar et al., 2017)
The nodal region of seedlings/ PLR1		*NptII*		Standardisation for gus gene expression	(Jayabalan, 2018)
Abiotic stress tolerance					
Leaf / Pusa Purple Long	*mtlD*	*NptII*	Tolerance against osmotic stress induced by salt, drought and chilling stress	First report of genetically engineered abiotic tolerance in eggplant	(Prabhavathi et al., 2002)
Leaf and cotyledon explants/ Pusa Purple Long	*adc*	*Hpt*	Stress tolerance	Increased tolerance levels to multiple abiotic stresses	(Prabhavathi and Rajam, 2007)

Shoot-tip /Utkal Anushree	*rd29A::DREB1A*	*NptII*	Tolerance to moisture stress	Transformation frequencies were compared	(Sagare and Mohanty, 2012)
Hypocotyledonary leaf/ PKM1	*HAL1*	*NptII*	Salt tolerance	Salt tolerant lines	(Kumar et al., 2014)
Other useful studies					
Leaf, cotyledon and hypocotyl/ female parent of the hybrid Rimina	*iaaM*and *DefH9* promoter		Parthenocarpic fruit development	Fruit set under unfavourable conditions	Rotino et al (1997)
Hypocotyls /pure lines E-8 and E-38	*Barnase and Cre* gene	*Bar, NptII*	Pollen fertility restoration	Use of Cre/loxP system for fertility restoration	(Cao et al., 2010))
Leaf and cotyledon/ DR2	amiRNA targeting *SmTAF10*and *SmTAF13*	*NptII*	Male sterility	Complete transgene containment in eggplant	(Toppino et al., 2011)
Leaf segments	antisense *SmePPO1*	*Hpt*	Reduction of enzymatic browning	Reduced browning	(Padma, 2012)

5. Genome editing

Genome editing, or genome engineering, includes a set of techniques that allow to edit, delete, replace or insert, in a targeted site, specific genomic sequences of interest in the genome of a living organism (Gaj et al., 2016). Unlike genetic transformation techniques that randomly insert a gene into a host genome, genome editing targets the insertions to site-specific locations. Availability of sequenced genomes and the emergence of highly efficient editing technologies based on complexes that guide endonucleases allowed scientists to alter the plant genomes in a site-specific manner (Komor et al., 2017). These technologies are based on the induction of cuts in double-strand DNA (DSB, double-strand breaks), in genomes of different organisms including plants, which are then repaired either with the non-homologous end-joining (NHEJ) or with homology-directed repair (HDR) (Rinaldo and Ayliffe, 2015). There are four nuclease systems which can be used for inducing the DSBs (i) meganucleases, (ii) zinc finger nucleases (ZFN), (iii) transcription activator-like effector nucleases (TALEN) and, (iv) Clustered Regularly Interspaced Short Palindromic Repeats /CRISPR-associated nucleases (CRISPR/Cas). ZFN, TALEN and CRISPR/Cas are more efficient to 'edit' the 'target' sequences compared to meganucleases (Figure 3). Recently, two excellent reviews presenting the importance of genome editing in vegetables especially in solanaceous food crops have been published (Cardi et al., 2017; Van Eck, 2018).

Efficacy of CRISPR/Cas9 has been tested in tomato successfully. (Brooks et al., 2014) targeted the neighbouring sequences in the second

exon of the tomato homolog of Arabidopsis ARGONAUTE7 (SlAGO7) using CRISPR/Cas9 constructs and obtained the mutants having distinctive phenotypes. In another study, using CRISPR/Cas9 system, (Lor et al., 2014) efficiently mutagenized the RIN gene of tomato, which encodes a MADS-box transcription factor regulating fruit ripening and confirmed the important role of RIN in ripening. In 2016, (Thomazella et al., 2016) generated mutants showing resistance against different pathogens, including *Pseudomonas syringae* pv. tomato and *Phytophthora capsici* and *Xanthomonas* spp by producing small deletions in the SlDMR6-1 gene using CRISPR/Cas-9 system. Hilioti et al., 2016developed ZFN-based technology for the *S. lycopersicum* seed system and induced mutations in LEAFY-COTYLEDON1-LIKE4 (L1L4) gene leading to phenotypic diversity including fruit organ. Day-length sensitivity in crops limits their geographical range of cultivation, if photoperiod response of a crop is modified then the crop can be grown over geographical regions. Soyk et al., 2017 used CRISPR/Cas9 system to induce the mutations in SELF-PRUNING 5G (SP5G) causing rapid flowering and enhancing the compact determinate growth habit of field tomatoes ultimately increasing the yield. CRISPR/Cas9–mediated knock-out of polyphenol oxidase genes in eggplant (*S. melongena* L.) has also been reported (Gianoglio et al., 2017).

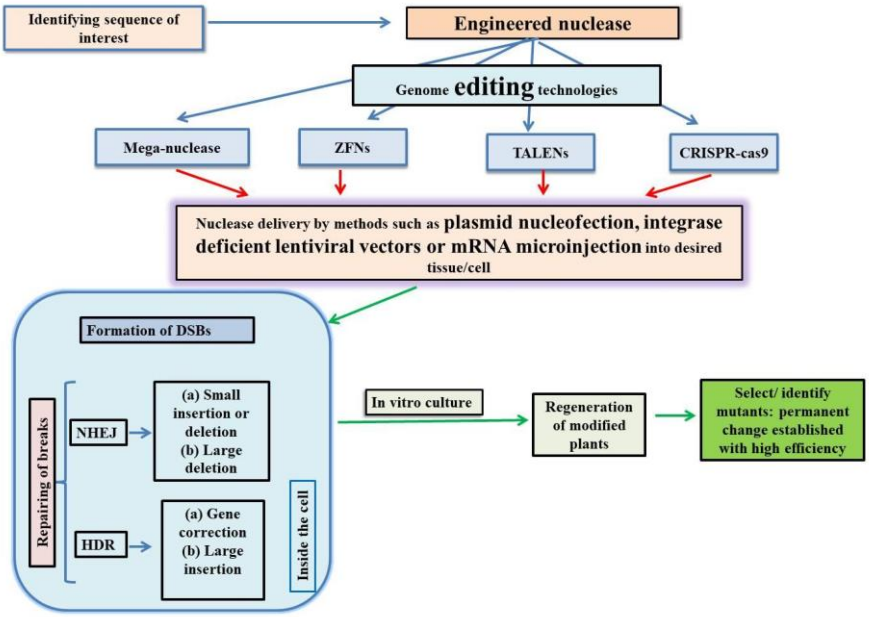

Figure 3: Schematic representation for steps related to the delivery of an engineered nuclease for editing the genomes.

6. Concluding remarks

Plant biotechnology has played a crucial rule in the improvement of vegetable crops. Plant biotechnology is a continuously evolving field and every day a new technology adds to the plant biotechnologist kit. Vegetables like tomato is a model for biotechnological studies. But eggplant is still discovered for various traits based on the traditional breeding or sexual gene transfer methods. Although, in the past decade eggplant breeding has made a significant improvement. Recently availability of molecular markers and the eggplant genome has helped plant breeder identify and incorporate better traits in the modern eggplant varieties. But, as soon as we compare it with biotechnological

advancement aspects, including the development of biotechnological tools it lags the other members of Solanaceae.

Most of the transformation studies in eggplant are by agrobacterium mediated transformation, covering some biotic challenges and abiotic challenges. There is an urgent need to eggplant varieties resistant of viral, fungal and bacterial pathogens along with a wider adaptability to climate change. Techniques like genome editing and molecular farming are yet to be explored in eggplant. Other methods for eggplant transformation have not been explored much. With a hiking trend in the global population the sole development on traditional long plant breeding methods to end with a trait-based variety could be detrimental. Being a large-fruited vegetables eggplants open the prospects of molecular farming.

Figure 4: Word cloud (bigger words means frequently used) based summary of tissue culture explants, and genes, markers, reporters, methods and traits.

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OBJECTIVES

The objectives of this thesis are the application of available tools to plant breeders, from the traditional conventional breeding appraoches to the modern biotechnological tools. The breeding tools are used against all three possible genepools of eggplant in order to develop the eggplant ideotype for morphological and fruit bioactive phenolics. Further, the conventional, biotechnological, and genomics approaches are chosen keeping in mind the eggplant (*Solanum melongena* L.) breeding behaviour and its suitability in crossing to its wild relatives. The specific objectives planned are as follows.

1. To study the morphological diversity of cultivated eggplant, wild relatives and their interspecific hybrids using conventional and fruit phenomics descriptors.

2. To evaluate the biochemical diversity of cultivated eggplant, wild relatives and their interspecific hybrids for fruit related biochemical composition of phenolics, fruit flesh colour and browning.

3. To understand the genetics of important morphological and fruit biochemical descriptors in eggplant using

Line × Tester matting design using wild relative as testers.

4. To deepen the understanding of genetics of important morphological and biochemical descriptors of eggplant using the genetically distant cultivated eggplant varities and a primary genepool species in a diallel matting design

5. To develop an agroinfiltration metod for eggplant fruits and thereby using that method for over-expressing the chlorogenic acid pathway gene.
   5.1. Development of Eggplant (SmHQT) gene construct with specific promoter in a plant transformation vector (pBIN19).
   5.2. Development of a p19 construct for using in co-infiltration experiments.
   5.3. Use of a control construct (GUS bearing; pCAMBIA1304) & protocol standardization for agroinfiltration in Eggplant fruit.
   5.4. Agroinfiltration of SmHQT construct in Eggplant

RESULTS

Chapter 1: Phenotyping of Eggplant Wild Relatives and Interspecific Hybrids with Conventional and Phenomics Descriptors Provides Insight for Their Potential Utilization in Breeding

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Abstract

Eggplant (*Solanum melongena*) is related to a large number of wild species that are a source of variation for breeding programmes, in particular for traits related to adaptation to climate change. However, wild species remain largely unexploited for eggplant breeding. Detailed phenotypic characterization of wild species and their hybrids with eggplant may allow identifying promising wild species and information on the genetic control and heterosis of relevant traits. We characterizated six eggplant accessions, 21 accessions of 12 wild species (the only primary genepool species *S. insanum* and 11 secondary genepool species) and 45 interspecific hybrids of eggplant with wild species (18 with *S. insanum* and 27 with secondary genepool species) using 27 conventional morphological descriptors and 20 fruit morphometric descriptors obtained with the phenomics tool Tomato Analyzer. Significant differences were observed among cultivated, wild and interspecific hybrid groups for 18 conventional and 18 Tomato Analyzer descriptors, with hybrids generally having intermediate values. Wild species were generally more variable than cultivated accessions and interspecific hybrids displayed intermediate ranges of variation and coefficient of variation (CV) values, except for fruit shape traits in which the latter were the most variable. The multivariate principal components analysis (PCA) reveals a clear separation of wild species and cultivated accessions. Interspecific hybrids with *S. insanum* plotted closer to cultivated eggplant, while hybrids with secondary genepool species generally clustered together with wild species. Many differences were observed among wild species for traits of agronomic

interest, which allowed identifying species of greatest potential interest for eggplant breeding. Heterosis values were positive for most vigour-related traits, while for fruit size values were close to zero for hybrids with *S. incanum* and highly negative for hybrids with secondary genepool species. Our results allowed the identification of potentially interesting wild species and interspecific hybrids for introgression breeding in eggplant. This is an important step for broadening the genetic base of eggplant and for breeding for adaptation to climate change in this crop.

Keywords: descriptors, genepools, intespecific hybrids, introgression breeding, phenomics, *Solanum melongena*, Tomato Analyzer.

Introduction

Eggplant (*Solanum melongena* L.) is one of the most important vegetables, in particular in tropical and subtropical regions, such as the South of Europe, North of Africa, Middle East, Southeast Asia and Eastern Asia, where it is a source of dietary fiber, micronutrients and bioactive compounds (Mennella et al., 2010; Niño-Medina et al., 2014; San José et al., 2014). At present eggplant is the sixth most important vegetable after tomato, watermelon, onion, cabbage and cucumber and the most important *Solanum* crop native to the Old World (FAO, 2016). At the global level, it has been one of the crops with a greater increase in production in the last years, with total production rising by 59% in a decade, from $31.0 \cdot 10^6$ t in 2004 to $49.3 \cdot 10^6$ t in 2013 (FAO, 2016).

The narrow genetic base of eggplant, probably a consequence of a genetic bottleneck during its domestication in Southeast Asia (Meyer et al., 2012), is a limitation to obtain major breeding advances. This limited genetic diversity contrasts with the large morphological and genetic variation present in the eggplant wild relatives (Meyer et al., 2012; Vorontsova et al., 2013; Vorontsova and Knapp, 2016). Phylogenetically, eggplant is a member of the so-called "spiny solanums" group (*Solanum* subgenus *Leptostemonum*), which contains many wild species from the Old World, most of them from Africa (Vorontsova et al., 2013; Vorontsova and Knapp, 2016). These wild species could represent a source of variation for developing a new generation of eggplant cultivars with dramatically improved yield and quality, as well as for addressing the challenges posed by adaptation to the climate change. In this respect, resistance and tolerance to several major diseases and pests is found among wild eggplant

relatives (Daunay and Hazra, 2012; Rotino et al., 2014) and they can also be found in a wide range of environmental conditions, including desertic and semi-desertic areas, environments with extreme temperatures (Knapp et al., 2013, 2016). Also, some eggplant wild relatives are known to possess high levels of chlorogenic acid and other bioactive compounds of interest for human health (Mennella et al., 2010; Meyer et al., 2015). However, with a few exceptions (Rotino et al., 2014; Liu et al., 2015), up to now eggplant breeders have largely neglected the potential of wild species for eggplant breeding, and contrarily to other crops like tomato (Díez and Nuez, 2008), wild relatives have not made a relevant contribution to the development of new eggplant cultivars.

Eggplant can be intercrossed with different degrees of success with a large number of wild relatives (Daunay and Hazra, 2012; Rotino et al., 2014; Plazas et al., 2016). In this respect, the closest wild relative of eggplant is *S. insanum* (Knapp et al., 2013; Vorontsova et al., 2013), which is naturally distributed in Southeast Asia, Madagascar and Mauritius (Knapp et al., 2013; Vorontsova and Knapp, 2016), where it is frequently found as a weed (Mutegi et al., 2015). *Solanum insanum* is considered as the wild ancestor of eggplant and is the only species forming part of the primary genepool of cultivated eggplant (Syfert et al., 2016). Hybrids of *S. melongena* with *S. insanum* are easily obtained; fruits from interspecific hybridization have many seeds, which have high germination rates, and the hybrid plants are fully fertile (Davidar et al., 2015; Plazas et al., 2016). Interspecific hybrids have also been obtained with many wild species from the secondary genepool (Daunay and Hazra, 2012; Rotino et al., 2014; Plazas et al., 2016), which includes some 50 African and Southeast Asian wild species from the Eggplant clade and the Anguivi grade (Vorontsova

et al., 2013; Syfert et al., 2016). The degree of success of interspecific sexual hybridization between *S. melongena* and secondary genepool species, as well as the hybrid fertility is very variable depending on the species and accessions involved and the direction of the cross (Plazas et al., 2016).

The characterization of wild species and interspecific hybrids for traits of interest for breeders is a fundamental step for the efficient utilization of crop wild relatives in breeding. Combined data on the cultivated and wild species and their interspecific hybrids, not only allows identifying sources of variation and materials of potential interest, but also provides information on the inheritance of some traits present in the wild species, as has been demonstrated in crosses between *S. incanum* and *S. melongena* (Prohens et al., 2013). Also, characterization of these materials for vigour traits may allow identifying materials potentially useful as rootstocks (Gisbert et al., 2011). In the case of eggplant wild relatives there are a number of studies on their taxonomic and phylogenetic relationships (Vorontosova et al., 2013; Vorontsova and Knapp, 2016), as well as screenings for resistance or tolerance to diseases and pests (Bubici and Cirulli, 2008; Daunay and Hazra, 2012; Naegele et al., 2014). However, to our knowledge there are no comprehensive studies on the morphological and agronomic traits of interest in a set of wild species of the primary and secondary genepools of eggplant and their interspecific hybrids with cultivated eggplant.

Several characterization studies in *S. melongena* with standardized morphological and agronomic descriptors developed by the European Eggplant Genetic Resources Network (EGGNET; van der Weerden and Barendse, 2007) and the International Board for Plant Genetic Resources

(IBPGR, 1990) have revealed that these descriptors are suited for providing a useful morphological and agronomic characterization for eggplant breeders (Prohens et al., 2005; Muñoz-Falcón et al., 2009; Boyaci et al., 2015). Also, EGGNET and IBPGR descriptors have been successfully used for evaluating segregating generations of interspecific crosses between eggplant and related species (Prohens et al., 2012, 2013). In addition to conventional morphological descriptors fruit phenomics data provide eggplant breeders with relevant information for evaluating the variation of the fruit morphology. In this respect, the phenomics tool Tomato Analyzer (Rodríguez et al., 2010) has revealed as useful for the detailed morphometric analysis of fruit size and shape of eggplant and related materials (Prohens et al., 2012; Hurtado et al., 2013).

In this work we characterize cultivated eggplant, wild relatives from the primary and secondary genepools and interspecific hybrids between cultivated eggplant and wild relatives using conventional and Tomato Analyzer descriptors. Apart from providing a characterization of the three types of materials studied and their differences, the aim is evaluating the interest for breeding of different wild relatives using characterization data of the wild relatives and of their interspecific hybrids with eggplant. The information obtained may also provide clues on the interest of wild species and interspecific hybrids as potential rootstocks for eggplant.

Material and Methods

Plant Material

The plant material used included six accessions of cultivated eggplant (*S. melongena*), twenty-one accessions of a total of twelve wild species, and forty-five interspecific hybrids between the *S. melongena* accessions and seven of the wild species (Table 1). The *S. melongena* accessions include materials from both the Occidental (Ivory Coast) and Oriental (Sri Lanka) cultivated genepools (Vilanova et al., 2012; Cericola et al., 2013). Among the wild relatives, three accessions belong to the primary genepool (GP1) *S. insanum*, and eighteen accessions to secondary genepool (GP2) species, namely *S. anguivi* (n=2), *S. campylacanthum* (n=3), *S. dasyphyllum* (n=1), *S. incanum* (n=1), *S. lichtensteinii* (n=2), *S. lidii* (n=2), *S. linnaeanum* (n=2), *S. pyracanthos* (n=1), *S. tomentosum* (n=1), *S. vespertilio* (n=2) and *S. violaceum* (n=1). All the accessions are deposited at the germplasm bank of the Universitat Politècnica de València (València, Spain). The forty-five interspecific hybrids were obtained after reciprocal crossings between cultivated eggplant and wild relatives (Plazas et al., 2016) resulting in eighteen hybrids between *S. melongena* and primary genepool species and twenty-seven hybrids between *S. melongena* and secondary genepool species (Table 1). Five plants per accession or interspecific hybrid were grown under open field conditions during the summer season of 2015 at the Universitat Politècnica de València (Valencia, Spain; GPS coordinates of the plot: 39º 28’ 55” N, 0º 22’ 11” W; altitude 7 m a.s.l.). Plants were spaced 1.2 m between rows and 1.0 m within the row. Drip irrigation was applied and 80 g plant^{-1} of a 10N–2.2P–24.9K plus micronutrients fertilizer (Hakaphos Naranja; Compo

Agricultura, Barcelona, Spain) was applied during the whole cultivation period through the irrigation system. Plants were trained with bamboo canes and pruned when needed. Weeds were removed manually and no phytosanitary treatments were needed.

Table 1. Accessions of cultivated eggplant (*Solanum melongena*) and wild relatives of the primary and secondary genepools, and interspecific hybrids between cultivated eggplant and wild relatives used for the morphological and phenomics characterization. For the interspecific hybrids, the first and second parentals included in the hybrid code correspond to the female and male, respectively.

Species	Accession	Germplasm collection code	Country of origin	Interspecific hybrids with *S. melongena* accessions					
				MEL1	MEL2	MEL3	MEL4	MEL5	MEL6
Cultivated eggplant									
S. melongena	MEL1	BBS-118/B	Ivory Coast						
	MEL2	BBS-146	Ivory Coast						
	MEL3	BBS-175	Ivory Coast						
	MEL4	07145	Sri Lanka						
	MEL5	8104	Sri Lanka						
	MEL6	Ampara	Sri Lanka						
Wild primary genepool (GP1)									
S. insanum	INS1	SLKINS-1	Sri Lanka	MEL1×INS1	MEL2×INS1	MEL3×INS1	MEL4×INS1	INS1×MEL5	MEL6×INS1
	INS2	SLKINS-1	Sri Lanka	MEL1×INS2	MEL2×INS2	MEL3×INS2	MEL4×INS2	MEL5×INS2	MEL6×INS2
	INS3	MM498	Japan	INS3×MEL1	INS3×MEL2	INS3×MEL3	INS3×MEL4	MEL5×INS3	INS3×MEL6
Wild secondary genepool (GP2)									
S. anguivi	ANG1	BBS119	Ivory Coast		MEL2×ANG1	MEL3×ANG1	MEL4×ANG1	MEL5×ANG1	
	ANG2	BBS125/B	Ivory Coast	MEL1×ANG2	MEL2×ANG2	ANG2×MEL3	ANG2×MEL4	MEL5×ANG2	ANG2×MEL6
S. campylacanthum	CAM5	MM680	Tanzania						
	CAM6	MM700	Kenya						
	CAM8	MM1426	Tanzania						
S. dasyphyllum	DAS1	MM1153	Uganda	MEL1×DAS1	MEL2×DAS1	MEL3×DAS1		MEL5×DAS1	
S. incanum	INC1	MM664	Israel	INC1×MEL1		MEL3×INC1		MEL5×INC1	MEL6×INC1
S. lichtensteinii	LIC1	MM674	South Africa	MEL1×LIC1				MEL5×LIC1	MEL6×LIC1
	LIC2	MM677	Iran	MEL1×LIC2		MEL3×LIC2	MEL4×LIC2		
S. lidii	LID1	4788	Spain						

	LID2	MM1005	Spain			
S. linnaeanum	LIN1	JPT0028	Spain			LIN1×MEL6
	LIN3	MM195	Tunisia			
S. pyracanthos	PYR1	SOLN-66	Unknown			
S. tomentosum	TOM1	MM992	South Africa	MEL2×TOM1	TOM1×MEL3	
S. vespertilio	VES1	4601A	Spain			
	VES2	BGV-3218	Spain			
S. violaceum	VIO1	SLKVIL-1	Sri Lanka			

Characterization

All plants were characterized using 27 conventional morphological descriptors based on EGGNET (van der Weerden and Barendse, 2007) and IBPGR (IBPGR, 1990) descriptors (Table 2). These morphological descriptors describe different traits of the whole plant (4), leaf (7), inflorescence and flower (7) and fruit (9). Except for descriptors concerning the whole plant (e.g., plant growth habit), for which one measurement was taken per plant, five measurements were taken from each individual plant in order to obtain individual plant averages for the conventional morphological descriptors. Additionally, five fruits per plant, collected at the commercially ripe stage (i.e., physiologically immature) for cultivated eggplant and at a similar physiological stage (when they had attained full size but was not physiologically mature) in the case of wild species and interspecific hybrids, were cut opened longitudinally and scanned using an HP Scanjet G4010 photo scanner (Hewlett Packard, Palo Alto, CA, USA) at a resolution of 300 dpi. Scanned images were subjected to fruit morphometric analysis with the fruit shape phenomics tool Tomato Analyzer version 4 software (Rodríguez et al., 2010). A total of 20 fruit morphometric descriptors were recorded using this tool (Table 2).

Table 2. Descriptors used for phenotyping. The list displays conventional morphological descriptors based on EGGNET (van der Weerden et al., 2007) and IBPGR (1990) descriptors list and phenomics fruit morphometric descriptors based on Tomato Analyzer software (Rodríguez et al., 2010) used for the characterization of accessions of cultivated *S.melongena* (n=6); wild relatives (n=21) and interspecific hybrids between cultivated eggplant and wild relatives (n=45).

Descriptors	Units/Scale/Description
Conventional morphological descriptors	
Plant growth habit	3=Upright; 7=Prostrate
Plant height	cm
Stem diameter	mm
Shoot tip anthocyanin intensity	0=Absent; 9=Very strong
Leaf blade lobing	1=Very weak (none); 9=Very Strong
Leaf prickles (upper surface)	0=None; 0=Very many (>20)
Leaf surface shape	1=Flat; 9=Very convex or bullate
Leaf blade tip angle	1=< 15º; 9=>160º
Leaf pedicel length	cm
Leaf blade length	cm
Leaf blade width	cm
Number of flowers per inflorescence	---
Corolla colour	1=Greenish white; 9=Bluish violet
Corolla diameter	mm
Number of flower prickles (calyx)	0=None; 9=Very many (>20)
Number of sepals	---
Number of petals	---
Number of stamens	---
Fruit pedicel length	mm
Fruit pedicel diameter	mm
Fruit length /breadth ratio	1=Broader than long; 9=Several times as long as broad
Fruit cross section	1=Circular, no grooves; 9=Very irregular
Fruit apex shape	3=Protruded; 7=Depressed
Fruit weight	g
Fruit flesh density	1=Very loose; 9=Very dense
Fruit calyx length (relative)	1 Very short (<10 %); 9=Very long (>75 %)
Fruit calyx prickles	0=None; 9=Very many (>30)
Tomato analyzer phenomics fruit morphometric descriptors	
Perimeter	cm
Area (A)	cm^2
Width Mid-height	The width measured at ½ of the fruit's height (cm)
Maximum Width	The maximum horizontal distance of the fruit (cm)
Height Mid-width	The height measured at ½ of the fruit's width (cm)
Maximum Height	The maximum vertical distance of the fruit (cm)
Curved Height	The height measured along a curved line through the fruit (cm).

Fruit Shape Index External I	The ratio of Maximum Height to Maximum Width
Fruit Shape Index External II	The ratio of Height Mid-width to Width Mid-height
Curved Fruit Shape Index	The ratio of Curved Height to the width of the fruit at mid-curved-height.
Proximal Fruit Blockiness	The ratio of the width at the upper blockiness position to Width Mid-height
Distal Fruit Blockiness	The ratio of the width at the lower blockiness position to Width Mid-height
Fruit Shape Triangle	The ratio of the width at the upper blockiness position to the width at the lower blockiness position.
Ellipsoid	The ratio of the error resulting from a best-fit ellipse to the area of the fruit. Smaller values indicate that the fruit is more ellipsoid.
Circular	The ratio of the error resulting from a best-fit circle to the area of the fruit. Smaller values indicate that the fruit is more circular.
Rectangular	The ratio of the area of the rectangle bounding the fruit to the area of the rectangle bounded by the fruit.
Shoulder Height	The ratio of the average height of the shoulder points above the proximal end point to Maximum Height.
Obovoid	Calculated according to the formula provided in the Tomato Analyzer Manual (Rodríguez et al., 2010). The higher the value, the greater is the area of the fruit below mid height
Ovoid	Calculated according to the formula provided in the Tomato Analyzer Manual (Rodríguez et al., 2010). The higher the value, the greater is the area of the fruit above mid height
Fruit Shape Index Internal	The ratio of the internal ellipse's height to its width.

Data Analyses

For each trait, the mean, range and coefficient of variation (CV, %) were calculated using average accession or hybrid values of cultivated eggplant (n=6), wild relatives (n=21) and interspecific hybrids (n=45). Means of each accession or hybrid were subjected to analyses of variance (ANOVA) to detect differences among the three groups considered. Significance of differences among group means was evaluated using the Student-Newman-Keuls multiple range test at P=0.05. Heterosis over mid parent (H; %) for the traits of greater agronomic importance was studied in the interspecific hybrids using formula H = 100 × ((F1-MP)/MP), where

F_1 = hybrid mean, and MP = mean of the parents. Principal components analyses (PCA) were performed using pairwise Euclidean distances among accession or hybrid means for characterization data. All the statistical analyses were performed using the Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA).

Results

Differences Between Eggplant, Wild Relatives and Interspecific Hybrids

Significant differences ($P<0.05$) were found among average values for the groups constituted by cultivated eggplant, wild relatives and interspecific hybrids for 18 out of the 27 conventional descriptors (Table 3). Traits for which no significant differences were found among the three groups included Plant growth habit, Leaf surface shape, Leaf blade tip angle, Corolla colour, Number of flower prickles, Fruit cross section, Fruit apex shape, Fruit calyx length, and Fruit calyx prickles (Table 3). The largest F-ratios were observed for descriptors related to leaf size, fruit weight, number of flower parts, fruit pedicel size, and fruit shape descriptors (Table 3). Generally, wild species and interspecific hybrids had larger plant size, more leaf prickles, greater number of flowers per inflorescence, and shorter fruit length/breadth ratio than the cultivated species. Also, the cultivated species and interspecific hybrids had more anthocyanin pigmentation, larger leaf size, and greater number of flower parts than the wild species. For the corolla diameter, fruit pedicel size and fruit weight the cultivated species had the greater average values, while the smaller ones were for the wild species, with the interspecific hybrids

having intermediate values. The three groups overlap in the ranges of variation for all conventional descriptors except for Leaf pedicel length, Corolla diameter, Fruit pedicel length, Fruit pedicel diameter, and Fruit weight, in which all the accessions of the cultivated species presented higher values than any of the wild species.

Table 3. Variation parameters for conventional morphological descriptors. Values represent the mean, range (between brackets), and coefficient of variation (CV; %) for the conventional morphological descriptors studied in accessions of cultivated eggplant (*S. melongena*; n=6), wild relatives (n=21) and interspecific hybrids between cultivated eggplant and wild relatives (n=45 except for fruit traits in which n=42) and significance of mean differences among the three groups.

	Cultivated eggplant (n=6)		Wild relatives (n=21)		Interspecific hybrids (n=45; 42 for fruit traits)			
Descriptors	Mean[a] (range)	CV (%)	Mean (range)	CV (%)	Mean (range)	CV (%)	F-ratio	Probability
Plant growth habit	5.33 a (5.00-7.00)	15.3	4.71 a (3.00-7.00)	24.3	5.00 a (5.00-5.00)	0.0	2.51	0.0883
Plant height	97.1 a (69.7-111.7)	16.5	124.8 b (91.0-160.5)	17.5	141.9 b (91.0-199.0)	19.5	9.81	0.0002
Stem diameter (mm)	22.6 a (15.3-28.0)	20.3	24.3 ab (12.0-34.7)	25.9	27.8 b (18.3-38.3)	16.8	5.09	0.0087
Shoot tip anthocyanin intensity	3.33 b (0.00-7.00)	86.3	0.57 a (0.00-3.00)	211.2	2.06 ab (1.00-7.00)	112.7	5.43	0.0064
Leaf blade lobing	4.33 a (3.00-5.00)	23.8	4.81 ab (1.00-9.00)	52.4	6.02 b (3.00-9.00)	19.5	5.42	<0.0065
Leaf prickles (upper surface)	0.11 a (0.00-0.67)	244.9	3.38 b (0.00-9.00)	95.6	4.45 b (0.00-9.00)	66.7	6.03	0.0039
Leaf surface shape	5.67 a (5.00-9.00)	28.8	5.29 a (1.00-9.00)	45.2	6.33 a (5.00-9.00)	30.1	1.96	0.1489
Leaf blade tip angle	5.00 a (3.00-7.00)	25.3	4.48 a (3.00-7.00)	32.8	4.58 a (2.00-7.00)	33.1	0.29	0.7484
Leaf pedicel length (cm)	6.91 c (5.80-8.28)	14.1	2.74 a (0.63-4.61)	41.1	5.70 b (2.67-9.05)	25.5	42.27	<0.0001
Leaf blade length (cm)	22.0 b (19.7-24.9)	7.9	13.8 a (5.2-20.9)	33.7	21.0 b (15.0-31.9)	19.3	23.65	<0.0001
Leaf blade width (cm)	15.8 b (12.5-19.5)	18.3	8.7 a (3.3-18.7)	38.1	15.9 b (10.8-25.7)	21.3	34.41	<0.0001

Number of flowers per inflorescence	3.49 a (1.07-5.00)	42.2	8.33 b (1.00-16.10)	57.9	6.77 b (2.00-14.44)	43.3	4.58	<0.0135
Corolla colour	5.67 a (5.00-7.00)	18.2	5.57 a (1.00-9.00)	37.9	6.02 a (3.00-7.00)	24.1	0.58	0.5620
Corolla diameter (mm)	43.3 c (37.2-49.9)	12.9	22.2 a (7.7-30.4)	30.4	35.8 b (20.4-49.9)	22.3	30.44	<0.0001
Number of flower prickles (calyx)	1.83 a (0.00-5.00)	100.1	3.62 a (0.00-9.00)	102.5	3.64 a (0.00-9.00)	85.2	0.86	0.4269
Number of sepals	5.57 b (5.00-7.00)	14.1	4.81 a (4.00-5.00)	8.4	5.25 b (5.00-6.00)	7.2	10.69	<0.0001
Number of petals	5.65 c (5.00-7.00)	13.1	4.81 a (4.00-5.00)	8.4	5.24 b (5.00-6.00)	6.5	13.24	<0.0001
Number of stamens	5.61 b (5.00-7.00)	13.7	4.80 a (4.00-5.00)	8.4	5.26 b (5.00-6.22)	7.9	10.70	<0.0001
Fruit pedicel length (mm)	43.8 c (33.0-52.2)	15.2	17.5 a (8.5-27.5)	30.1	28.2 b (8.6-50.3)	44.9	16.13	<0.0001
Fruit pedicel diameter (mm)	10.2 c (7.0-12.2)	20.7	2.84 a (1.0-5.1)	42.8	5.4 b (1.0-10.3)	51.3	23.92	<0.0001
Fruit length/breadth ratio	6.50 b (1.00-8.00)	42.1	2.71 a (1.00-5.00)	35.2	3.90 a (1.00-7.00)	44.1	12.81	<0.0001
Fruit cross section	5.67 a (5.00-7.00)	18.2	6.05 a (1.00-9.00)	47.5	5.45 a (2.00-9.00)	41.7	0.43	0.6537
Fruit apex shape	5.33 a (3.00-7.00)	36.9	5.19 a (3.00-7.00)	32	5.33 a (3.00-7.00)	30.9	0.05	0.9485
Fruit weight (g)	244.7 c (94.4-354.5)	36.0	10.5 a (0.4-35.7)	111.6	58.4 b (0.6-224.2)	111.2	39.43	<0.0001
Fruit flesh density	6.33 b (5.00-7.00)	16.3	3.95 a (1.00-9.00)	63.2	5.38 ab (1.00-9.00)	44.4	3.60	0.0328
Fruit calyx length (relative)	2.67 a (1.00-3.00)	30.6	4.62 a (1.00-9.00)	57.5	4.05 a (1.00-9.00)	51.5	1.85	0.1647
Fruit calyx prickles	2.00 a (1.00-3.00)	54.8	3.48 a (0.00-9.00)	91.3	3.19 a (0.00-9.00)	95.0	0.58	0.5646

[a]Means within rows separated by different letters are significantly different according to the Student-Newman-Keuls

All Tomato Analyzer descriptors evaluated, except two (Rectangular and Shoulder Height) displayed significant ($P<0.05$) and in most cases (12) highly significant ($P<0.0001$) differences among average values for the three groups (Table 4). Traits with larger F-ratios were those related to fruit shape indexes (Fruit Shape Index External I, Fruit Shape Index External II, Curved Fruit Shape Index, and Fruit Shape Index Internal), Distal Fruit Blockiness, related to fruit height (Height Mid-width, Maximum Height and Curved Height), Circular, and related to fruit size (Perimeter and Area). For the eight Tomato Analyzer descriptors related to fruit size (from Perimeter to Curved Height), the four fruit shape indexes, Circular, and Obovoid the cultivated eggplant presented significantly higher values than wild species, while for Ovoid it had lower values; interspecific hybrids presented intermediate values, in most cases being significantly different from both cultivated eggplant and wild species (Table 4). Cultivated eggplant had greater Distal Fruit Blockiness and Ellipsoid values than either wild species or interspecific hybrids, while wild species had higher values for Triangular than either cultivated species or interspecific hybrids. Similarly to conventional descriptors, the three groups overlap in the ranges of variation for all Tomato Analyzer descriptors except for Perimeter, Area, Height Mid-width, Maximum Height, Curved Height and Circular, in which there is no overlap between the range of variation of cultivated and wild species, with the values of the former being larger than those of the latter (Table 4).

Table 4. Variation parameters for Tomato Analyzer phenomics fruit descriptors. Mean, range (between brackets), and coefficient of variation (CV; %) for the Tomato Analyzer phenomics fruit morphometric descriptors studied in accessions of cultivated eggplant (*S. melongena*; n=6), wild relatives (n=21) and interspecific hybrids between cultivated eggplant and wild relatives (n=42) and significance of mean differences among the three groups.

	Cultivated eggplant (n=6)		Wild relatives (n=21)		Interspecific hybrids (n=42)			
Descriptors	Mean[a] (range)	CV (%)	Mean (range)	CV (%)	Mean (range)	CV (%)	F-ratio	Probability
Perimeter	24.1 c (20.2-28.0)	12.1	6.1 a (2.1-16.2)	70.0	12.7 b (2.4-28.2)	73.0	13.45	<0.0001
Area	35.4 c (24.4-42.2)	20.5	3.8 a (0.3-17.2)	129.5	15.4 b (0.4-46.9)	109.0	13.47	<0.0001
Width Mid-height	5.21 b (4.01-7.03)	22.8	1.87 a (0.63-4.93)	68.2	3.08 a (0.70-7.37)	66.0	8.80	0.0004
Maximum Width	5.35 b (4.06-7.07)	21.7	1.88 a (0.64-4.96)	68.1	3.11 a (0.86-7.43)	66.1	9.22	0.0003
Height Mid-width	8.17 c (6.39-10.51)	18.4	1.69 a (0.54-3.78)	68.9	4.09 b (0.74-10.41)	77.2	15.60	<0.0001
Maximum Height	8.28 c (6.55-10.64)	18.1	1.72 a (0.55-3.90)	69.5	4.15 b (0.75-10.53)	77.0	15.57	<0.0001
Curved Height	8.47 c (6.93-10.81)	17.2	1.95 a (0.85-4.52)	60.0	4.34 b (0.99-10.62)	73.2	15.53	<0.0001
Fruit Shape Index External I	1.64 c (0.93-2.23)	30.0	0.90 a (0.75-1.04)	8.4	1.22 b (0.75-1.91)	22.5	21.66	<0.0001
Fruit Shape Index External II	1.67 c (0.91-2.30)	31.3	0.89 a (0.74-1.03)	8.8	1.22 b (0.71-1.96)	23.2	21.99	<0.0001
Curved Fruit Shape Index	1.72 c (0.99-2.36)	29.9	1.13 a (0.91-1.41)	13.2	1.35 b (0.89-1.99)	17.7	14.32	<0.0001
Proximal Fruit Blockiness	0.62 a (0.55-0.71)	9.1	0.66 a (0.58-0.78)	7.7	0.61 a (0.36-0.74)	12.4	5.04	0.0092
Distal Fruit Blockiness	0.73 b (0.65-0.77)	9.3	0.60 a (0.52-0.65)	6.5	0.64 a (0.52-0.75)	8.5	16.30	<0.0001
Fruit Shape Triangle	0.86 a (0.74-1.10)	16.6	1.12 b (0.91-1.49)	12.6	0.97 a (0.52-1.31)	16.5	9.91	0.0002
Ellipsoid	0.05 b (0.03-0.07)	29.7	0.02 a (0.01-0.03)	22.0	0.03 a (0.01-0.07)	39.8	10.98	<0.0001
Circular	0.16 c (0.08-0.25)	52.0	0.05 a (0.02-0.10)	41.7	0.09 b (0.03-0.21)	54.2	14.92	<0.0001
Rectangular	0.51 a (0.49-0.54)	3.7	0.51 a (0.48-0.54)	3.2	0.50 a (0.41-0.53)	5.3	2.75	0.0711
Shoulder Height	0.01 a (0.00-0.02)	56.7	0.01 a (0.00-0.03)	68	0.01 a (0.00-0.03)	74.1	0.23	0.7985
Obovoid	0.18 b (0.04-0.29)	55.5	0.05 a (0.00-0.18)	105.6	0.10 a (0.00-0.31)	74.4	8.63	0.0005
Ovoid	0.03 a	160.0	0.09 b	62.6	0.05 ab	97.2	5.65	0.0054

	(0.00-0.11)		(0.00-0.21)		(0.00-0.17)			
Fruit Shape	1.67 c	31.4	0.90 a	8.5	1.22 b	23.3	21.71	<0.0001
Index Internal	(0.91-2.30)		(0.76-1.02)		(0.72-1.96)			

[a]Means within rows separated by different letters are significantly different according to the Student-Newman-Keuls test

Variation in Eggplant, Wild Relatives and Interspecific Hybrids

A large variation for the conventional and Tomato Analyzer descriptors was found in the materials studied (Tables 3 and 4). For most traits, a larger variation both in terms of range and CV was found in the wild species, compared to the cultivated eggplant accessions. In this respect, for all conventional descriptors the range of variation was larger in the wild species than in the cultivated eggplant, except for Shoot tip anthocyanin intensity, the number of flower parts (Number of sepals, Number of petals and Number of stamens), and traits related to fruit size and shape such as Fruit pedicel length, Fruit pedicel diameter, Fruit length/breadth ratio, Fruit apex shape and Fruit weight (Table 3). Also, the CV was larger in the wild species than in the cultivated eggplant for all the conventional descriptors except for Leaf prickles, Number of sepals, Number of petals, Number of stamens and Fruit shape apex. Conversely, in the case of Tomato Analyzer descriptors, the range of variation was greater in wild species than in the cultivated eggplant for only six out of the 20 descriptors evaluated (Perimeter, Width Mid-height, Maximum Width, Rectangular, and Ovoid), while for the CV the wild species had a greater value than cultivated eggplant for nine of the descriptors, of which seven are related to fruit size (Perimeters, Area, Width Mid-height, Maximum Width, Height Mid-Width, Maximum height and Curved height), plus Shoulder Height and Obovoid (Table 4).

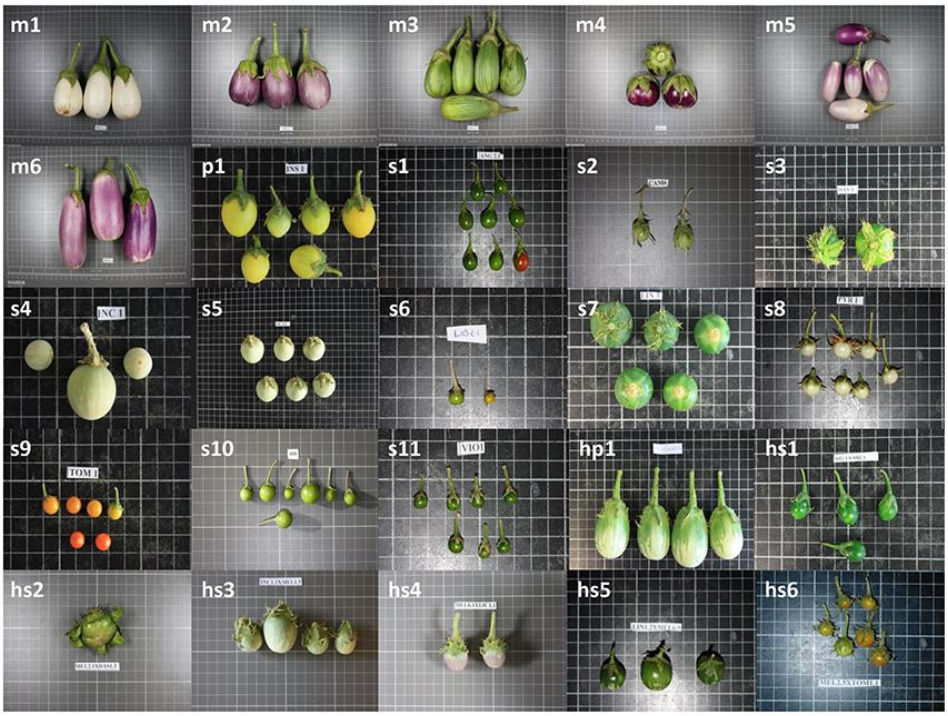

Figure 1. Fruit samples of the materials used. This include: *Solanum melongena* accessions MEL1 (m1) to MEL6 (m6); wild species of primary genepool *S. insanum* (p1); wild species of secondary genepool *S. anguivi* (s1), *S. campylacanthum* (s2), *S. dasyphyllum* (s3), *S. incanum* (s4), *S. lichtensteinii* (s5), *S. lidii* (s6), *S. linnaeanum* (s7), *S. pyracanthos* (s8), *S. tomentosum* (s9), *S. vespertilio* (s10) and *S. violaceum* (s11); interspecific hybrids between *S. melongena* and primary genepool species *S. insanum* (hp1); and, interspecific hybrids between *S. melongena* and secondary genepool species S. *anguivi* (hs1), *S. dasyphyllum* (hs2), *S. incanum* (hs3), *S. lichtensteinii* (hs4), *S. linnaeanum* (hs5) and *S. tomentosum* (hs6). Fruits are not depicted at the same scale; the size of the grid cells is 1 cm × 1cm.

When considering the interspecific hybrids a large range of variation was found for many conventional descriptors, with variation parameters generally larger than those of the cultivated species and smaller than those of the wild species. In this respect, the range of variation was larger than that of the cultivated eggplant for all but nine conventional descriptors (Plant growth habit, Shoot tip anthocyanin intensity, Leaf surface shape, Number of sepals, Number of petals, Number of stamens, Fruit length/breadth ratio, Fruit apex shape and Fruit weight), while compared to wild species it was larger for eleven descriptors (Plant height, Shoot tip anthocyanin intensity, Leaf blade tip angle, Leaf pedicel length, Leaf blade length, Corolla diameter, Number of stamens, Fruit pedicel length, Fruit pedicel diameter, Fruit length/breadth ratio, Fruit cross section, and Fruit weight) (Table 3). The coefficient of variation for conventional descriptors was also larger than in the cultivated species for all traits except nine (Plant growth habit, Stem diameter, Leaf blade lobing, Leaf prickles, Number of flower prickles, Number of sepals, Number of petals, Number of stamens, and Fruit apex shape) and larger than that of the wild species for eight descriptors (Plant height, Leaf blade tip angle, Fruit pedicel length, Fruit pedicel diameter, Fruit length/breadth ratio, fruit cross section, fruit apex shape, and Fruit calyx prickles) (Table 3).

Regarding the variation for Tomato Analyzer traits, the range of variation in the interspecific hybrids was greater than those of cultivated eggplant and wild species for all traits except five in the case of cultivated eggplant, which correspond to fruit shape indexes (Fruit Shape Index External I, Fruit Shape Index External II, Curved Fruit Shape Index, and Fruit Shape Index Internal) and Circular, and only one (Ovoid) in the case of wild species (Table 4). Also, larger values were obtained in the CV for

Tomato Analyzer descriptors in the interspecific hybrids compared to the cultivated species for all traits but seven, including the four fruit shape indexes, Distal Fruit Blockiness, Fruit Shape Triangle, and Ovoid. When compared to wild species the interspecific hybrids also presented higher CV for all traits, except four (Area, Width Mid-height, Maximum Width, and Obovoid) (Table 4).

Multivariate Analysis

The three first components of the principal components analysis made with all conventional and Tomato Analyzer descriptors on the *S. melongena*, wild related species and interspecific hybrids globally accounted for 58.8% accounted of the total variation among accession means, with the first, second and third component accounting, respectively for 37.2%, 12.0% and 9.5% of the total variation (Table 5). The first principal component was positively correlated to Corolla diameter, and to multiple traits related to fruit size as well as to elongated fruit shape (Table 5). The second principal component was positively correlated to Plant height and to obovoid fruit shape (positive correlations with Distal Fruit Blockiness and Obovoid and negative correlations to Proximal Fruit Blockiness, Fruit Shape Triangle, Rectangular and Ovoid). The third principal component was positively correlated to Plant growth habit (i.e., prostrate habit), to multiple plant, leaf and corolla size traits, to a higher number of flower parts (sepals, petals and stamens) and to an increased prickliness in leaves, and flower and fruit calyces (Table 5).

Table 5. Correlation coefficients between morphological conventional and phenomics descriptors. Values represent the correlation coefficients

for the three first principal components in the collection of eggplant (*S. melongena*), wild relatives and interspecific hybrids evaluated. Only correlations with absolute values ≥0.150 have been listed.

Descriptors	First principal component	Second principal component	Third principal component
Plant growth habit			0.151
Plant height (cm)		0.154	0.176
Stem diameter (mm)			0.266
Leaf blade lobing			0.258
Leaf prickles (upper surface)		-0.165	0.184
Leaf surface shape			0.236
Leaf blade length (cm)			0.291
Leaf blade width (cm)			0.306
Corolla diameter (mm)	0.184		0.153
Number of flower prickles (calyx)		-0.170	0.226
Number of sepals			0.275
Number of petals			0.267
Number of stamens			0.266
Fruit pedicel length (mm)	0.218		
Fruit pedicel diameter (mm)	0.218		
Fruit length/breadth ratio	0.191		
Fruit weight (g)	0.212		
Fruit calyx prickles		-0.190	0.253
Perimeter (cm)	0.225		
Area (cm^2)	0.219		
Width Mid-height (cm)	0.204		
Maximum Width (cm)	0.206		
Height Mid-width (cm)	0.231		
Maximum Height (cm)	0.231		
Curved Height (cm)	0.231		
Fruit Shape Index External I	0.209		
Fruit Shape Index External II	0.209		
Curved Fruit Shape Index	0.167		
Proximal Fruit Blockiness		-0.371	
Distal Fruit Blockiness	0.163	0.204	

Fruit Shape Triangle		-0.349	
Circular	0.189		
Rectangular		-0.245	
Shoulder Height		0.159	
Obovoid		0.328	
Ovoid		-0.312	
Fruit Shape Index Internal	0.208		
Eigenvalue	17.50	5.65	4.48
Variance explained (%)	37.23	12.04	9.53
Cumulative variance explained (%)	37.23	49.27	58.80

The projection of *S. melongena*, wild species and interspecific hybrids in the PCA plot reveals that although considerable diversity exists in both *S. melongena* (black squares) and wild species (white symbols), the interspecific hybrids (grey symbols) present a more scattered distribution in the PCA plot (Figures 2 and 3). Amazingly, interspecific hybrids with the primary genepool species *S. insanum* plot closer to the cultivated eggplant and are intermingled with it the PCA graphs. On the contrary, interspecific hybrids with secondary genepool species plot closer to the wild species and are also intermingled with them (Figures 2 and 3). The first component clearly separates the group formed by *S. melongena* and the interspecific hybrids with the primary genepool species *S. insanum*, which present positive values (above 3 and 2, respectively) for this component, from the group formed by all the wild species and interspecific hybrids with secondary genepool species, which present negative values or below 1.5, respectively. Among the interspecific hybrids with secondary genepool species, those with *S. incanum* and *S. lichtensteinii* are the closest ones to *S. melongena* in this first component (Figures 2 and 3). When considering the second component all *S.*

melongena accessions but one have positive values, while interspecific hybrids with *S. insanum* are equally distributed in the positive and negative values of this second component (Figure 2). Primary genepool wild species *S. insanum* and all secondary genepool species, except *S. campylacanthum*, *S. pyracanthos*, *S. tomentosum* and one accession of each of *S. anguivi* and *S. lidii* have negative values for this second component. When considering interspecific hybrids with secondary genepool species, although they are intermingled with the wild species for this second component most of the hybrids present positive values for this second component, with the exceptions being the hybrids with *S. lichtensteinii* (four out of five), *S. linnaeanum* and one of each of the interspecific hybrids with each of the species *S. anguivi* and *S. incanum* (this latter with a value very close to 0). Amazingly, the highest values for this second component correspond to interspecific hybrids with *S. anguivi* (Figure 2). For the third component both *S. melongena* and the interspecific hybrids with *S. insanum* are scattered and display positive or negative values (Figure 3). Most wild species accessions have negative values for this third component, except the accessions of *S. dasyphyllum*, *S. linnaeanum*, *S. pyracanthos* and *S. violaceum*, as well as one accession of *S. incanum* (with values close to 0). The lowest values for this component are those of *S. lidii*, *S. vespertilio* and *S. tomentosum* (Figure 3). On the other hand all interspecific hybrids with secondary genepool species, with the exception of two interspecific hybrids with *S. anguivi*, present positive values for this third component. In this case, the highest values for the third component correspond to interspecific hybrids with *S. dasyphyllum*, *S. lichtensteinii* and *S. incanum* (Figure 3).

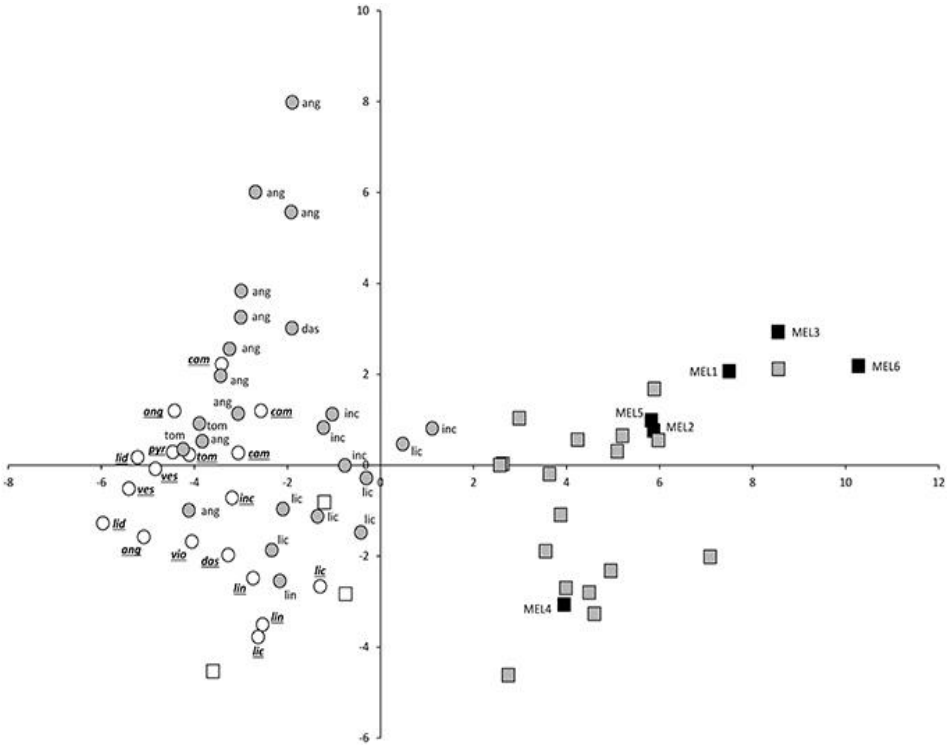

Figure 2. First (X-axis) and second (Y-axis) principal components (37.2% and 12.0% of the total variation explained, respectively) scatterplot of cultivated eggplant, wild relatives and interspecific hybrids based on 27 conventional and 20 Tomato Analyzer morphological descriptors. Cultivated eggplant (*S. melongena*) is represented by black squares, primary genepool species *S. insanum* by white squares, interspecific hybrids between *S. melongena* and *S. insanum* by grey squares, secondary genepool species by white circles (with species codes in normal font), and interspecific hybrids between *S. melongena* and secondary genepool species by grey circles (with wild species codes in underlined italics). For secondary genepool species and their hybrids with

S. melongena, the following codes are used: ang (*S. anguivi*), cam (*S. campylacanthum*), das (*S. dasyphyllum*), inc (*S. incanum*), lic (*S. lichtensteinii*), lid (*S. lidii*), lin (*S. linnaeanum*), pyr (*S. pyracanthos*), tom (*S. tomentosum*), ves (*S. vespertilio*), vio (*S. violaceum*).

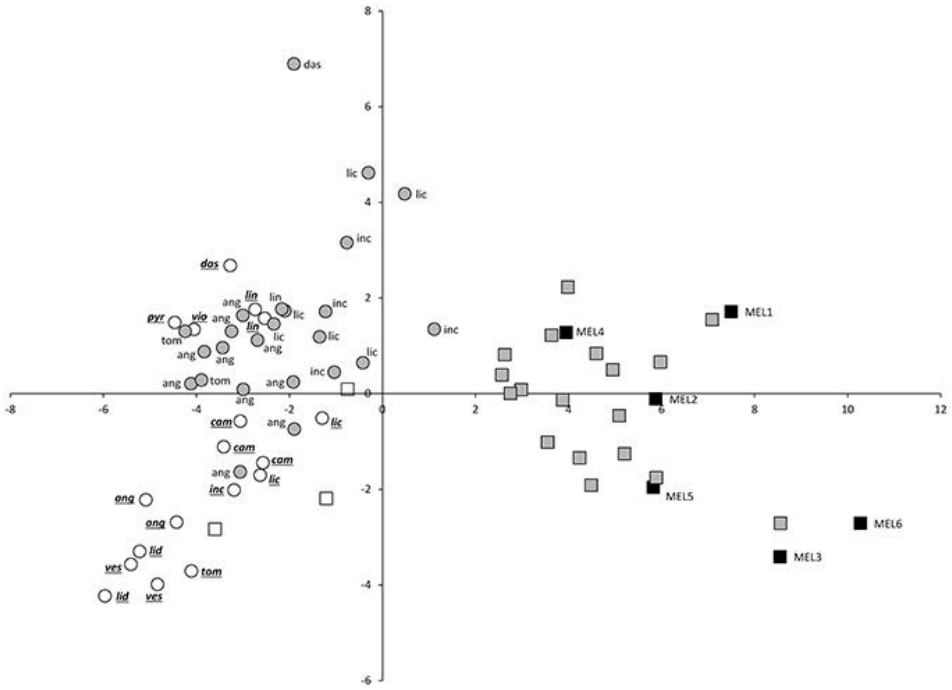

Figure 3. First (X-axis) and thrid (Y-axis) principal components (37.2% and 9.5% of the total variation explained, respectively) scatterplot of cultivated eggplant, wild relatives and interspecific hybrids based on 27 conventional and 20 Tomato Analyzer morphological descriptors. Cultivated eggplant (*S. melongena*) is represented by black squares, primary genepool species *S. insanum* by white squares, interspecific hybrids between *S. melongena* and *S. insanum*

by grey squares, secondary genepool species by white circles (with species codes in normal font), and interspecific hybrids between *S. melongena* and secondary genepool species by grey circles (with wild species codes in underlined italics). For secondary genepool species and their hybrids with *S. melongena*, the following codes are used: ang (*S. anguivi*), cam (*S. campylacanthum*), das (*S. dasyphyllum*), inc (*S. incanum*), lic (*S. lichtensteinii*), lid (*S. lidii*), lin (*S. linnaeanum*), pyr (*S. pyracanthos*), tom (*S. tomentosum*), ves (*S. vespertilio*), vio (*S. violaceum*).

Traits of Agronomic Interest in Wild Species

The 12 wild species evaluated presented considerable differences for traits of agronomic interest (Table 6). For example, regarding vegetative traits the tallest plants were those of *S. anguivi*, S. *campylacanthum* and *S. violaceum*, with values above 150 cm, while *S. dasyphyllum* did not reach 100 cm (Table 6). The thicker stem diameters were those of *S. anguivi* and *S. pyracanthos*, with average values above 30 mm, while those of *S. lidii*, *S. tomentosum* and *S. violaceum* did not reach 20 mm. The species with greatest leaf lobing were *S. dasyphyllum*, *S. linnaeanum* and *S. pyracanthos*, with a value of 9, while *S. anguivi* and *S. campylacanthum* presented values below 3 (Table 6). The species with a greater number of prickles in the leaves were *S. dasyphyllum*, *S. pyracanthos* and *S. violaceum*, with values of 7 or higher, while *S. anguivi* and *S. tomentosusm* did not present prickles in the leaves. The longest leaf pedicel lengths (>4 cm) were found in *S. pyracanthos* and *S. vespertilio*, while the smallest ones (<1.5 cm) were those of *S. dasyphyllum* and *S. tomentosum*. The largest leaf blades were those of *S. dasyphyllum* and *S.*

campylacanthum, both of which had a Leaf blade length above 19 cm; the smallest leaf blades were those of *S. tomentosum*, with a Leaf blade length of 5.2 cm (Table 6).

When considering flower and fruit traits, the two species with a larger number of flowers per inflorescence were *S. lidii* and *S. vespertilio*, with more than 13 flowers/inflorescence, while the smaller number was *S. insanum*, with an average of only 2 flowers per inflorescence (Table 6). The most pigmented flowers were those of *S. campylacanthum*, *S. incanum*, *S. lidii*, *S. pyracanthos* and *S. violaceum*, with a value of 7 or higher, while the less pigmented were those of *S. anguivi* and *S. tomentosum*, with values of 3 or lower. All wild species had five petals (and sepals and stamens), except *S. lidii* and *S. vespertilio*, which had only four. The largest fruits were those of *S. incanum* and *S. lichtensteinii*, with average values above 25 g, more than 10-fold heavier than those of *S. anguivi*, *S. lidii*, *S. pyracanthos*, *S. tomentosum*, *S. vespertilio* and *S. violaceum*. The highest calyx prickliness was observed in *S. linnaeanum*, *S. pyracanthos* and *S. violaceum*, with values of 7 or larger, while *S. anguivi*, *S. lidii* and *S. vespertilio* did not present calyx prickles (Table 6). The most elongated fruit were those of *S. incanum* with a value of Fruit Shape Index External I higher than 1, while the most flattened ones were those of *S. dasyphyllum* and *S. lidii*, with values below 0.8 (Table 6).

Table 6. Average (±SE) values based on accession means for selected traits in the 12 wild species (one from the primary genepool GP1, *S. insanum*; and 11 from the secondary genepool GP2) evaluated. Traits were selected so that they were relevant for breeding and useful to distinguish the different wild species.

	GP1	*GP2*										
Descriptors	*S. insanum*	*S. anguivi*	*S. campylacanthum*	*S. dasyphyllum*	*S. incanum*	*S. lichtensteinii*	*S. lidii*	*S. linnaeanum*	*S. pyracanthos*	*S. tomentosum*	*S. vespertilio*	*S. violaceum*
n	3	2	3	1	1	2	2	2	1	1	2	1
Plant height (cm)	108.7±9.8	153.7±6.3	150.2±6.9	95.0	120.0	130.5±12.5	108.6±3.0	107.0±0.0	141.7	104.0	115.5±1.5	154.0
Stem diameter (mm)	22.8±4.6	31.3±2.0	21.5±1.8	23.5	28.0	22.3±2.4	14.0±2.0	29.8±0.2	34.7	19.5	28.6±0.9	19.8
Leaf blade lobing	5.00±0.00	2.00±1.00	2.33± 0.67	9.00	3.00	5.00±0.00	5.00±0.00	9.00±0.00	9.00	3.00	3.00±0.00	7.00
Leaf prickles (upper surface)	3.33±1.67	0.00±0.00	0.67±0.67	9.00	1.00	0.50±0.50	6.00±1.00	6.00±1.00	7.00	0.00	4.00±1.00	9.00
Leaf pedicel length (cm)	2.27±0.51	1.98±0.10	2.75±0.71	1.2	2.3	2.75±0.89	3.07±0.33	2.99±0.71	4.61	0.63	4.11±0.13	3.95
Leaf blade length (cm)	8.9±1.5	10.9±1.6	19.1± 1.7	22.1	11.3	13.4± 2.8	14.7±3.9	13.9±2.4	16.9	5.2	14.3±0.6	15.7
Leaf blade width (cm)	7.0±1.0	7.4±1.1	8.4±1.4	18.7	7.8	9.3±2.1	7.9±3.5	9.5±0.8	7.4	3.3	9.5±0.7	12.9
Number of flowers per inflorescence	2.0±1.0	8.2±2.2	9.4±1.7	10.6	9.1	5.1±1.9	13.7±0.7	3.0±0.2	13.3	5.0	16.0±0.2	10.7
Corolla colour	5.67±0.67	2.00±1.00	7.67±1.33	5.00	7.00	4.00±1.00	7.00±0.00	5.00±0.00	9.00	3.00	5.00±0.00	7.00
Number of petals	5.00±0.00	5.00±0.00	5.00±0.00	5.00	5.00	5.00±0.00	4.00±0.00	5.00±0.00	5.00	5.00	4.00±0.00	5.00
Fruit weight (g)	26.5±5.0	1.3±0.5	4.6±1.2	19.3	11.6	28.7±6.2	0.4±0.0	16.2±3.3	1.0	0.5	1.2±0.1	0.4

Fruit prickles	calyx	3.00±1.15	0.00±0.00	1.67± 0.67	9.00	5.00	5.00±0.00	0.00±0.00	8.00±1.00	7.00	5.00	0.00±0.00	7.00
Fruit Shape External I	Index	0.97±0.39	0.95±0.047	0.93±0.04	0.79	1.04	0.93±0.08	0.78±0.04	0.94±0.07	0.86	0.94	0.87±0.01	0.85

Heterosis in Interspecific Hybrids

Interspecific hybrids between eggplant and its wild relatives generally displayed positive heterosis for plant size traits, with average heterosis values of up to 90.5% for Plant height and 46.2% for Stem diameter in the hybrids of *S. melongena* with *S. dasyphyllum* (Table 7). The only negative value observed for these traits was for Stem diameter in the interspecific hybrid with *S. linnaeanum*. Hybrids with primary genepool species *S. insanum* were heterotic for Shoot tip anthocyanin intensity, while those with secondary genepool species had negative or non-significant values for heterosis (Table 7). Most interspecific hybrids presented higher prickliness than their parent species, and in consequence, very high average values for heterosis for Leaf prickles are observed, with values between 91.0% for *S. dasyphyllum* and 800.0% for *S. tomentosum*. Leaf size traits were also, in general, heterotic in the interspecific hybrids, with the exception of Leaf pedicel length in *S. dasyphyllum* and *S. linnaeanum*. The same phenomenon was observed for the Number of flowers per inflorescence, with values of up to 87.7% in the hybrids with *S. tomentosum* (Table 7). The pigmentation of the corolla (Corolla colour) also presented average positive heterosis values in the hybrids of *S. melongena* with five out of the seven wild species, the exception being interspecific hybrids with *S. anguivi* and *S. tomentosum*. The number of flower parts, represented by the Number of petals presented low absolute values for heterosis in all cases (Table 7).

Regarding Fruit weight, considerable differences were observed between the hybrids with the primary genepool species (*S. insanum*) on one hand,

and the hybrids with secondary genepool species on the other. In this respect, while the hybrids with *S. insanum* displayed small negative average heterosis (-5.5%), not significantly different from 0, in the case of secondary genepool species, the heterosis for Fruit weight is highly negative, with values between -60.4% for hybrids with *S. dasyphyllum* to -98.6% in hybrids with *S. tomentosum* (Table 7). As occurred for Leaf prickles, positive heterosis values, although of smaller magnitude, were observed for Fruit calyx prickles, with the exception of the hybrids with *S. anguivi*, which did not present prickles in the calyx, and in consequence had a heterosis value of -100%. Finally, for fruit shape, the hybrids with primary genepool species *S. insanum* presented positive heterosis, while those with secondary genepool species had negative heterosis values (Table 7).

Table 7. Heterosis over mid parent values (%; ±SE) based on accession and interspecific hybrid means. Values are presented for traits of agronomic interest in the interspecific hybrids of eggplant with seven wild relatives (one from the primary genepool, *S. incanum*; and six from the secondary genepool).

Descriptors	*S. insanum*	*S. anguivi*	*S. dasyphyllum*	*S. incanum*	*S. lichtensteinii*	*S. linnaeanum*	*S. tomentosum*
n	18	10	4/1[a]	4	6	1	2
Plant height (cm)	16.7±4.6	34.4±7.1	90.5±7.6	36.8±11.3	38.1±4.4	2.3	23.3±4.2
Stem diameter (mm)	10.5±4.3	10.4±3.8	46.2±12.3	29.1±11.0	39.8±10.3	-18.7	23.8±3.8
Leaf prickles (upper surface)	155.1±34.5	260.0± 173.9	91.0±5.4	733.3±100.0	144.4±92.9	100.0	800.0±800.0
Leaf pedicel length (cm)	39.7±6.5	22.5±7.8	-21.6±1.2	19.5±2.7	24.9±9.2	-13.3	56.3±23.9
Leaf blade length (cm)	24.9±4.1	22.2±5.5	34.8±5.7	47.6±6.6	30.6±6.3	3.9	22.8±1.6
Leaf blade width (cm)	27.7±4.5	38.2±9.5	32.9± 5.0	67.7±9.6	41.7±8.5	7.1	22.4±14.0
Number of flowers per inflorescence	70.1±16.0	75.9±16.3	36.9±13.1	21.0±9.4	42.7±15.7	-1.8	87.7±35.5
Corolla colour	15.9±4.3	-2.5±4.6	18.9± 10.4	19.2±3.0	16.2±4.8	7.5	-0.1±8.6
Number of petals	1.3±2.1	-4.8±1.6	1.9±5.4	-4.4±2.4	-2.2±3.4	-3.2	-1.0±1.0
Fruit weight (g)	-5.5±6.9	-98.2±0.3	-60.4	-86.6±2.8	-89.4±1.5	-89.9	-98.6±0.3
Fruit calyx prickles	32.9±25.2	-100.0±0.0	80.0	27.1±42.4	56.9±27.6	80.0	29.1±104.1
Fruit Shape Index External I	13.7±3.5	-16.7±6.9	-26.4	-13.6±0.8	-15.0±4.3	-40.8	-27.4±8.0

[a]For *S. dasyphyllum* data are available for four accessions for plant traits and only for one accession for fruit traits.

Discussion

Crop wild relatives are widely recognized as an invaluable genetic resource for breeding, in particular for broadening the genetic base of crops with narrow genetic diversity, and as sources of variation for traits of interest in breeding crops, including adapting them to the challenges posed by climate change (Dempewolf et al., 2014). Modern varieties of many important crops carry introgressions from wild species resulting from breeding programmes performed in the last hundred years (Hajjar and Hodjkin, 2007). One of the most outstanding examples is tomato, where modern commercial hybrids carry different combinations of 15 different introgressions from different wild species (Díez and Nuez, 2008; Sabatini et al., 2013). However, in the case of eggplant, despite being one of the most important vegetables and being intercrossable with many wild relatives, there are few reports on the use of the variation available in the wild species for eggplant breeding (Daunay and Hazra, 2012; Rotino et al., 2014; Liu et al., 2015) and no modern commercial varieties of eggplant carrying introgressions from wild species are known to us.

In our study we have evaluated six accessions of cultivated eggplant, 21 accessions of 12 wild species, and 45 interspecific hybrids of cultivated eggplant with seven wild species. This represents the largest study up to now on morphological and agronomic traits for breeding of this type of materials. As expected, many differences were found within and among cultivated eggplant, wild relatives and the interspecific hybrids for the conventional descriptors used, confirming the utility of the EGGNET (van der Weerden and Barense, 2007) and IPGRI (1990)

conventional morphological descriptors and Tomato Analyzer traits (Rodríguez et al., 2010) used for evaluating eggplant wild relatives and interspecific hybrids (Prohens et al., 2013).

Also, many differences were found for the traits studied among cultivated eggplant, wild species and interspecific hybrids. Although many of the wild species of eggplant thrive in arid and semi-arid conditions (Knapp et al., 2013; Vorontsova and Knapp, 2016), when grown under the favourable conditions of cultivated environments, the wild species and their interspecific hybrids generally display a high vigour, expressed as average values for plant height and stem diameter above those of cultivated eggplant. This is of interest for developing new rootstocks, which generally require having high vigour (Gisbert et al., 2011). Another important trait of agronomic interest for which there were considerable differences among groups was prickliness, which was much greater in wild species and interspecific hybrids, confirming that alleles from the cultivated *S. melongena* are recessive (Doganlar et al., 2002; Gramazio et al., 2014; Portis et al., 2015). The number of flowers per inflorescence was also much greater in wild species and interspecific hybrids. This trait is very important in eggplant breeding, as a reduced value of this trait results in increased fruit size uniformity (Sękara and Bieniasz, 2008). Also, fruit size and shape, which are of great relevance for breeding (Daunay and Hazra, 2012; Portis et al., 2015), also differed considerably among the three groups, with the interspecific hybrids presenting intermediate values, although on most cases they were closer to those of the wild species, indicating dominance of the genes of the latter (Doganlar et al., 2012).

The much higher variation observed in wild species and interspecific hybrids for vegetative, flower and inflorescence traits

compared to cultivated eggplant was expected, as we were comparing a single species with an admixture of different wild species or hybrids, which present a much higher genetic diversity (Meyer et al., 2012; Särkinen et al., 2013; Vorontsova et al., 2013). However, for traits related to the fruit size and shape much higher variation was observed in the cultivated eggplant than in the wild species, confirming the general observation that the morphological variation in the organ for which a crop is domesticated (in this case the fruit) increases during domestication (Meyer and Purugganan, 2014). Amazingly, in the case of interspecific hybrids a larger variation was found for most fruit size and shape traits than in the cultivated *S. melongena*. Although most interspecific hybrids were more similar to the wild species, in some cases they were intermediate, revealing that different genic control mechanisms must exist for fruit size and shape among the wild relatives of eggplant. In this respect, the multivariate analysis clearly shows that interspecific hybrids with the primary genepool species *S. insanum* are morphologically closer to the cultivated *S. melongena*, while the hybrids with secondary genepool species present a general morphology closer to that of the wild species. These results may support the hypothesis that *S. insanum* is the wild ancestor of cultivated eggplant (Knapp et al., 2013), as domestication should be easier when genes for domestication traits from the wild species display intermediate dominance rather than full dominance.

The study of individual wild species suggests that *S. anguivi*, *S. campylacanthum*, *S. pyracanthos* and *S. violaceum* may be of interest for increasing the vigour of cultivated eggplant or for being used as rootstocks. Also, wild eggplant species use to have undesirable traits (e.g., prickliness,

small fruit size, etc.) that have to be removed during the breeding (Rotino et al., 2014). In this case, the most desirable wild species are those that are most similar to the crop for these traits. For example, the lack of prickles or very low prickliness of *S. anguivi*, *S. campylacanthum* and *S. tomentosum* is a very favourable trait for breeders, as one of the most important breeding objectives in modern eggplant breeding is an absolute lack of prickles in the plant and in the fruit calyx (Daunay and Hazra, 2012). Regarding fruit weight, the wild species with greater fruit weight should be the most interesting for breeders in order to recover fruit size in few backcross generations. In this case, *S. insanum*, *S. dasyphyllum* and *S. lichtensteinii* should be the most interesting candidates if a rapid recovery of fruit size is desired. In any case, Prohens et al. (2013) showed that fruit size recovers quickly even in first backcrosses with the wild species *S. incanum*, which has an intermediate fruit size among wild species.

Although differences were observed among interspecific hybrids from different wild species, hybrids were in general vigorous, displaying heterosis for vigour traits. This phenomenon had already been described in interspecific hybrids with *S. incanum* (Gisbert et al., 2011; Prohens et al., 2013), and our results suggest that this is a common phenomenon in the hybrids between eggplant and wild relatives. Amazingly, most interspecific hybrids were highly heterotic for prickliness, with heterosis values over 100%, which indicates overdominance for this traits. Prickles even appeared in interspecific hybrids with wild species that were not prickly, like *S. tomentosum*. In previous works, heterosis for prickliness had already been described in interspecific crosses in eggplant (Prohens et al., 2012; Devi et al., 2015; Plazas et al., 2016). Several studies with segregating populations of *S. linnaeanum* and *S. insanum* show that

differences in prickliness between cultivated eggplant and wild relatives is under the control of a few QTL (Doganlar et al., 2002; Gramazio et al., 2014) and therefore prickliness should be easily removed in backcross generations. Although for fruit size traits negative heterosis was generally observed in the interspecific hybrids, indicating a greater similarity to the wild species, interspecific hybrids with primary genepool species *S. insanum* presented values close to zero, similarly to intraspecific hybrids of *S. melongena* (Rodríguez-Burruezo et al., 2008), indicating intermediate dominance and values intermediate between both parental species. However, hybrids with wild species from the secondary genepool displayed highly negative heterosis, in some cases close to 100% like in interspecific hybrids with *S. anguivi* and *S. tomentosum*, suggesting that in these materials it may be more difficult to recover fruit size in the backcross generations.

In conclusion, the characterization with conventional descriptors and the Tomato Analyzer phenomics tool has allowed a detailed characterization of eggplant, close wild relatives and their interspecific hybrids. The high variation among wild species allowed identifying sources of variation and most promising species for traits of interest for eggplant breeding. The fact that interspecific hybrids with primary genepool species *S. insanum* are intermediate or close to eggplant for many traits, may facilitate the use of this species in introgression breeding and supports previous evidence that this species is the ancestor of cultivated eggplant. Also, the high vigour of most interspecific hybrids may be directly exploited by using them as rootstocks. The information obtained here on phenotypic characteristics and heterosis of wild species and interspecific hybrids is of interest for eggplant breeding. Given the

adaptation of many wild species to stressful conditions, their utilization in eggplant breeding may result in the development of a new generation of cultivars adapted to climate change challenges.

Author Contributions

JP, SV, PG and MP conceived and designed the research; PK and MP performed the phenotypic and phenomics characterization; PK, JP, and PG analysed the data. JP, SV, PG and MP wrote the manuscript. All authors read and approved the manuscript.

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Chapter 2: Phenolics Content, Fruit Flesh Colour and Browning in Cultivated Eggplant, Wild Relatives and Interspecific Hybrids: Implications for Fruit Quality Breeding

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Abstract

Increasing the content in bioactive phenolics in the eggplant (*Solanum melongena*) fruit is of interest, but may result in enhanced browning. We evaluated six varieties of *S. melongena*, 22 accessions of wild related species and 42 interspecific hybrids for phenolics content, fruit flesh colour, polyphenol oxidase (PPO) activity, and fruit flesh browning. Wild relatives generally had a higher content in phenolics and a broader range of variation than cultivated eggplant. Chlorogenic acid was the predominant (>65.0%) phenolic acid in cultivated eggplant and its primary genepool wild ancestor *S. insanum*, while for the other wild species on average represented less than 50% of the chromatogram peak area. Fruit flesh colour was lighter in *S. melongena* than in the wild species, while PPO activity and browning was much higher in wild species of the secondary and tertiary genepools. Interspecific hybrids between *S. melongena* and *S. insanum* were intermediate in their characteristics, while those with secondary and tertiary genepool species were more similar to the wild species. No significant correlations were found between total phenolics or chlorogenic acid contents and fruit flesh browning, but PPO activity was correlated to both the degree of browning (r=0.404) and colour difference (r=0.458). The results indicate that wild species can contribute to improving the bioactive properties of eggplant without affecting negatively fruit flesh colour and browning.

Keywords: browning, chlorogenic acid, diversity, hybrids, phenolics, *Solanum melongena*, wild species

1. Introduction

Eggplant (*Solanum melongena* L.) has recently received increased attention for its various beneficial bioactive properties, mostly derived from the high contents in phenolics in the fruit flesh (Mennella et al., 2010; Plazas et al., 2013a; Docimo et al., 2016a). Although anthocyanins are present in the skin of purple-pigmented eggplants (Stommel and Dumm, 2015), most of the phenolics of eggplant are present in the fruit flesh, mainly as phenolic acids (Docimo et al., 2016a). Among them, chlorogenic acid (5-*O*-caffeoyl-quinic acid), a derivative of cinnamic acid, is generally the most abundant phenolic compound in the flesh of eggplant (Stommel and Whitaker, 2003; Mennella et al., 2012; Prohens et al., 2013). Chlorogenic acid has been found to have anti-carcinogenic, anti-immflamatory, anti-microbial, anti-obesity, cardioprotective, hypotensive, and neuroprotective effects (Plazas et al., 2013a; Heleno et al., 2015). Because of this, interest in the development of vegetable crop varieties with high contents in chlorogenic acid and other phenolic acids has increased in the last years (Kaushik et al., 2015).

An important a drawback of increasing the content in chlorogenic acid in eggplant fruits is that it may result in enhanced fruit flesh browning (Prohens et al., 2007; Plazas et al., 2013b; Mishra et al., 2013; Prohens et al., 2013). Enzymatic browning of the eggplant fruit flesh is caused by the action of polyphenol oxidases (PPOs), which catalyze the conversion of phenolic acids, which are stored in vacuoles, to quinones, which subsequently further react with oxygen to give brown coloured compounds

(Mishra et al., 2013; Docimo et al., 2016b). Because of this, it has been hypothesized that selection for reduced browning has resulted in the indirect selection for lower content in phenolics acids in the eggplant fruit flesh of modern varieties, so that modern varieties have on average less chlorogenic acid content than old landraces (Prohens et al., 2007; Meyer et al., 2015). As a main player in the browning process, variation in the PPO activity, which can be variable in the eggplant fruit flesh (Plazas et al., 2013b), may influence the degree of browning. Up to six different PPO genes have been described in eggplant, all of which are expressed in the fruits, although they have different levels of expression, depending on the physiological stage of development (Shetty et al., 2011).

Various traits related to content in phenolics and fruit flesh browning have been studied in different eggplant materials (Mennella et al., 2010; Mishra et al., 2011; Mennella et al., 2012; Mishra et al., 2013; Plazas et al., 2013b; Prohens et al., 2013). Moreover, the genes of the chlorogenic acid pathway and PPOs, as well as QTLs related to chlorogenic acid content have been mapped (Gramazio et al., 2014; Toppino et al., 2016). In several crops, it has been found that browning and concentration in phenolics are positively correlated (Urbany et al., 2011; Di Guardo et al., 2013; Nayak et al., 2015). However, for eggplant, the correlation between fruit flesh phenolics concentration, and particularly chlorogenic acid content, and fruit flesh browning has been found to be moderate (Prohens et al., 2007; Plazas et al., 2013b; Docimo et al., 2016a, 2016b), suggesting that other physiological or cell morphology factors may be involved in the browning process (Prohens et al., 2007; Mishra et al., 2011; Docimo et al., 2016b).

Eggplant crop wild relatives from the primary, secondary and tertiary genepools contain a tremendous amount of underexploited genetic diversity for eggplant breeding (Knapp et al., 2013; Vorontsova et al., 2013; Plazas et al., 2016; Syfert et al., 2016). Recently breeders have emphasized the interest of exploiting them for their use for breeding against several biotic and abiotic stresses (Daunay and Hazra, 2012; Rotino et al., 2014). In this sense, many eggplant wild relatives can be hybridized with eggplant with different degrees of hybrid fertility (Daunay and Hazra, 2012; Rotino et al., 2014). Recently, Plazas et al. (2016) and Kouassi et al. (2016) have developed interspecific hybrids of six eggplant accessions with 14 wild species belonging to the primary, secondary and tertiary genepools, with varying numbers of hybrid combinations depending on the wild species concerned, many of which have been morphologically characterized (Kaushik et al., 2016). Also, a number of backcrosses of these hybrids to the cultivated *S. melongena* have been obtained (Kouassi et al., 2016).

Some wild relatives of eggplant are reported to have high contents in phenolic acids in the fruit flesh (Stommel and Whitaker, 2003; Ma et al., 2010; Mennella et al., 2012; Prohens et al., 2013; Meyer et al., 2015) and could be a source of variation for improved content in phenolics of the cultivated eggplant (Prohens et al., 2013). However, little is known on the diversity among wild species for the content of phenolics, and to our knowledge there are no studies related to either the fruit flesh colour and browning and PPO activity, or the relationship between content in phenolics and fruit flesh browning in collections of wild eggplants.

In this work we characterize cultivated eggplant, wild relatives from the primary, secondary and tertiary genepools, along with interspecific

hybrids between eggplant and some of the crop wild relatives for various traits related to content in phenolics and fruit flesh browning. Our work will provide relevant information on the potential of wild relatives for the development of eggplant cultivars with improved content in bioactive phenolics coupled with low fruit flesh browning.

2. Material and Methods

2.1. Plant material

Phenotypically diverse material (Kaushik et al., 2016) consisting of eggplant accessions, wild relatives and interspecific hybrids were used for this study. The cultivated eggplant was represented by six eggplant (*S. melongena*) accessions (Table 1). For wild species, a total of 22 accessions from 12 species from the three eggplant genepools (Syfert et al., 2016) were also used (Table 1). Of these, three accessions belong to the primary genepool species *S. insanum*, 13 to secondary genepool species *S. anguivi* (n=2), *S. campylacanthum* (n=2), *S. dasyphyllum* (n=1), *S. incanum* (n=1), *S. lichtensteinii* (n=2), *S. linnaeanum* (n=2), *S. pyracanthos* (n=1), *S. tomentosum* (n=1), and *S. violaceum* (n=1), and six to tertiary genepool species *S. elaeagnifolium* (n=2), *S. sisymbriifolium* (n=2), and *S. torvum* (n=2). The cultivated eggplant and wild related species accessions from primary and secondary genepools were used to generate interspecific hybrids based on reciprocal crossing (Plazas et al., 2016; Kaushik et al., 2016), of which we used 18 hybrids with primary genepool wild species *S. insanum* and 24 hybrids with secondary genepool wild species, respectively. Five plants per accession or hybrid were grown under open

field conditions at the agricultural experimental farm of Universitat Politècnica de València (Valencia, Spain: latitude, 39° 28' 55" N; longitude, 0° 22' 11" W; altitude: 4 masl) during the summer season of 2015 using standard horticultural practices for the eggplant crop.

Table 1

Accessions of cultivated eggplant (*Solanum melongena*) and wild relatives of the primary secondary and tertiary genepools, and interspecific hybrids between cultivated eggplant and wild relatives of primary and secondary genepool used for dry matter abd phenolics composition, flesh colour and browning related characterization. For the interspecific hybrids, the first and second parentals included in the hybrid code correspond to the female and male, respectively.

Species	Accession	Germplasm collection code	Country of origin	Interspecific hybrids with *S. melongena* accessions					
				MEL1	MEL2	MEL3	MEL4	MEL5	MEL6
Cultivated eggplant									
S. melongena	MEL1	BBS-118/B	Ivory Coast						
	MEL2	BBS-146	Ivory Coast						
	MEL3	BBS-175	Ivory Coast						
	MEL4	7145	Sri Lanka						
	MEL5	8104	Sri Lanka						
	MEL6	Ampara	Sri Lanka						
Wild primary genepool (GP1)									
S. insanum	INS1	SLKINS-1	Sri Lanka	MEL1×INS1	MEL2×INS1	MEL3×INS1	MEL4×INS1	INS1×MEL5	MEL6×INS1
	INS2	SLKINS-1	Sri Lanka	MEL1×INS2	MEL2×INS2	MEL3×INS2	MEL4×INS2	MEL5×INS2	MEL6×INS2
	INS3	MM498	Japan	INS3×MEL1	INS3×MEL2	INS3×MEL3	INS3×MEL4	MEL5×INS3	INS3×MEL6
Wild secondary genepool (GP2)									
S. anguivi	ANG1	BBS119	Ivory Coast		MEL2×ANG1	MEL3×ANG1	MEL4×ANG1	MEL5×ANG1	
	ANG2	BBS125/B	Ivory Coast	MEL1×ANG2	MEL2×ANG2	ANG2×MEL3	ANG2×MEL4	MEL5×ANG2	ANG2×MEL6
S. campylacanthum	CAM5	MM680	Tanzania						

	CAM6	MM700	Kenya						
	CAM8	MM1426	Tanzania						
S. dasyphyllum	DAS1	MM1153	Uganda	MEL1×DAS1					
S. incanum	INC1	MM664	Israel	INC1×MEL1		MEL3×INC1		MEL5×INC1	MEL6×INC1
S. lichtensteinii	LIC1	MM674	South Africa	MEL1×LIC1				MEL5×LIC1	MEL6×LIC1
	LIC2	MM677	Iran	MEL1×LIC2		MEL3×LIC2	MEL4×LIC2		
S. lidii	LID1	4788	Spain						
	LID2	MM1005	Spain						
S. linnaeanum	LIN1	JPT0028	Spain						LIN1×MEL6
	LIN3	MM195	Tunisia						
S. pyracanthos	PYR1	SOLN-66	Unknown						
S. tomentosum	TOM1	MM992	South Africa		MEL2×TOM1	TOM1×MEL3			
S. vespertilio	VES1	4601A	Spain						
	VES2	BGV-3218	Spain						
S. violaceum	VIO1	SLKVIL-1	Sri Lanka						
Wild tertiary genepool (GP3)									
S. elaeagnifolium	ELE1								
	ELE2								
S. sisymbriifolium	SIS1								
	SIS2								
S. torvum	TOR2								
	TOR3								

2.2. Sample preparation

For each accession, three samples were used, each one consisting of five fruits collected at the developmental stage considered as commercially ripe (i.e., physiologically immature) for cultivated eggplant. Fruits were processed by cutting them transversally with a knife at halfway between the distal and proximal part of the fruit. One half of the fruit was used to measure fruit flesh browning, while for the other half a transversal slice (or the whole half for small fruits) was cut from the middle part of the fruit, peeled, frozen immediately with liquid N_2 and kept at −80 °C until lyophilized.

2.3. Dry matter and phenolics

Homogenized tissue of each sample, consisting in an equivalent weight of each of the five fruits that make up a sample, was used for the chemical analyses except for dry matter analysis. Dry matter was measured for fresh fruit samples as the change of weight before and after lyophilisation based on the formula 100 × (dry weight/fresh weight) and expressed as percentage of dry weight (dw). Total phenolics content (mg/g dw) was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965) after extraction with acetone (70% v/v) and acetic acid (0.5% v/v). Absorbance was measured at 750 nm with a spectrophotometer (Jenway, Essex, UK) and chlorogenic acid (Sigma-Aldrich Chemie, Germany) was used as a standard, as this is

the most common phenolic compound of the eggplant fruit flesh (Stommel and Whitaker, 2003; Docimo et al., 2016a). For chlorogenic acid content determination, powdered samples (0.1 g) were homogenized in 1.8 mL of methanol:water (80:20, v/v) plus 0.1% (w/v) of 2,3-tert-butyl-4-hydroxyanisole (BHT) and subsequently filtered through 0.2-μm polytetrafluoroethylene (PTFE) membrane filters. A standard solution of chlorogenic acid was used as control. High-performance liquid chromatography (HPLC) was performed for determination of chlorogenic acid content (mg/g dw) according to the protocol of Plazas et al. (2014). Extracts were analyzed on a 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) operated by the OpenLAB CDS ChemStation Edition software package (Agilent Technologies) using manufacturer's instructions. The chlorogenic acid peak area and the total peak area of other phenolic acids (hydroxycinnamic acid conjugates) were used to calculate the percentage of peak area in the chromatogram corresponding to chlorogenic acid.

2.4. Fruit flesh colour

Fruit flesh colour parameters were measured with a CR-300 chromameter (Minolta, Osaka, Japan) at midpoint between the center of the fruit and the pericarp for each of the five fruits that constitute one sample. For small-fruited samples (some wild species and interspecific hybrids) the fruit flesh colour measurement had to be done including the central part of the fruit. The fruit flesh primary colour

values obtained were based on the CIELAB 1976 colour coordinates L* (0=black; 100=white), a* (positive values=red; negative values=green) and b* (positive values=yellow; negative values=blue) immediately after the fruit was cut L^*_0, a^*_0, b^*_0). The distance to pure white colour (DW) was calculated as $DW= [(100-L^*_0)^2 + a^{*2}_0 + b^{*2}_0)]^{0.5}$ (Prohens et al., 2007) and used to determine the DW value just after the cut of the fruit (DW_0) in order to have a relative measure of the whiteness of the fruit flesh (DW_0).

2.5. Fruit flesh browning

For traits related to browning, the polyphenol oxidase (PPO) activity was determined according to Bellés et al. (2006). Basically, 0.1 g of lyophilized tissue was homogenized in 4 mL of 0.1 M sodium phosphate buffer (pH 6.0), and centrifuged at 12,000 rpm for 15 min at 4 °C. Thereafter, a five-fold dilution of supernatant was carried out with extraction buffer solution. The control contained 50 μL of buffer instead of enzyme extract. The enzymatic reaction was followed colourimetrically at 420 nm in a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Montchain, DE, USA). One unit of enzyme activity was defined as the increase in 0.1 absorbance unit per minute per milligram of dry weight. For determining the liquid extract browning (LEB), we used the protocol described in Plazas et al. (2013). A sample of 0.25 g of lyophilized tissue was homogenized with 2.5 mL water and kept for 10 min at room temperature. Subsequently, 2.5 mL of a 4% metaphosphoric solution

was added to stop the oxidizing reaction. The control was prepared with 0.25 g of lyophilized tissue homogenized with 2.5 mL of 4% metaphosphoric acid and after 10 min, 2.5 mL of water was added to the solution. After that the sample and the corresponding control solutions were centrifuged at 8000 rpm for 5 min before measuring the absorbance at 420 nm using a ND-1000 spectrophotometer. One unit of LEB was defined as a difference of 0.01 absorbance units between the sample and the control. For the degree of browning (DB) fruit flesh colour parameters (L*, a*, b*) were measured 10 min after the fruit was cut (L^*_{10}, a^*_{10}, b^*_{10}) at the same position where measurements were taken just after the cut (0 min) and DB was calculated as $DB=DW_{10}-DW_0$, where DW_{10} and DW_0 are, respectively DW values measured at 0 min and at 10 min after fruits were opened. The fruit flesh colour difference (CD) after 10 min compared to 0 min was calculated as $CD=[(L^*_{10}-L^*_0)^2+(a^*_{10}-a^*_0)^2(b^*_{10}-b^*_0)^2]^{0.5}$ (Prohens et al., 2007).

2.6. Data analysis

Average values for each accession or interspecific hybrid was used to determine, the mean, range and coefficient of variation (CV, %) of each trait for the six groups of cultivated eggplant (n = 6), wild relatives of the primary genepool (n = 3), wild relatives of the secondary genepool (n = 13), wild relatives of the tertiary genepool (n = 6), interspecific hybrids with primary genepool relatives (n = 18) and interspecific hybrids with secondary genepool relatives (n = 27).

Means were subjected to multifactor analyses of variance (ANOVA) analysis to detect differences among the six groups studied. Significance of differences among group means was evaluated using the Duncan´s multiple range test at $P = 0.05$. Pearson linear intra-group correlations (to avoid biased results due to differences among group means) based on within-group residuals of accession means was estimated. Mid-parent heterosis value was estimated for all the traits studied using the formula $H=100\times[(F1 - MP)/MP]$, where F_1 is the interspecific hybrid mean, and MP is the mean of the two parents. All the statistical analyses were performed using the Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA).

3. Results

3.1. Differences among groups

3.1.1. Dry matter and phenolics

The average values for dry matter content among the different groups studied were of more than two-fold, with a range between 10.5% (in cultivated eggplant) and 21.3% in wild species or the tertiary genepool (Table 2). For total phenolics content the average differences were almost of two-fold, from 9.8 mg/g in cultivated eggplant to 18.8 mg/g in the wild species of the tertiary genepool, while for chlorogenic acid content the range varied from 2.52 mg/g in the cultivated eggplant

to 3.56 mg/g in the wild species of the primary genepool. When considering the area of the chromatogram accounted by chlorogenic acid, the lowest average values were in the wild species of the secondary genepool (44.3%), while the highest was in the cultivated eggplant (79.2%).

Highly significant differences ($P < 0.001$) were observed among average values of cultivated eggplant, wild relatives and interspecific hybrids for dry matter and phenolics traits (Table 2). For all traits the largest differences in average values were observed between the cultivated eggplant and hybrids of cultivated eggplant with primary genepool species on one side and the wild secondary and tertiary genepools on the other. In addition, no significant differences were detected between cultivated eggplant and hybrids with the wild primary genepool species or between the wild species of the secondary and tertiary genepool for any of the traits (Table 2). Cultivated eggplant and its hybrids with the primary genepool species had significantly lower contents of dry matter content, phenolics and chlorogenic acid and higher percentage of chromatogram area corresponding to chlorogenic acid than wild species of the secondary and tertiary genepool (Table 2). The wild species from the primary genepool had a significantly higher dry matter, total phenolics and chlorogenic acid contents than the cultivated eggplant, as well as a significantly higher total phenolics content and chlorogenic acid content than the hybrids with the primary genepool species. The hybrids with the secondary genepool species had values for dry matter, total phenolics, and

chlorogenic acid content not significantly different to the cultivated species (Table 2).

Table 2. Mean, range (between brackets), and coefficient of variation (CV; %) for dry matter, total phenolics; chlorogenic acid content; percentage area % of chlorogenic acid content under the HPLC chromatogram curve in accessions of cultivated eggplant (*S. melongena*; n=6), wild relatives of the primary (n=3), secondary (n=18) and tertiary (n=6) genepools and interspecific hybrids between cultivated eggplant and wild relatives from the primary genepool (n=18) and secondary genepool (n=24) and significance of mean differences among the six groups.

Accessions	Dry matter (%)	Total phenolics (mg/g)	Chlorogenic acid content (mg/g)	Chlorogenic acid peak area (%)
Cultivated eggplant				
Mean	10.5 a	9.8 a	2.52 ab	79.2 b
Range	(6.8-13.6)	(6.2-12.2)	(1.43-2.93)	(67.4-86.8)
CV	21.8	23.4	21.6	8.9
Wild primary genepool (GP1)				
Mean	16.0 bc	16.3 b	3.56 c	73.0 b
Range	(14.6-17.7)	(14.6-17.3)	(3.00-3.97)	(65.1-79.2)
CV	9.8	9.0	14.0	9.9
Wild secondary genepool (GP2)				
Mean	19.7 cd	18.4 b	3.25 bc	44.3 a
Range	(11.8-29.5)	(9.6-27.6)	(1.25-4.71)	(25.3-65.0)
CV	28.7	33.6	30.4	28.1
Wild tertiary genepool (GP3)				
Mean	21.3 d	18.8 b	3.09 abc	49.5 a
Range	(15.6-29.4)	(11.9-26.8)	(1.82-4.48)	(17.9-80.1)
CV	26.9	27.9	36.1	50.6
Hybrids with primary genepool (GP1)				
Mean	12.8 ab	9.9 a	2.34 a	73.9 b
Range	(9.4-17.9)	(7.2-15.4)	(1.32-3.87)	(64.7-83.1)
CV	15.5	23.1	28.3	7.8
Hybrids with secondary genepool (GP2)				
Mean	12.3 ab	15.6 ab	3.39 b	54.2 a
Range	(7.4-17.4)	(8.5-30.25)	(2.50-4.32)	(36.8-79.7)
CV	23.0	34.7	13.8	18.5
F-ratio	13.7	7.25	5.71	16.63
Probability of *F*	<0.0001	<0.0001	0.0002	<0.0001

[a]Means within rows separated by different letters are significantly different according to the Duncan's multiple range test at P<0.05.

3.1.2. Fruit flesh colour

An important range of variation was observed for the L^*_0 parameter (for a scale for L^*_0 going from 0 to 100), with mean values among the different groups with values ranging from 46.1 for the tertiary genepool wild species to 82.7 for the cultivated eggplant (Table 3). However, for the a^*_0 parameter all the group averages were similar, while for b^*_0 the differences ranged from 13.7 in the hybrids with the primary genepool species to 20.6 in the wild species of the secondary genepool (Table 3). Finally, for the DW_0 value the differences were also important, with a minimum average value of 23.0 in the cultivated eggplant to 57.6 in the wild species of the tertiary genepool.

Highly significant differences ($P<0.001$) were observed among average values of cultivated eggplant, wild relatives and interspecific hybrids for all fruit flesh colour traits except for a^*_0 (Table 3). The cultivated eggplant group and the hybrids with the wild primary genepool had the whitest flesh. These two last groups differed significantly for L^*_0, b^*_0 and DW_0 from the wild species of the secondary and tertiary genepools, and also differed from the wild species of the primary genepool and hybrids with the secondary genepool for L^*_0 and DW_0, and from the hybrids with the secondary genepool for b^*_0 (Table 3).

Table 3. Mean, range (between brackets), and coefficient of variation (CV; %) for flesh colour CIELAB colour parameters L^*_0, a^*_0, b^*_0, degree of whiteness (DW_0), polyphenol oxidase activity (PPO), liquid extract browning (LEB), degree of browning (DB) and colour difference (CD) in accessions of cultivated eggplant (*S. melongena*; n=6), wild relatives of the primary (n=3), secondary (n=18) and tertiary (n=6) genepools and interspecific hybrids between cultivated eggplant and wild relatives from the primary genepool (n=18) and secondary genepool (n=24) and significance of mean differences among the six groups.

Accessions	L^*_0	a^*_0	b^*_0	DW_0	PPO	LEB	DB	CD
Cultivated eggplant								
Mean	82.7 d	-2.65 a	14.6 ab	23.0 a	1.92 a	3.35 a	4.09 a	5.91 a
Range	(78.7-85.7)	(-6.66-(-1.12)	(10.2-20.7)	(17.8-29.8)	(1.34-2.87)	(0.80-5.67)	(2.71-5.64)	(4.08-7.98)
CV	3.2	79.0	30.5	20.8	27.4	48.0	28.8	27.2
Wild primary genepool (GP1)								
Mean	67.3 c	-1.36 a	16.5 bc	36.9 b	2.71 ab	5.52 ab	9.28 b	11.29 ab
Range	(62.2-76.5)	(-2.19-(-0.67)	(14.7-18.9)	(27.8-42.4)	(1.48-4.79)	(4.70-6.25)	(7.93-11.37)	(9.46-12.9)
CV	11.9	56.9	13.4	21.5	66.8	14.1	19.8	15.4
Wild secondary genepool (GP2)								
Mean	55.1 b	-2.4 a	20.6 d	49.8 c	9.63 bc	4.23 ab	5.80 ab	9.33 ab
Range	(47.4-60.2)	(-6.49-4.21)	(17.6-24.0)	(46.0-55.8)	(1.61-41.26)	(2.80-5.95)	(3.12-10.43)	(5.50-15.89)
CV	8.4	149.1	9.9	7.6	121.3	25.4	49.1	40.9
Wild tertiary genepool (GP3)								
Mean	46.1 a	-1.71 a	18.8 cd	57.6 d	16.59 c	6.19 b	7.01 ab	11.36 ab
Range	(41.5-55.0)	(-9.40-6.94)	(16.6-22.5)	(51.0-60.1)	(4.11-38.79)	(0.95-14.41)	(2.07-12.96)	(2.72-20.16)
CV	10.0	311.4	11.6	6.1	82.3	66.8	60.6	54.3
Hybrids with primary genepool (GP1)								
Mean	80.5 d	-1.84 a	13.7 a	24.4 a	2.29 a	3.94 ab	5.33 ab	6.93 a
Range	(69.7-85.8)	(-7.09-4.28)	(11.1-17.3)	(18.4-40.6)	(1.42-4.45)	(1.57-6.87)	(2.30-12.36)	(3.11-13.74)
CV	7.4	153.6	15.3	22.7	40.1	26.7	56.2	44.0
Hybrids with secondary genepool (GP2)								
Mean	62.5 bc	-2.70 a	19.6 c	43.0 b	6.07 ab	5.95 b	9.42 b	12.84 b
Range	(42.2-75.5)	(-7.50-0.62)	(15.3-23.8)	(30.9-60.3)	(0.57-34.02)	(1.70-11.10)	(4.17-30.53)	(6.63-39.38)
CV	15.4	87.0	11.8	19.0	117.1	40.6	57.1	52.1
F-ratio	38.72	0.29	18.58	47.39	5.28	3.59	3.47	3.95
Probability of *F*	<0.0001	0.9153	<0.0001	<0.0001	0.0004	0.0064	0.0078	0.0035

[a]Means within rows separated by different letters are significantly different according to the Duncan's multiple range test at P<0.05.

3.1.3. Fruit flesh browning

The average value differences for PPO activity among the different groups studied were of more than 8.7-fold (Table 3). For LEB, DB and CD the relative differences were much lower, with differences of around 2-fold (Table 4). Highly significant differences ($P<0.001$) were observed among average values of cultivated eggplant, wild relatives and interspecific hybrids for all flesh browning related traits. For PPO activity several significant differences were observed among groups, with the cultivated eggplant and hybrids with primary genepool species displaying values significantly lower than those of the wild species of the secondary and tertiary genepools. Also the wild species of the primary genepool and the hybrids with the secondary genepool presented values significantly lower than those of the tertiary genepool (Table 3). For LEB, the only significant differences detected were among average group values were among the cultivated eggplant on one side (with lower values) and the tertiary genepool and hybrids with secondary genepool species (with higher values). For the DB, the only significant differences were among cultivated eggplant, with lower values, and the wild species of the primary genepool and hybrids with secondary genepool species, with higher values, on the other. Finally, for CD, the only significant differences were among the cultivated eggplant and hybrids with primary genepool species on one side and hybrids with secondary genepool on the other (Table 3).

3.2. Differences within groups

3.2.1. Dry matter and phenolics

A large range of variation was found for accession average values for most traits within each of the groups considered, in some cases with differences of several fold among accession values (Table 2). In this respect, the largest relative difference was observed for the percentage of chromatogram peak area corresponding to chlorogenic acid, with differences of almost 4.5-fold in the wild species of the tertiary genepool. For the rest of groups also large differences were observed, although in some cases, like dry matter content, phenolics and chlorogenic acid in the wild species of the primary genepool the range of variation was generally low. Also, wide variation was observed within the interspecific hybrids and the values of the hybrids exceeded those of the accessions of the cultivated species with highest values, except for the percentage of the area under the curve accounted by chlorogenic acid. In any case, the wide variation observed within most of the groups resulted in overlap of the ranges of variation in most cases (Table 2).

The values for the coefficient of variation were very variable depending on the group and trait considered (Table 2). It is remarkable that for all traits the wild species of the secondary and tertiary genepools displayed larger values of the coefficient of variation than the cultivated species, which nonetheless presented values for the coefficient of variation of up to 23.4% for the total phenolics. The

interspecific hybrids presented a value for the coefficient of variation values in some cases similar to those of the cultivated species and in other cases similar to some of the wild species groups (Table 2).

3.2.2. Fruit flesh colour

Within each of the groups considered variation was found among accessions for the traits, studied, although there were differences in the range both among groups and among traits (Table 3). For L^*_0 the largest variation was observed in the hybrids with secondary genepool species with a difference among accessions of 33.3 units, while the lowest was for cultivated eggplant with a difference among accessions of 7.0 units. The range of variation of cultivated eggplant did not overlap with those of any of the other groups, except with the hybrids with the primary genepool species. For a^*_0 and b^*_0 the ranges of variation were considerably lower (Table 3). In all cases, the ranges of variation for a^*_0 for the different groups overlapped. Similarly, for b^*_0, the ranges of variation overlapped in all groups except between the hybrids of the primary genepool species on one side (with lower values) and the wild species of the secondary genepool on the other (with higher values). For DW_0, the greatest range of variation was found for the hybrids with secondary genepool species, and the lowest was found among wild species of the tertiary genepool species. The ranges of variation for DW_0 of the cultivated species, wild species of the primary genepool, and hybrids with primary genepool species overlapped, but these three groups did not overlap with the wild species

of the secondary or tertiary genepool species, which had larger DW_0 values than the former (Table 3). The hybrids with secondary genepool species overlapped in the range of variation with the rest of groups, except with the cultivated species, which had lower values for DW_0. The values of coefficient of variation for a^*_0 were much larger than for the rest of traits (Table 3), due to the fact that values of a^*_0 are close to 0.

3.2.3. Fruit flesh browning

A considerable variation was found within each group studied for the average values for the fruit flesh browning related traits studied (Table 3). The largest differences were found for the PPO activity, with up to 56-fold differences in case of hybrids of secondary genepool and almost 26-fold for the secondary genepool species. Even for the cultivated eggplant there were differences between 1.96-fold (for CD) to 7.0-fold (LEB) for the all the traits studied (Table 3). In contrast, the differences were lowest in the case of primary genepool species. For rest of the groups considered also large differences were observed (Table 3). When considering each of the traits evaluated, the ranges of variation of the six groups considered overlapped, except for PPO activity between the cultivated eggplant (lower values) and the wild tertiary genepool species (higher values) and for DB and CD between the cultivated eggplant (lower values) and the wild species of the primary genepool (higher values) (Table 3).

The coefficient of variation was highly variable, ranging from 14.1% for the LEB in the primary genepool species to 121.1% for PPO activity in the secondary genepool species (Table 3). In most of the cases the secondary and tertiary genepool species along with interspecific hybrids of secondary genepool displayed higher values for coefficient of variation than rest of the groups, although in some cases, like LEB relatively high values were also observed in the cultivated eggplant (Table 3).

3.3. Correlations among traits

A total of 17 pairwise correlation within-group residuals of accessions means were found to be significant ($P<0.05$) (Table 4). Two of these correlations presented very high absolute values (above 0.75), and corresponded to positive correlations between L^*_0 and DW_0, and between DB and CD (Table 4). Also, the content of total phenolics presented a moderate positive correlation with the content in chlorogenic acid, but these two traits were not correlated with browning traits. The content of chlorogenic acid was correlated with the total area under the curve, but not with browning traits (Table 4). However, the percentage of chlorogenic acid area in the chromatogram was positively correlated with a^*_0 and negatively correlated with browning traits (LEB, DB and CD). Also, a^*_0 was negatively correlated with PPO activity, LEB and CD, while b^*_0 was positively correlated with DB and CD. Finally, PPO activity was found to be positively

correlated with both DB and CD, and LEB was also positively correlated to both DB and CD (Table 4).

When considering the relationship between the degree of browning in the different materials studied with the different traits related to concentration in phenolic compounds (Fig. 1), it can be observed that there are several accessions of the secondary genepool and hybrids of eggplant with this secondary genepool species that present low values for browning and high levels for total phenolics, and chlorogenic acid content (Fig. 1). Also, it can be observed that many of the hybrids with the primary genepool species have similar values for browning and for the rest of traits than the cultivated eggplant, although one of them presents low browning and high content in chlorogenic acid. Remarkably, some wild species and interspecific hybrids displayed a high DB but values for the phenolics content traits similar to those of cultivated eggplant (Fig. 1).

Table 4. Pairwise Pearson linear correlations based on within-group residuals of total accession and hybrid means (n = 70) for the traits studied: dry matter content, chlorogenic acid content, (CGA), percentage (%) of CGA peak area, CIELAB fruit flesh colour parameters L^*_0, a^*_0, b^*_0, degree of whiteness (DW_0), polyphenol oxidase activity (PPO), liquid extract browning (LEB), degree of browning (DB), and colour difference (CD).

	Total phenolics	CGA	% CGA peak area	L^*_0	a^*_0	b^*_0	DW_0	PPO	LEB	DB	CD
Dry matter	0.032^{ns}	-0.218^{ns}	-0.188^{ns}	0.019^{ns}	0.077^{ns}	-0.126^{ns}	-0.052^{ns}	-0.233^{ns}	0.006^{ns}	-0.074^{ns}	-0.102^{ns}
Total phenolics		0.401^{***}	-0.263^{*}	-0.070^{ns}	-0.107^{ns}	-0.161^{ns}	0.032^{ns}	-0.218^{ns}	0.111^{ns}	0.082^{ns}	-0.003^{ns}
CGA			0.004^{ns}	-0.140^{ns}	-0.168^{ns}	-0.118^{ns}	0.113^{ns}	0.109^{ns}	0.099^{ns}	-0.030^{ns}	-0.040^{ns}
% CGA peak area				-0.135^{ns}	0.337^{**}	-0.134^{ns}	0.117^{ns}	-0.139^{ns}	-0.263^{*}	-0.393^{***}	-0.393^{***}
L^*_0					-0.118^{ns}	0.125^{ns}	-0.977^{***}	-0.197^{ns}	0.089^{ns}	0.151^{ns}	0.157^{ns}
a^*_0						-0.191^{ns}	0.053^{ns}	-0.247^{*}	-0.241^{*}	-0.197^{ns}	-0.284^{**}
b^*_0							0.079^{ns}	0.050^{ns}	0.170^{ns}	0.241^{*}	0.327^{**}
DW_0								0.217^{ns}	-0.066^{ns}	-0.114^{ns}	-0.100^{ns}
PPO									0.190^{ns}	0.404^{***}	0.458^{***}
LEB										0.319^{***}	0.346^{***}
DB											0.964^{***}

^{ns}, *, **,*** indicate non-significant, or significant at P = 0.05, 0.01 and 0.001, respectively

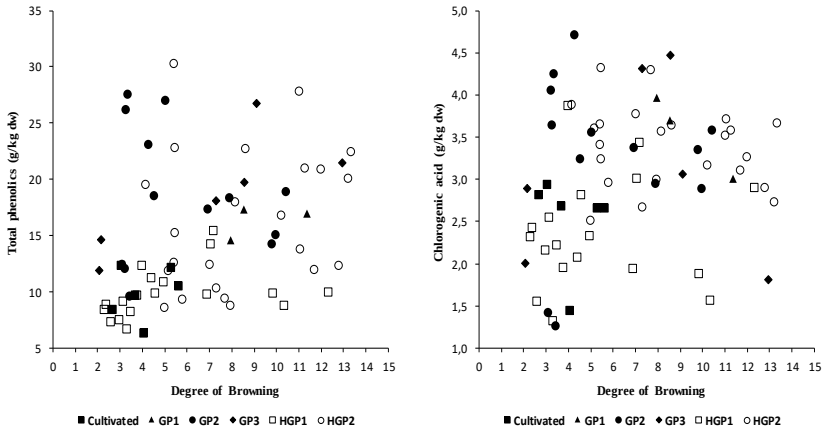

Fig. 1

Relationships between degree of browning (*x*-axis) with: a) total phenolics content (g/kg dw, y-axis; left graph), and b) chlorogenic acid content (g/kg dw, y-axis, right graph), in a collection of cultivated eggplant (■), wild relatives of the primary (GP1;▲), secondary (GP2;●) and tertiary genepools (GP3;◆) , and interspecific hybrids between cultivated eggplant and wild relatives of primary (□) and secondary genepool (○) respectively. One outlier data with DB above 30 was not included in the figure.

3.4. Heterosis

Different species belonging to different gene pools performed differently regarding heterosis (Table 5). Interspecific hybrids with cultivated eggplant primary gene pool species *S. insanum*, displayed negative or non-significant heterosis for almost all the characters studied except for L^*_0 and PPO activity, in which heterosis values were

positive. The highest absolute values for heterosis in the hybrids with *S. insanum* were for phenolics content (-23.3%) and the content of chlorogenic acid (-21.8%). Regarding interspecific hybrids involving the secondary gene pool species, the general trend was opposite, with positive heterosis for most traits and few traits displaying negative heterosis, like the percentage of area under the curve corresponding to chlorogenic acid in hybrids with all species. Also, dry matter content and L^*_0 presented negative values for heterosis in hybrids with several wild species. For the rest of traits heterosis was generally positive or non-significant (Table 5). Most of the interspecific hybrids with secondary genepool species demonstrated heterosis for total phenolics content with values of up to 73.9% in hybrids involving *S.tomentosum*. Regarding chlorogenic acid content all secondary gene pool interspecific hybrids presented positive heterosis with highest values in *S. anguivi*, with an heterosis of 76.5%. Amazingly, in general high positive values for heterosis, in some cases above 100%, were found for PPO activity and browning related traits (LEB, DB and CD) in interspecific hybrids with secondary genepool species (Table 5).

Table 5. Heterosis in interspecific hybrids over mid parent values (%; ±SE) based on parental accession (cultivated eggplant vs. wild species) and interspecific hybrids with primary (GP1; *S. insanum*) and secondary (GP2; rest of species) genepool species means for the traits studied: dry matter content, soluble refractrometric residue (SRR), chlorogenic acid content, (CGA), CGA peak area (%), CIELAB fruit flesh colour parameters L^*_0, a^*_0, b^*_0, degree of whiteness (DW_0), polyphenol oxidase activity (PPO), liquid extract browning (LEB), degree of browning (DB), and colour difference (CD).

	GP1	GP2					
Traits	*S. insanum*	*S. anguivi*	*S. dasyphyllum*	*S .incanum*	*S .lichtensteinii*	*S. linnaeanum*	*S. tomentosum*
n	18	10	1	4	6	1	2
Dry matter	-2.9±3.6	-35.8±6.6	0.0	12.4±12.8	-16.6±7.4	41.6	-31.6±14.1
Total phenolics content	-23.3±5.2	16.9±10.2	-24.6	52.5±17.1	19.9±9.3	21.8	73.9±25.1
CGA	-21.8±5.7	76.5±17.8	4.6	1.5±6.9	11.5±3.5	4.9	38.5±8.2
% CGA peak area	-2.8±1.4	-6.4±5.8	-35.8	-20.5±3.9	-25.7±5.0	-27.5	-17.8±11.8
L^*_0	7.4±1.6	-16.0±3.3	-0.1	6.6±1.6	-2.3±2.7	-17.7	-36.4±2.2
a^*_0	-9.5±41.4	118.7±203.2	159.6	-3.3±25.1	646.3±591.9	-19.1	-27.2±51.3
b^*_0	-9.7±4.4	3.0±6.4	12	27.7±8.0	17.9±5.8	43.9	-7.4±5.3
DW_0	-18.2±3.8	27.4±5.3	4.9	-4.6±3.5	8.5±5.0	39.9	55.4±2.1
PPO	16.5±17.8	65.6±27.9	221	50.3±81.1	23.8±32.5	-13.7	187.3±42.1
LEB	-7.8±7.0	93.2±24.6	51.5	68.0±47.9	33.1±22.2	46.6	35.8±47.7
DB	-16.8±12.8	85.9±20.2	287.8	224.6±48.1	138.6±26.6	143.9	-9.1±8.5
CD	-19.1±8.6	55.9±10.6	242.3	175.9±37.9	124.4±19.5	106.6	-10.9±6.8

4. Discussion

Eggplant has high levels of phenolics, particularly phenolic acids (Stommel and Whitaker, 2003; Mennella et al., 2012; Prohens et al., 2013; Docimo et al., 2016a) and the development of varieties with an enhanced content of phenolic acids is a current breeding objective (Plazas et al., 2013a; Kaushik et al., 2015). However, increases in phenolic acids content can result in a greater degree of fruit flesh browning due to the phenolic oxidation mediated by PPOs (Prohens et al., 2007; Plazas et al., 2013b), which reduces the visual quality of the fruit both for the fresh market and for the processing industry (Mishra et al., 2013).

Some wild relatives of eggplant have been reported as having significantly higher contents (of several-fold) in phenolic acids than cultivated eggplant (Stommel and Whitaker, 2003; Ma et al., 2010, Mennella et al., 2012; Prohens et al., 2013; Plazas et al., 2014; Meyer et al., 2015). Therefore, eggplant wild relatives may represent new sources of variation for increasing the bioactive properties of cultivated eggplant (Plazas et al., 2013a; Prohens et al., 2013). Our results reveal that, as occurs for morphological traits (Kaushik et al., 2016) and molecular markers (Vorontsova et al., 2013), there is a great diversity among wild species for the composition and fruit flesh colour and browning traits studied. The generally higher contents in phenolics in the wild species, together with the wide ranges of variation observed among them, indicates that there is large potential among wild relatives for breeding for bioactive properties of eggplant. In this way, some of

the wild species have shown contents in total phenolics and chlorogenic acid contents several fold higher than those of the cultivated accessions. In this respect, Prohens et al. (2013) found individuals in the first backcross towards the cultivated eggplant of an interspecific hybrid between the latter and *S. incanum* with considerably higher levels of phenolic acids that the cultivated recurrent eggplant parent. On the other hand, Mennella et al. (2010) did not find increased contents in phenolic acids in introgression lines of *S. sodomaeum* (=*S. linnaeanum*); however, these lines had been selected for tolerance for Verticillium wilt and not for phenolics acid content. In tomato it has been possible to introgress the higher antioxidant activity and the content in some phenolic acids from the wild *S. pennellii* to the cultivated tomato (Rigano et al., 2016), suggesting that the same possibility may exist in eggplant.

In eggplant, chlorogenic acid is the predominant phenolic acid in the fruit flesh (Stommel and Whitaker, 2003; Mennella et al., 2012; Prohens et al., 2013) and the same occurs in the primary genepool species *S. insanum*, which is its wild ancestor (Knapp et al., 2003). A similar result was found by Meyer et al. (2015). Amazingly, in the wild relatives of the secondary and tertiary genepool the percentage of the chromatogram peak accounted by chlorogenic acid is on average much lower, indicating that other derivatives of hydrocinnamic acid represented an important part of the phenolic acids content. In other studies, Plazas et al. (2014) found that in the secondary genepool species *S. dasyphyllum*, chlorogenic acid peak only accounted for around 50% of the HPLC chromatogram area, while Meyer et al.

(2015) found that in the secondary genepool species *S. violaceum* chlorogenic acid was a minor constituent in the phenolic acids fraction. On the other hand, in secondary genepool species *S. incanum* (Prohens et al., 2013) and *S. linnaeanum* (Meyer et al., 2015) it has been found that chlorogenic acid was the most important phenolic acid. Some of these other phenolic acids and their derivatives also have important bioactive properties (Heleno et al., 2015), and therefore may also be of interest for introgression in eggplant.

A white fruit flesh colour is desirable for most eggplant markets (Daunay and Hazra, 2012), and the cultivated eggplant had much higher luminosity (L^*_0) and therefore a lower distance to pure white (DW_0) than the wild species. Wild species of *Solanum* crops usually have chlorophylls and carotenoids in the fruit flesh (Acosta-Quezada et al., 2015; Herraiz et al., 2016), which as in the case of eggplant result in a less white flesh. In this case, the primary genepool species presented better characteristics, with a fruit flesh colour closer to pure white than those of secondary and tertiary genepool species. Regarding browning traits, wild relatives generally had much higher PPO activity than the cultivated species and had higher fruit flesh browning. The higher PPO activity in wild eggplant relatives compared to cultivated species may be related to the fact that PPOs are involved in plant defence (Shetty et al., 2011), and therefore may be enhanced in the wild species.

The interspecific hybrids with the primary genepool species *S. insanum* are morphologically intermediate between the two parents, while those with secondary genepool species are more similar to the

wild species (Kaushik et al., 2016). A very similar result has been obtained with the traits measured here, with interspecific hybrids with *S. insanum* more similar to cultivated eggplant in phenolic composition, fruit flesh and browning characteristics, while interspecific hybrids with secondary genepool species were more similar to the wild parents for these characteristics. This seems to indicate that, generally, for secondary wild species there is dominance of the alleles of the wild species over those of the cultivated eggplant, and therefore are heterotic, while those of *S. insanum* have intermediate dominance. This suggests that secondary and tertiary genepool species may be of greater interest than the closely related *S. insanum* to improve the content of phenolics in eggplant, but not for the fruit flesh colour and browning traits. However, studies with segregating generations will be helpful to confirm the inheritance mode from these secondary genepool species.

Association between target traits is important for breeding. In this respect, the high values for the correlations between the luminosity and high degree of whiteness and also between the degree of browning and colour difference were expected (Prohens et al., 2007), as they represent different measures of a same phenomenon (fruit flesh colour and browning, respectively). Similarly, the correlation between total phenolics and chlorogenic acid content is a common phenomenon in eggplant (Plazas et al., 2013b), although the values obtained by us here have been lower than in this latter study, probably due to the fact that we are dealing with materials that are genetically very different (wild species) to the ones used by these authors (local Spanish landraces).

Most interestingly, no significant correlations have been observed between total phenolics content or chlorogenic acid content with any of the fruit flesh colour or browning traits, which suggests that these traits may be independent. In fact, some of the interspecific hybrids had high content in total phenolics and chlorogenic acid content and limited browning. In this respect, genetic and QTL mapping studies reveal that QTLs for chlorogenic acid content as well as the genes involved in the accumulation of chlorogenic acid pathway are not linked to the PPO genes cluster (Gramazio et al., 2014; Docimo et al., 2016a). This is important, as in our materials PPO activity has shown to have a positive correlation with browning traits, which indicates that by selecting for low PPO activity it is possible to develop materials with reduced browning.

Overall, our results reveal that wild relatives of eggplant are highly variable for traits related to phenolics content, and fruit flesh colour and browning and represent a source of variation of interest, in particular in the case of wild species from the secondary and tertiary genepools, for improving the content in phenolics of cultivated eggplant. However, for the fruit flesh colour and browning traits the characteristics present in the wild species are detrimental. In addition, the lack of correlation between phenolics content traits on one side and fruit flesh colour and browning on the other suggest that the wild relatives can make an effective contribution to the improvement of the bioactive properties of eggplant, while keeping a white fruit colour and low browning.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3: Line × Tester Analysis for Morphological and Fruit Biochemical Traits in Eggplant (*Solanum melongena* L.) Using Wild Relatives as Testers

1. Introduction

The global demand of vegetables is increasing, and this trend is expected to continue in the future [1]. Vegetables, being well adapted to crop rotation, rich in nutrient and minerals, and also highly diverse by nature, can make and effective contribution to address the challenges of food security [2]. Eggplant (*Solanum melongena* L.; Solanaceae) is a highly diverse vegetable with a large array of phenotypically variable local varieties. Several studies show that eggplant can be hybridized with many wild related species, opening the way for introgression breeding by using wild relatives as donors of variation [3,4]. Although the centre of origin of eggplant is the Indo-Chinese region [5], the greatest diversity in its wild relatives is found in Africa [6]

Undoubtedly, crop wild relatives are important reservoirs of useful genes and underexploited variation [7]. Wild relatives of eggplant are a source of variation for important traits, such as pest and disease resistance, drought tolerance, and for some quality traits, like a high content in bioactive phenolic acids [8]. Although eggplant is one of the

vegetables with highest concentrations in phenolic acids [9], wild relatives can contribute to a further dramatic increase in these bioactive compounds highly beneficial for human health. Most of the phenolic acids content (usually above 90%) in the eggplant flesh correspond to chlorogenic acid, while in the wild species other phenolic acids such as caffeic acid conjugates may also be present in significant proportions [10,11]. However, most eggplant wild relatives are prickly and generally produce small fruits, which are undesirable traits [12,13]. The eggplant fruit ideotype is variable depending on the final market niche and is based on several morphological and biochemical traits [14]. However, in general a high content in phenolic acids seems desirable due to their antioxidant activity and their properties in preventing several diseases [15].

Information on the inheritance of important traits and their gene action is very important in order to proceed with an efficient genetic improvement of plants. There are several mating designs for obtaining such information, and among these the Line × Tester (L × T) mating design introduced by Kempthorne [16] allows gaining a better insight on the performance of lines and testers in a series of cross combinations. In this design, the line is basically the female parent which in addition to contributing with 50% of the nuclear genes has a cytoplasmic effect on the hybrid, while the tester is the male parent in the cross [17,18]. For lines, the information regarding cytoplasmic inheritance is obtained [19].

The Line × Tester design provides an estimation of the general (GCA) and specific (SCA) combining abilities. GCA is the estimate of the average performance of a line in a series of cross combinations and SCA is the performance of a specific cross better or worse than expected GCA. GCA and SCA estimates are important to understand the genetic architecture of quantitative traits, and therefore of great relevance to the establishment of efficient breeding programmes [20]. In this way, the usefulness of wild species and cultivated varieties in a breeding program largely depends on the combining ability estimates for traits of interest. Also, the heterotic performance of cross combinations depends on the combining ability of the parents involved in the cross [21,22]. In eggplant, the earliest reports of the estimation of combining ability effects date back to late 1940s [23]. However, few studies have dealt with the estimations of CGA and SCA in crosses with wild relatives. In a recent study using a diallel cross in which one accession of the wild eggplant relative *S. insanum* was included, we found that GCA and SCA estimates were significant for most of the morphological traits. Also, the wild relative *S. insanum* had the low values for GCA fruit related morphological traits [21].

Heterosis is commonly used to measure the superiority of hybrids with respect to their parents [24,25]. In eggplant, the first success in the development of heterotic hybrids for agronomic traits was recorded in 1890s [23,26]. Thereafter, heterosis breeding has become an important routine in eggplant improvement [26]. Previously, we have evaluated the heterosis for the agronomical and biochemical traits in eggplant,

using crosses with wild relatives as well as with cultivated parents [11,13,21]. However, to our knowledge, up to now there are no studies using the L × T breeding design in eggplant using wild species as testers. Therefore, the overall objectives with this study were to determine the combining ability, gene action, heterosis and heritability of important morphological, morphometric, and biochemical traits by using four eggplant wild relatives as testers against two eggplant lines, one from the Occidental group and another one from the Oriental group [27].

2. Material and Methods

2.1.Plant material and growing conditions

Two cultivated eggplant (*S. melongena*) lines, one from Ivory Coast (MEL3; Occidental group), and one from Sri Lanka (MEL4; Oriental group) were used as the female parent lines (Table 1). Four accessions of eggplant wild relatives, of which two were from the primary gene pool species *Solanum insanum* (INS1 and INS2), and two from the secondary genepool species *S. anguivi* (ANG1) and *S. lichtensteinii* (LIC2) were used as male parents (testers) (Table 1). The mating of lines by testers has produced eight interspecific hybrids (Table 1). The lines, testers and the L × T interspecific crosses were grown in an experimental field at the Universitat Politècnica de València (Valencia, Spain; GPS coordinates of the plot: 39° 28′ 55″ N, 0° 22′ 11″ W; altitude 7 m a.s.l.). Five plants (each plant was a

replication) of each of the lines, testers and L × T interspecific hybrids were distributed in a randomised complete block design in the open field plot. The plant to plant and row to row spacings were 1.2 m and 1.0 m respectively. The plants were irrigated with drip irrigation system and fertilized using 80 g plant^{-1} of a 10N–2.2P–24.9K plus micronutrients fertilizer (Hakaphos Naranja; Compo Agricultura, Barcelona, Spain), which was distributed throughout the cultivation period with the drip irrigation system.

Table 1. Accessions of cultivated eggplant (lines) and wild relatives (testers) used for the line by tester analysis.

Species	Accession	Germplasm collection code	Country of origin	Interspecific hybrids	
				With MEL3	With MEL4
Cultivated eggplant					
S. melongena	MEL3	BBS-175	Ivory Coast		
	MEL4	7145	Sri Lanka		
Wild primary genepool (GP1)					
S. insanum	INS1	SLKINS-1	Sri Lanka	MEL3×INS1	MEL4×INS1
	INS2	SLKINS-1	Sri Lanka	MEL3×INS2	MEL4×INS2
Wild secondary genepool (GP2)					
S. anguivi	ANG1	BBS119	Ivory Coast	MEL3×ANG1	MEL4×ANG1
S. lichtensteinii	LIC2	MM677	Iran	MEL3×LIC2	MEL4×LIC2

2.2.Characterisation and data analysis

Line and tester parents and their resultant interspecific hybrids were characterised for the 12 conventional morphological descriptors as defined by the EGGNET and IBPGR [28,29]. Five measurements were recorded in each replication except for plant height and stem diameter. Five plants per replicate were collected at the commercial ripe stage for the fruit morphometric and biochemical characterization. Eight fruit morphometric traits were also scored using popular the Tomato Analyzer version 4 software [30]. For the fruit morphometric analysis, the fruits were cut opened longitudinally and scanned with the help of a HP Scanjet G4010 photo scanner (Hewlett Packard, Palo Alto, CA, USA) at 300 dpi.

Snap frozen tissues of fruit flesh samples were lyophilized and grounded to the fine powder consistency. This fine powder was used for the estimation of three biochemical traits (dry matter, total phenolics, and chlorogenic acid content). Dry matter was estimated as the change of weight in the fresh sample before and after lyophilization based on the formula 100 × (dry weight / fresh weight) and expressed as dry matter percentage. The total phenolics were estimated using the Folin-Ciocalteu method defined elsewhere [11,31]. The chlorogenic acid (CGA) content was determined with help of high-performance liquid chromatography (HPLC) system using a standard solution of CGA as control. The analysis was performed on to an 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA,

USA). The results were computed by the OpenLAB CDS ChemStation Edition software package (Agilent Technologies) following the manufacturer instructions.

Average values for lines, testers and L × T hybrids is provided in the Table S1. The estimation of general combining ability (GCA) and the specific combining ability (SCA) including the variance and its contribution effects were performed based on the traditional linear model of L × T analyses [16]. The heterosis was estimated over the mid-parent values (H; %) hybrids using the formula as H = 100 × ((F1 − MP)/MP), where F_1 = hybrid mean, and MP = mean of the parents. All these calculations were performed with the help of the software package AGD-R version 5.0 [32].

3. Results

Analysis of variance for line, tester and L × T effects and GCA and SCA estimates

The average of parents and their hybrids were different significantly and a wide range of variation was present Table S1. The analysis of variance for combining abilities of the twenty-three descriptors studied in a L × T (2 x 4) design is presented in Table 2. The mean squares due to treatments were highly significant for all the traits (Table 2). But, the mean squares due to lines (female) were

significant for only nine traits out of the total twenty-three. However, fourteen traits of testers and nineteen of L × T were determined to be significant (Table 2). The parents vs hybrid components were significant for fourteen out of twenty-three traits showing heterotic effects of more than half of the studies traits. There were larger values of the SCA effect as compared to the GCA. Moreover, the GCA/SCA ratio was less than equal to 0.5 for all the traits except for the number of flowers per inflorescence (Table 2).

Table 2. Analysis of variance for general combining ability (GCA) and specific combining ability (SCA) for the descriptors studied for the characterisation.

Source of variation	Replicates	Treatments	Parents	Lines	Testers	Lines X testers	Parents vs hybrids	Hybrids	Lines	Testers	Lines X testers	Error	s ² GCA	s ² SCA	GCA/SCA
d.f	2	13	5	1	3	1	1	7	1	3	3	26			
Phenolics	10.86	46.95***	54.31***	4.58	50.43***	115.67	15.15	46.25***	1.69	0.96	106.40***	4.84	0.07	53.20	0.001
GCA	0.06	1.56***	0.59	0.07	0.27	2.11**	0.97	2.35***	1.15	1.29	3.81***	0.24	0.05	1.91	0.025
Dry Matter	0.04	46.35***	7.21	19.3	3.7	5.67	0.09	80.92***	99.35	8.09	147.60***	5.01	4.14	73.80	0.056
Fruit Pedicel Length	2.34	488.86 ***	477.05***	60.17**	118.64***	1969.14***	5.98	566.28***	598	3.5	1118.50***	5.89	24.91	559.24	0.045
Fruit Pedicel Diameter	0.09	28.82***	25.81***	1.5	14.81***	83.11***	1.04	34.94***	23.21	16.96	56.83***	0.39	0.96	28.41	0.034
Fruit Weight	152.26	29905.50***	20384.54***	411.35	154.91	101046.60***	16454.45***	38627.77***	56326.5	15950.7	55405.25***	558.62	2346.93	27702.63	0.085
Stem Diameter	5.22	51.15***	18.55	10.67	13.46	41.71*	78.77**	70.49***	93.02	61.89	71.57**	9.82	3.87	35.78	0.108
Plant Height	16.67	1879.98***	1715.83***	32.67	948.75***	5700.25***	6396.44***	1352.02***	3762.52	742.32	1158.23***	83.22	156.77	579.11	0.271
Leaf blade length	0.23	77.67***	26.58***	21.09***	37.24***	0.07	157.58***	102.76***	13.28	10.37	224.97***	1.47	0.55	112.48	0.005
Leaf Blade Lobing	0.01	5.27***	3.20***	6.00***	3.00***	1.00***	4.57***	6.86***	6	12	2.00***	0.01	0.25	1.01	0.248
Leaf breath width	0.56	48.60***	21.45***	0.98	35.36***	0.18	140.26***	54.91***	13.37	6.93	116.74***	0.65	0.55	58.37	0.009
Number of flower prickles	0.87	16.17***	11.30***	1.5	17.00***	4.00*	27.86***	17.99***	21.09	21.84	13.10***	0.88	0.87	6.54	0.133
Number of flowers per infloresence	0.39	22.41***	23.68***	0.02	14.43***	75.10***	84.26***	12.67***	50.85**	12.03*	0.57	0.34	2.11	0.28	7.536
Corolla color	0.07	8.40***	11.60***	6.00***	16.00***	4.00***	0.45	7.23***	0.38	10.38	6.38***	0.07	0.02	3.18	0.005
Corolla Diameter	15.98	328.41***	96.97***	7.48	81.68***	232.31***	301.63***	497.56***	106.26	57.77	1067.77***	6	4.42	533.88	0.008
Perimeter	0.45	277.04***	335.48***	5.66	2.33	1664.76***	209.84***	244.90***	43.63	84.2	472.70***	6.75	1.81	236.35	0.008

Area	23.02	877.33***	1166.98***	15.06	0.93	5817.05***	328.38*	748.87***	150.87	229.38	1467.70***	56.89	6.28	733.84	0.009
Height Mid-width	0.12	30.77***	37.84***	4.24*	0.19	184.40***	17.70***	27.60**	1.01	4.52	59.52***	0.8	0.04	29.75	0.001
Maximum Height	0.11	31.76***	38.96***	3.60*	0.2	190.61***	18.42***	28.52***	1.16	4.92	61.25***	0.8	0.05	30.62	0.002
Curved Height	0.05	31.23***	36.90***	3.39	0.19	180.55***	19.47***	28.88***	1.66	5.19	61.63***	0.83	0.07	30.81	0.002
Fruit Shape Index External I	0.01	0.30***	0.14***	0.19***	0.05*	0.36***	0.01	0.46***	0.01	0.24	0.83***	0.01	0.01	0.41	0.024
Fruit Shape Index External II	0.01	0.34***	0.16***	0.27***	0.05*	0.39***	0.01	0.52***	0	0.28	0.93***	0.01	0.01	0.46	0.022
Distal Fruit Blockiness	0.01	0.02***	0.01***	0.01**	0.01	0.03***	0	0.03***	0.02	0.01	0.05***	0.01	0.01	0.02	0.500

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively

3.1.*Contribution to total variance*

The proportional contributions to the total variance of lines, testers and their interspecific hybrids (L × T) is provided in Table 3. The interspecific hybrids, showed the greatest contribution in the expression of the traits, thereafter testers and lines, as there were higher value of SCA variance for the traits. Except for leaf blade lobbing, number of flower prickles and number of flowers per inflorescence the interspecific hybrids contributed the largest portion of the variance in the expression of traits. Subsequently, tester contributed more than the lines for all the traits except for fruit related traits i.e., fruit weight, fruit length, and fruit diameter (Table 3).

Table 3. Contribution of lines, testers and their cross (L × T) in the expression of characters studies.

Traits	Lines	Testers	L × T
Phenolics	0.52	0.89	98.59
GCA	7.02	23.45	69.54
Dry Matter	17.54	4.29	78.17
Fruit Pedicel Length	15.09	0.26	84.65
Fruit Pedicel Diameter	9.49	20.8	69.71
Fruit Weight	20.83	17.7	61.47
Stem Diameter	18.85	37.63	43.52
Plant Height	39.76	23.53	36.71
Leaf blade length	1.85	4.32	93.83
Leaf Blade Lobbing	12.5	75.02	12.5
Leaf breath width	3.48	5.41	91.11
Number of flower prickles	16.75	52.05	31.2
Number of flowers per inflorescence	57.36	40.71	1.94
Corolla colour	0.74	61.48	37.78
Corolla Diameter	3.05	4.98	91.97
Perimeter	2.54	14.73	82.72
Area	2.88	13.13	83.99
Height Mid-width	0.52	7.02	92.46
Maximum Height	0.58	7.39	92.03
Curved Height	0.82	7.71	91.47
Fruit Shape Index External I	0.18	22.41	77.4
Fruit Shape Index External II	0.02	23.00	76.98
Distal Fruit Blockiness	8.26	15.84	75.9

3.2.*GCA and SCA*

For GCA estimates of the parental genotypes for all the three biochemical traits studied only one genotype was determined to be significant i.e., ANG1 was found significant for the phenolic and CGA content; and MEL4 for the dry matter content. But none of the

accession was found to be significant for the area. Interestingly, both of the lines i.e., MEL3 and MEL4 were determined to be reverse complementary to each other for all the twenty-three traits studied. While, the oriental accession MEL3 was determined highly significant for fruit pedicel length and the occidental accession MEL 4 was determined to be positively highly significant for the number of flowers per inflorescence. For all the remaining eighteen traits the testers were more positively significant than the lines (Table 4). The secondary genepool species LIC2 was the only significant accession for the height mid-width and maximum height (Table 4).

The SCA variation with respect to the mean is provided in Table 5. The lowest fluctuations i.e., below 12% were determined by the traits, plant height, leaf blade lobbing and the number of flowers per inflorescence. While the highest fluctuations i.e., above 75% were observed for the fruit weight, height mid-width, and maximum height. While for all the remaining traits the SCA varied from -17% to 73.36% (Table 5). For eight out of the twenty-three traits, the fluctuation ranged between -40% to 50.

Table 4. Estimates of the general combining ability effect (GCA) for the descriptors studied.

	Lines		**Testers**			
Traits/Characters	**MEL3**	**MEL4**	**INS1**	**INS2**	**ANG1**	**LIC2**
Phenolics	-0.27	0.27	-0.28	0.24	0.43*	-0.4
GCA	0.22	-0.22	-0.44	-0.23	0.63*	0.05
Dry Matter	-2.03**	2.03**	-0.46	1.45	0.3	-1.29
Fruit Pedicel Length	4.99***	-4.99***	-0.53	0.71	-0.78	0.6
Fruit Pedicel Diameter	0.98***	-0.98***	-0.82***	2.39***	-1.45***	-0.12
Fruit Weight	48.45***	-48.45***	26.96*	57.12***	-57.06***	-27.02*
Stem Diameter	-1.97	1.97	4.24*	-3.14*	-1.68	0.57
Plant Height	-12.52***	12.52***	16.6***	-6.9*	-4.23	-5.48
Leaf blade length	-0.74	0.74	0.84	0.79	-1.94**	0.31
Leaf Blade Lobing	-0.50***	0.50***	-2.00***	0.01***	1.00***	1.00***
Leaf breath width	-0.75*	0.75*	-0.46	0.22	-1.14**	1.38**
Number of flower prickles	-0.94*	0.94*	-2.81***	1.19*	1.19*	0.44
Number of flowers per inflorescence	-1.46***	1.46***	0.47	1.38***	-1.97***	0.12
Corolla colour	0.13	-0.13	-1.13***	-1.13***	0.88***	1.38***
Corolla Diameter	2.1*	-2.1*	-3.54*	0.4	-0.8	3.95**
Perimeter	1.35	-1.35	-2.82	2.97*	-3.63*	3.48*
Area	2.51	-2.51	-3.44	5.84	-6.94	4.53
Height Mid-width	0.21	-0.21	-0.32	0.03	-0.88	1.17*
Maximum Height	0.22	-0.22	-0.35	0.04	-0.91	1.22*
Curved Height	0.26	-0.26	-0.36	0.2	-1.02*	1.18*
Fruit Shape Index External I	-0.02	0.02	0.16***	-0.29***	0.06	0.07
Fruit Shape Index External II	-0.01	0.01	0.20***	-0.3***	0.05	0.05
Distal Fruit Blockiness	0.03	-0.03	0.04	-0.02	-0.05*	0.04

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively

Table 5. Range of specific combining ability estimates with respect to mean.

Traits	Minimum	Maximum
Phenolics	-49.35	49.35
GCA	-44.73	44.73
Dry Matter	-34.69	34.69
Fruit Pedicel Length	-45.08	45.08
Fruit Pedicel Diameter	-43.38	43.38
Fruit Weight	-86.33	86.33
Stem Diameter	-17.02	17.02
Plant Height	-9.77	9.77
Leaf blade length	-40.49	40.49
Leaf Blade Lobing	-10.00	10.00
Leaf breath width	-43.55	43.55
Number of flower prickles	-73.36	73.36
Number of flowers per inflorescence	-11.52	11.52
Corolla color	-21.97	21.97
Corolla Diameter	-55.35	55.35
Perimeter	-60.53	60.53
Area	-70.71	70.71
Height Mid-width	-78.14	78.14
Maximum Height	-77.37	77.37
Curved Height	-71.51	71.51
Fruit Shape Index External I	-43.33	43.33
Fruit Shape Index External II	-47.00	47.00
Distal Fruit Blockiness	-17.57	17.57

3.3 Heterosis

The lowest value for the overall mid-parent heterosis was noticed for the number of flowers per inflorescence (-41.9%), whereas, the

highest mid-parent heterosis was noticed for the number of flower prickles (141.1%). The negative mid-parent heterosis was determined for the traits like phenolics, CGA, stem diameter, plant height, leaf blade length, leaf blade lobbing, leaf blade width, corolla colour, corolla diameter and distal fruit blockiness (Figure 1). In contrast, the positive value for mid-parent heterosis was determined for the dry matter, fruit pedicel diameter, fruit weight, perimeter, area, height mid-width, maximum height, curved height, and fruit shape index external I and II, respectively. The mid-parent heterosis for the dry matter was less than 1%. Whereas, it was around 3% for the fruit shape index external I and II. Significantly negative heterosis was determined for all the leaf based traits i.e., leaf blade length (-20.4%), leaf blade lobbing (-11.8%), and leaf blade width (-25.2%).

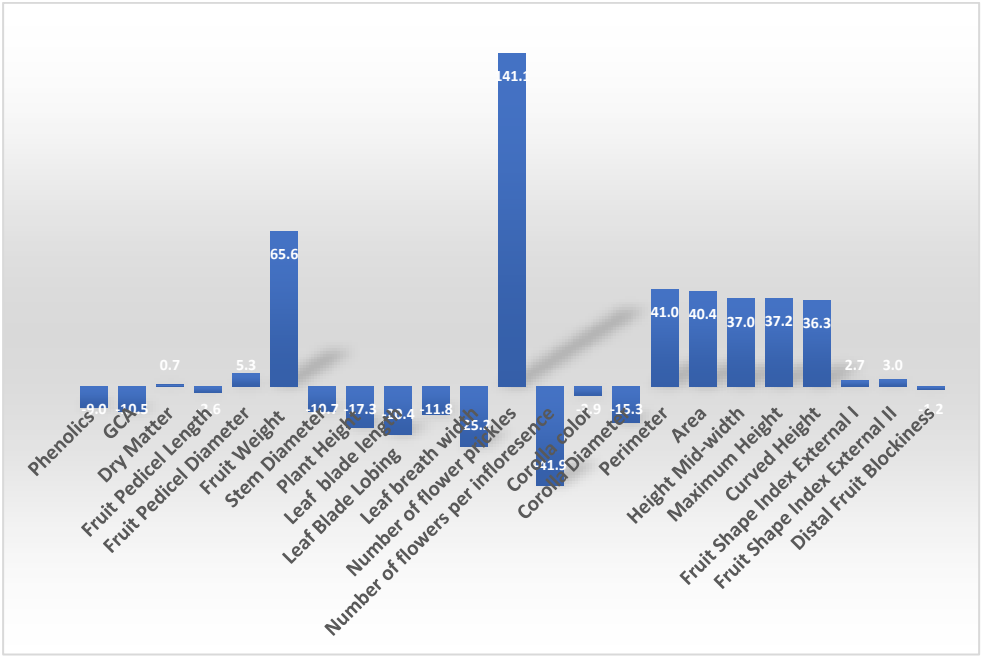

Figure 1. Heterosis over mid-parent values for all the descriptors studies

4. Discussions

The phenotypic selection of parents is still mainly key to the improvement of many vegetables for quantitative traits, especially in resource-limited circumstances[33,34]. The Line × Tester a well-established biometrical genetics-based approach gives a better estimate and sure prediction the important quantitative traits as seen for other solanaceous vegetables including eggplant[35–37]. Any kind of improvement of traits would ultimately depend on the genetic nature and magnitude of gene action[38]. The mean square due to GCA, SCA, and GCA/SCA ratio points out the magnitude of gene action, this further aids in developing an appropriate breeding strategy for the future breeding programs. [20].

In our study, the two lines one with oriental and another with occidental cytoplasm were crossed with four testers representing three wild species this diverse germplasm has helped in the precise estimation of the basis of inheritance of 3 biochemical, 12 morphological and 8 tomato analyser based descriptors. The significant amount of variation was noticed for all the 23 traits studied.

Overall, larger values for the SCA component as compared to GCA were noticed. This can be due to the larger genetic distances as only wild species were used as the testers [39,40]. The higher SCA values have resulted in low GCA/SCA pointing out the presence of non-additive effects governing all the traits studied except for number of flowers per plant [22] Among all the genotypes studied only the accession of secondary genepool wild relative of eggplant *S. anguivi* was found to be significant for the biochemical traits. The eggplant has a huge diversity in shape based on its local landraces and wild species cultivated in the different countries. The popular variety is based on local preferences[37]. The secondary genepool species are the reserve of useful genes for the improvement of present-day varieties, because of breeding barriers they are not exploited to their full potential [4,41,42]. Therefore, most of the times the local germplasm is used that might have resulted in the lower genomic diversity of eggplant thereby resulted in the yield stagnation and susceptibility to diseases [43]. Similarly, for most of other traits testers were more significant in values than the cultivated lines although both of the lines were having different cytoplasm.

The information of GCA effects provides a relative picture of genotypes are important for the selection and further exploitation in the breeding programs. The positive and negative SCA and their values are also important for some characters as some need to be more positive than negative. The lowest fluctuation was noticed for the plant height to the maximum for fruit weight. Recently, a studyfound that SNPs are

not the replacement of biometrical study in case of eggplant[21]. It was revealed that there was positive heterosis for the 12 traits and negative heterosis for the 11 traits. The positive heterosis was determined mostly in case of all tomato analyzer based descriptors and negative values for most of the biochemical and morphological descriptors. Earlier heterosis is well reported and exploited in eggplant with respect to several traits[26]. Overall, in our study, most of the traits are shown to be governed by non-additive gene actions. Earlier studies reported both additive and non-additive gene actions governing several important traits of eggplant [21,44].

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Chapter 4: Diallel Genetic Analysis for Multiple Traits in Eggplant and Assessment of Genetic Distances for Predicting Hybrids Performance

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Abstract

Evaluation and prediction of the performance of hybrids is important in eggplant (*Solanum melongena*) breeding. A set of 10 morphologically highly diverse eggplant parents, including nine inbred *S. melongena* and one weedy *S. insanum* accessions, were intercrossed according to a half-diallel mating design without reciprocals to obtain 45 hybrids. Parents and hybrids were evaluated for 14 morphological and agronomic conventional descriptors and 14 fruit morphometric traits using Tomato Analyzer. Genetic distances among parents were estimated with 7,335 polymorphic SNP markers. Wide ranges of variation and significant differences were observed in the set of 55 genotypes for all traits, although the hybrids group had significantly higher vigour and yield than parents. General and specific combining abilities (GCA and SCA) were significant for most (GCA) or all (SCA) traits, although a wide variation was obtained for GCA/SCA ratios. Many relevant traits associated to vigour and yield had low GCA/SCA ratios and narrow-sense heritability (h^2) values, while the reverse occurred for most fruit shape descriptors. Broad-sense heritability (H^2) values were generally high, irrespective of GCA/SCA ratios. Significant correlations were found between traits related to size of leaf, flower and fruit, as well as among many fruit morphometric traits. Genetic distances (GD) among parents were coherent with their phylogenetic relationships, but few significant and generally low correlations were found between GD and hybrid means, heterosis or

SCA. The results provide relevant information for developing appropriate strategies for parent selection and hybrid development in eggplant and suggest that GD among parents have limited value to predict hybrid performance in this crop.

Keywords

breeding, combining ability, genetic distances, heritability, heterosis, hybrids, *Solanum melongena*

Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop of tropical and subtropical regions of the world, being cultivated in more than 1.79 million ha and having a global production of 51.28 million tons [1]. Eggplant production has increased by 50 % in the last decade and the demand is expected to increase, in part due to its high content in bioactive compounds beneficial for human health [2,3]. Despite its economic importance, eggplant breeding has lagged behind other major vegetable solanaceous crops like tomato or pepper [4,5]. Due to the swift growth of human population and increased demand of vegetables coupled with limited availability of cultivable land, it is necessary to develop improved vegetable cultivars to increase yields and meet the demands of consumers [6]. The use of heterosis for yield and other traits of agronomic interest in F1 hybrids has made, and can continue making, major contributions to developing new vegetable crop varieties with improved yield and other characteristics of agronomic interest [7,8]. In this respect, the productive advantages of hybrids in eggplant are known from long time ago [9,10]. Although hybrid breeding in eggplant is expanding and many new F1 hybrid varieties are available [11], a large part of the production still relies in non-hybrid varieties [12].

Eggplant is mostly autogamous [5,13,14] and local varieties display low levels of observed heterozygosity for molecular markers [15–17]. Therefore, pure lines are easy to develop through selection

within local varieties [18]. Eggplant flowers are large, easy to emasculate and pollinate by hand and each fruit can give a large number of seeds, typically between 200 and 2000 seeds [19,20]. In addition, male-sterility systems have been described [21,22], which might facilitate the development of hybrids.

Selection of parents giving good hybrids is a critical step for hybrid breeding programs [23,24]. The identification of parents with good general combining ability (i.e., generally giving good hybrids), as well as specific combinations of parents that result in exceptionally good hybrids, allows breeders selecting parents for obtaining hybrids [25–27]. Among the available biometrical procedures for determining general combining ability (GCA) and specific combining ability (SCA), as well as the nature and magnitude of gene actions and heritability of traits, the diallel analysis proposed by [25] has been widely used in different types of crops [28–32]. In this analysis, GCA is due to additive effects and additive × additive interactions, while SCA to dominance effects and additive × dominant and dominant × dominant interactions [27]. Among the different types of diallel crosses, the half-diallel cross including one-directional crosses makes the overall layout more manageable for breeders than with a doubled number of reciprocal crosses with the full diallel analysis [33].

Several studies have used a variable number of parents (four to 10) for half-diallel analysis to evaluate GCA and SCA for yield and several traits of agronomic interest in different eggplant parents and

their hybrids from the Mediterranean region [34], southeast Asia [35,36], or Africa [37]. These works have revealed that both GCA and SCA are generally significant in the parents and hybrids evaluated, although their magnitude and relative importance is variable. However, these works lack general information on several parameters of interest for eggplant breeding, like narrow-sense (h^2) and broad-sense (H^2) heritabilities, as well as correlations among traits [38], and in addition the number of traits evaluated is limited. Furthermore, up to now there have been no studies in which diallel analyses and molecular marker genotyping are coupled to evaluate the reliability of molecular markers for the selection of eggplant parents giving good hybrids. Although Rodríguez-Burruezo et al. [39] found that genetic distances based on AFLP molecular markers were positively correlated with the yield of hybrids as well as with the heterosis of hybrids, these authors based their conclusions on only 10 hybrids obtained among Spanish local varieties. In other crops, the relationships between genetic distances based on molecular markers and agronomic performance, heterosis and SCA of hybrids has been studied in half-diallel crosses, although results have been contrasting depending on the crop, accessions, markers used, and traits evaluated [28,31,32,40].

In this work we evaluate a large number of traits (28) of interest for eggplant breeding, including conventional descriptors [41–43] and fruit morphometric descriptors using the phenomics tool Tomato Analyzer [44,45] in 10 parents, which is considered as an appropriate number for obtaining valid estimates of genetic parameters [46,47], and their respective 45 hybrids. Parents encompass a wide morphological

diversity and different origins, including an edible accession of the weedy ancestor of eggplant (*S. insanum* L.) [48]. Experimental hybrids between *S. insanum* and *S. melongena* have been found to be intermediate between parents in characteristics [43] and with potential for commercial utilization in specific markets. Among the markers available in eggplant, we have used SNPs obtained by a high throughput genotyping-by-sequencing platform, which allows scoring thousands of polymorphic SNPs [17,49], and therefore obtaining reliable estimates of genetic distances among eggplant genotypes. Our approach is unprecedented in eggplant in the combination of a large number of parents, number of traits evaluated, genetic parameters and trait correlations studied, and also in the use of genetic distances for predicting the performance of hybrids, their heterosis and SCA in a half-diallel mating design. The results obtained will provide relevant information for eggplant breeding, in particular for developing new eggplant hybrid varieties.

Material and methods

Plant materials

Nine inbred cultivated eggplant (*S. melongena*) accessions plus one weedy accession of *S. insanum* were used as parents for the present study (Table 1). These accessions were selected based on their differing morphological features, especially in relation to fruit size, shape and colour (Fig. 1). The parents used consist of materials from the Occidental and Oriental cultivated eggplant groups [15] including two eggplant accessions (MM1597 and MEL5) from the primary center for diversity in Southeast Asia [50], three (ANS26, H15, and IVIA371) from the Spanish secondary center of diversity [51], one (MEL1) from West Africa, one of unknown origin (A0416), one breeding line (DH621), which is a doubled haploid of the commercial hybrid Ecavi (Rijk Zwaan Ibérica, Almería, Spain), as well as a *S. insanum* accession (INS2) originating from Sri Lanka (Table 1). The accession names, their origin and main fruit characteristics are indicated in Table 1. The 10 parental genotypes were intercrossed during the summer season of 2015 using a diallel mating design excluding reciprocals [25] to obtain 45 F1 hybrids.

Table 1. Materials of cultivated (*S. melongena*) and weedy (*S. insanum*) eggplant used in the present study. Information also includes their origin and main characteristics.

Accession	Origin	Group	Fruit size	Fruit shape	Stripes	Primary fruit colour
S. melongena						
A0416	Unknown	Unknown	Intermediate	Flattened	No	White
ANS26	Spain	Occidental	Intermediate	Obovate	No	Purple
ASIS1	Spain	Oriental	Intermediate	Round	No	Black
DH621	Doubled haploid breeding line	Occidental	Large	Semi long	No	Black
H15	Spain	Occidental	Intermediate	Semi long	No	Purple
IVIA371	Spain	Occidental	Large	Long	Yes	Purple
MEL1	Ivory Coast	Occidental	Intermediate	Semi long	No	White
MEL5	Sri Lanka	Oriental	Intermediate	Semi long	No	Pale purple
MM1597	India	Oriental	Large	Very Long	No	Green
S. insanum						
INS2	Sri Lanka	Oriental	Small	Round	Yes	Green

Fig. 1. Fruits of the 10 eggplant parentals used in the diallel analysis. Materials include nine cultivated *S. melongena* (A0416, ANS26, ASIS1, DH621, H15, IVIA371, MEL1, MEL5, and MM1597) and one weedy *S. insanum* (INS2) accessions. Fruits are not depicted at the same scale; the size of the grid cells is 1 cm × 1 cm.

Growing conditions

Seeds of the parents and hybrids were germinated and transplanted into an open field plot situated in the campus of the Universitat Politècnica de València (Valencia, Spain; GPS coordinates of the plot: 39° 28′ 55″ N, 0° 22′ 11″ W; altitude 7 m a.s.l.) on May 2016. A randomized block-design with three replications and three plants was established. Plants were grown using a spacing of 1.2 m between rows and 1.0 m within the row. Irrigation was applied using drip irrigation; fertilization was provided through the irrigation system and consisted of 80 g·plant^{-1} of a 10N–2.2P–24.9K plus micronutrients fertiliser (Hakaphos Naranja; Compo Agricultura, Barcelona, Spain) distributed through the entire period of cultivation. Plants were trained with bamboo canes. Weeds were removed manually, and no phytosanitary treatments were performed throughout the cultivation period (May-October 2016), as pest levels were below treatment limits.

Characterization of plants and fruits

Plants were characterized using 14 morphological and agronomic descriptors based on EGGNET [42,43] and [41] descriptors: Plant Height (cm), Stem Diameter (mm), Leaf Pedicel Length (cm), Leaf Blade Length (cm), Leaf Blade Width (cm), Number of Flowers per Inflorescence, Corolla Diameter (mm), Fruit Pedicel Length (mm), Fruit Pedicel Diameter (mm), Fruit Length (cm), Fruit Width (cm),

Fruit Calyx Prickles (measured in a scale from 0=none to 9 = more than 30 prickles), Fruit Weight (g), and Yield (measured as the total weight of commercial fruits; kg/plant). Except for Plant Height and Stem Diameter, where only one data could be obtained per plant, for the remaining characters, at least five measurements were taken per plant. For fruit morphometric analysis, five fruits per replication were collected at a commercially ripe stage (i.e., physiologically immature) and were cut longitudinally and scanned using an HP Scanjet G4010 Photo Scanner (Hewlett-Packard, Palo Alto, CA, USA) at a resolution of 300 dpi. Scanned images were processed for fruit morphometric analysis with the fruit shape phenomics tool Tomato Analyzer version 4 software [44]. A total of 14 fruit morphometric descriptors were recorded using this tool: Perimeter (cm), Area (cm^2), Width Mid-height (cm), Maximum Width (cm), Height Mid-width (cm), Maximum Height (cm), Curved Height (cm), Fruit Shape Index External I, Fruit Shape Index External II, Curved Fruit Shape Index, Proximal Fruit Blockiness, Distal Fruit Blockiness, Fruit Shape Triangle, and Fruit Shape Index Internal. A full description of the Tomato Analyzer traits measured can be found in Kaushik et al. [43] and Hurtado el al. [45].

Morphological and agronomic data analysis

For each trait measured, the mean and range were calculated for the parental (n = 10) and hybrid (n = 45) groups. Mean values of parents and hybrids were compared with *t*-tests to detect differences among the two groups. The significance of differences among group means was evaluated using at $p < 0.05$ using the Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). The general combining ability (GCA) of parents and specific combining ability (SCA) of individual hybrids, along with the variance components, and narrow (h^2) and broad (H^2) sense heritabilities were estimated based on the Griffing's [25] Method 2 Model 1 (fixed effects) using AGD-R (Analysis of Genetic Designs with R) software package [52]. The relative importance of GCA over SCA (GCA/SCA ratio) was estimated as GCA/SCA = $2 \times s^2_{GCA} / ((2 \times s^2_{GCA}) + s^2_{SGA})$ [26], Relative SCA values of individual hybrids were expressed in percentage (%) over the average of the trait. Pair-wise Pearson linear coefficients of correlation (*r*) were calculated using the Statgraphics Centurion XVI software, and significance of correlations was evaluated using the Bonferroni test [53]. The F1 hybrids heterosis over mid parent (*Het*; %) was calculated using formula $Het = 100 \times ((F1 - MP)/MP)$, where F1 = hybrid mean, and MP = mean of the parents.

Genetic distances and correlation with hybrid performance and genetic parameters

Polymorphism information for the parental accessions used in this study was retrieved from genotyping data obtained by Acquadro et al. [17], where the 10 accessions used here were genotyped using a modified RAD sequencing approach targeting coding sequences. The VCF (Variant Call Format) file of Acquadro et al. [17], which consisted of 75,399 polymorphic sites, was filtered selecting only our 10 accessions. Subsequently, all missing data were excluded when individual accessions were compared to the reference genome of accession '67/3' developed by the Italian Eggplant Genome Sequencing Consortium [54]. Finally, all the non-polymorphic SNPs among the accessions were removed, yielding a total of 7,335 polymorphic SNPs in our set of accessions. The genetic distance (GD) among parents was calculated based on identity-by-state (IBS) as GD=1-IBS using the TASSEL software version 5.0 Standalone [55]. The VCF file was exported to R software (version 1.1.383) using the vcfR package [56] and transformed into a genlight object using a vcfR2genlight function. A dendrogram with 1,000 bootstrap replicates was calculated using the aboot function of the popper package (version 2.6.1, https://cran.r-project.org/web/packages/poppr/index.html) using a UPGMA hierarchical clustering method and Hamming distance (bitwise distance). The relationship among GD of parents of individual hybrids

was used to estimate pairwise Pearson linear correlations between GD and hybrid trait values, heterosis, and SCA.

Results

Variation in parents and hybrids

A wide variation was found for most of the traits evaluated both in the parents and their respective hybrids (Table 2, S1 Table). In this way, differences over four-fold both in parents and hybrids groups were found for four conventional descriptors (Number of Flowers per Inflorescence, Fruit length, Fruit weight, and Yield), and for five Tomato Analyzer fruit descriptors (Area, Fruit Shape Index External I, Fruit Shape Index External II, Curved Fruit Shape Index, and for Fruit Shape Index Internal). For all traits an overlap in the ranges of variation was found between parent and hybrid groups (Table 2). Significant differences among averages of parents and hybrids ($p < 0.05$) were found only for Plant Height, Stem Diameter, and Yield, with higher values in hybrids (Table 2).

Table 2. Average values and ranges of variation. Traits evaluated include conventional morphological and Tomato Analyzer descriptors in eggplant parents and their hybrids. Probability of the *t*-test for comparison between parent and hybrid means is also included.

	Parents (n=10)		Hybrids (n=45)		
Descriptors	Mean	Range	Mean	Range	Prob. *t*
Conventional descriptors					
Plant Height (cm)	72.71	(65.80-85.63)	86.26	(59.80-121.50)	0.0069
Stem Diameter (mm)	14.95	(11.43-17.33)	17.90	(12.83-23.67)	0.0021
Leaf Pedicel Length (cm)	8.13	(4.83-12.04)	8.31	(5.45-12.24)	0.7573
Leaf Blade Length (cm)	23.91	(14.96-28.47)	25.34	(18.85-30.80)	0.1902
Leaf Blade Width (cm)	16.4	(10.80-22.83)	17.44	(14.22-23.22)	0.1940
Number of Flowers per Inflorescence	3.28	(1.00-5.33)	4.24	(1.00-7.00)	0.0551
Corolla Diameter (mm)	31.80	(19.37-43.00)	35.93	(23.50-47.17)	0.0603
Fruit Pedicel Length (mm)	50.92	(23.40-93.43)	42.80	(21.67-70.10)	0.0816
Fruit Pedicel Diameter (mm)	13.70	(7.50-21.33)	13.87	(7.83-20.37)	0.8819
Fruit Length (cm)	9.64	(4.40-19.20)	9.26	(3.73-18.87)	0.7647
Fruit Width (cm)	6.61	(3.83-9.83)	6.74	(4.13-10.10)	0.8081
Fruit Weight (g)	160.1	(55.2-245.7)	205.8	(63.6-353.3)	0.1123
Fruit Calyx Prickles[a]	1.00	(0.00-5.00)	1.25	(0.00-5.00)	0.6050
Yield (kg/plant)	2.38	(0.93-4.55)	3.28	(1.69-6.91)	0.0181
Tomato Analyzer descriptors					
Perimeter (cm)	26.76	(16.43-37.63)	30.88	(19.51-47.26)	0.0665
Area (cm^2)	43.26	(17.48-78.88)	58.11	(24.13-100.91)	0.0464
Width Mid-height (cm)	6.20	(2.27-10.56)	6.83	(4.03-11.60)	0.3633
Maximum Width (cm)	6.50	(3.53-10.60)	7.00	(4.22-11.61)	0.4475
Height Mid-width (cm)	8.47	(4.32-13.66)	10.33	(4.95-19.66)	0.0847
Maximum Height (cm)	8.76	(4.68-13.90)	10.54	(5.04-19.93)	0.1006
Curved Height (cm)	9.15	(4.92-14.76)	11.06	(5.28-20.28)	0.0792
Fruit Shape Index External I	1.51	(0.69-3.33)	1.62	(0.78-3.40)	0.6683
Fruit Shape Index External II	1.70	(0.99-5.01)	1.65	(0.75-3.74)	0.9040
Curved Fruit Shape Index	1.84	(0.75-5.70)	1.76	(0.90-3.80)	0.7901
Proximal Fruit Blockiness	0.60	(0.46-0.76)	0.60	(0.30-0.75)	0.9462
Distal Fruit Blockiness	0.74	(0.64-0.97)	0.72	(0.61-0.90)	0.4744
Fruit Shape Triangle	0.83	(0.60-1.19)	0.85	(0.42-1.13)	0.7568
Fruit Shape Index Internal	1.69	(0.66-5.05)	1.66	(0.75-3.74)	0.9022

GCA and SCA

The analysis of variance performed on the 55 genotypes (10 parents and 45 hybrids) detected no significant ($p < 0.05$) block effects except for Leaf Pedicel Length, Leaf Blade Length, Leaf Blade Width, Corolla Diameter, and Yield (Table 3). However, highly significant differences ($p < 0.001$) were found among genotypes for all traits. Similarly, highly significant ($p < 0.001$) effects were found for general combining ability (GCA) and specific combining ability (SCA) for all the traits evaluated, although higher values for the mean squares were observed for GCA than for SCA (Table 3). The GCA/SCA ratio was very variable, ranging from 0.15 for Yield to 4.08 for Fruit Shape Index External I. High (above 2) GCA/SCA ratios were found for conventional descriptors Leaf Blade Width, Fruit Pedicel Diameter, Fruit Length, and Tomato Analyzer descriptors Width Mid-height, Maximum Width, Fruit Shape Index External I, Fruit Shape Index External II, Curved Fruit Shape Index, and Fruit Shape Index Internal, while low (below 0.5) GCA/SCA ratios were found for conventional descriptors Stem Diameter, Fruit Calyx Prickles and Yield and for Tomato Analyzer descriptors Proximal Fruit Blockiness and Fruit Shape Triangle (Table 3). Narrow sense heritability (h^2) values ranged between 0.11 for Proximal Fruit Blockiness and 0.83 for three fruit shape descriptors (Fruit Shape Index External I, Fruit Shape Index External II and Fruit Shape Index Internal). Traits related to plant

vigour (like Plant Height, and Stem Diameter), Fruit Calyx Prickles, and Yield had low h^2 values, while most fruit size and shape traits, either using conventional or Tomato Analyzer descriptors, had h^2 values above 0.5 (Table 3). Broad sense heritability (H^2) had values above 0.5 for all traits, except for Proximal Fruit Blockiness (0.42) and Fruit Shape Triangle (0.35). Most traits related to vigour, yield and fruit size and shape had values above 0.85 for H^2 (Table 3). For traits with higher GCA/SCA ratios, the h^2 and H^2 values were much more similar than those with low GCA/SCA ratios (Table 3).

Table 3. Mean squares, GCA/SCA ratio [26], and narrow sense (h^2) and broad sense heritabilities (H^2) for the ANOVA for conventional morphological and Tomato Analyzer fruit descriptors. Materials evaluated include 10 parents and 45 hybrids of eggplant. Griffing's (1956) Method 2 Model 1 (fixed effects) of diallel analysis was used.

	Mean squares							
Descriptors	Block[a]	Genotypes[a]	GCA[a]	SCA[a]	Error	GCA/SCA	h^2	H^2
d.f.	2	54	9	45	108			
Conventional descriptors								
Plant Height (cm)	105.07	643.47***	2035.18***	365.13***	71.41	0.56	0.39	0.74
Stem Diameter (mm)	4.72	23.65***	55.94***	17.20***	1.84	0.29	0.30	0.82
Leaf Pedicel Length (cm)	7.80*	8.39***	32.85***	3.50**	1.95	1.66	0.41	0.53
Leaf Blade Length (cm)	24.70***	28.85***	129.03***	8.82***	3.25	1.88	0.58	0.73
Leaf Blade Width (cm)	14.40*	15.61***	68.37***	5.05	3.71	4.01	0.46	0.52
Number of Flowers per Inflorescence	0.042	6.23***	20.70***	3.33***	0.02	0.52	0.51	0.99
Corolla Diameter (mm)	28.11**	120.00***	403.55***	63.29***	6.24	0.58	0.47	0.87
Fruit Pedicel Length (mm)	11.26	534.34***	2027.87***	235.63***	8.36	0.74	0.57	0.96
Fruit Pedicel Diameter (mm)	1.97**	33.30***	171.44***	5.67***	1.17	3.15	0.78	0.90
Fruit Length (cm)	1.29	38.47***	204.26***	5.31***	0.72	3.69	0.83	0.95
Fruit Width (cm)	0.18	7.24***	34.16***	1.86***	0.39	1.92	0.68	0.86
Fruit Weight (g)	73.45	20241.37***	86296.35***	7030.37***	457.60	1.09	0.64	0.94

Fruit Calyx Prickles	0.01	5.67***	16.63***	3.48***	0.01	0.40	0.44	1.00
Yield (kg/plant)	1.05**	3.68***	5.73***	3.26***	0.17	0.15	0.21	0.89
Tomato Analyzer descriptors								
Perimeter (cm)	1.04	124.13***	476.98***	53.56***	8.67	0.87	0.52	0.83
Area (cm)	9.03	1377.23***	5035.12***	645.65***	125.62	0.79	0.48	0.78
Width Mid-height (cm)	0.03	11.67***	59.14***	2.18***	0.57	3.04	0.75	0.87
Maximum Width (cm)	0.02	10.65***	53.21***	2.13***	0.58	2.82	0.73	0.86
Height Mid-width (cm)	0.39	28.51***	123.36***	9.54***	1.15	1.21	0.63	0.89
Maximum Height (cm)	0.37	28.79***	125.40***	9.47***	1.23	1.26	0.63	0.89
Curved Height (cm)	0.34	28.86***	120.65***	10.50***	1.22	1.07	0.61	0.89
Fruit Shape Index External I	0.01	1.44***	7.67***	0.19***	0.03	4.08	0.83	0.93
Fruit Shape Index External II	0.00	2.21***	11.79***	0.30***	0.05	3.93	0.83	0.94
Curved Fruit Shape Index	0.01	2.42***	12.52***	0.40***	0.06	3.07	0.80	0.93
Proximal Fruit Blockiness	0.01	0.03***	0.04***	0.02***	0.01	0.18	0.11	0.42
Distal Fruit Blockiness	0.00	0.02***	0.07***	0.01***	0.00	1.23	0.45	0.63
Fruit Shape Triangle	0.00	0.07***	0.12***	0.06***	0.03	0.26	0.12	0.35
Fruit Shape Index Internal	0.00	2.25***	11.96***	0.30***	0.05	3.87	0.83	0.94

[a]***, **, * indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively.

For all traits, a significant GCA effect was detected in most of the parents, except for Leaf Blade Width and Proximal Fruit Blockiness where five and eight parents, respectively, did not present GCA effects (Table 4). Regarding outstanding GCA values, the flattened accession A0416 was characterized by strikingly low GCA values for Plant height, Stem Diameter, and for high absolute GCA values for fruit traits associated to flattened fruits (Table 4); the Spanish landrace AN-S-26 for high GCA values of Fruit Pedicel Diameter; the round accession ASI-S-1 for high GCA values for Fruit Area and for traits associated to fruit width, as well as for high GCA absolute values for fruit shape traits associated to elongated shape, and low GCA values for Yield and for Number of Flowers per Inflorescence; the élite background accession DH621 for high GCA values for Fruit Weight and fruit Area; the pickling accession H15 for high GCA for Fruit Pedicel Length; the striped accession IVIA371 for high GCA values for Fruit Calyx Prickles and Yield, and low GCA values for Plant Height; the white accession MEL1 did not present particularly high or low levels for any trait; the mauve-colored accession MEL5 had high GCA levels for the Number of Flowers per Inflorescence, and low GCA values for Fruit Weight and fruit Area; the elongated accession MM1597 for its high GCA values for Plant Height, leaf size traits, fruit length, and for fruit shape traits associated to elongated fruits, and Yield, while it had low GCA values for traits associated to fruit width; finally, the weedy

accession INS2 in general displayed low GCA levels for Corolla Diameter, traits related to leaf and fruit size, and Yield (Table 4).

Table 4. General combining ability estimates of parents for conventional morphological and Tomato Analyzer fruit descriptors for the 10 eggplant parents evaluated.

	S. melongena									*S. insanum*
Descriptors[a]	A0416	ANS26	ASIS1	DH621	HI5	IVIA371	MEL 1	MEL5	MM1597	INS2
Conventional descriptors										
Plant Height (cm)	-13.26***	5.56***	3.13*	5.87***	4.11**	-9.75***	-4.96***	-1.71	10.87***	0.15
Stem Diameter (mm)	-1.79***	0.08	-1.18***	-0.27	-0.47	-0.18	0.55*	-0.65**	1.35***	2.55***
Leaf Pedicel Length (cm)	-1.07**	0.96***	-0.23	0.74**	0.66*	1.07***	-0.7**	0.22	0.18	-1.84***
Leaf Blade Length (cm)	-1.65***	0.55	1.50***	2.44***	-0.09	0.28	0.03	-1.00**	1.97***	-4.01***
Leaf Blade Width (cm)	-1.38***	-0.07	0.55	0.38	0.05	0.37	0.27	-0.96**	2.98***	-2.18***
Number of Flowers per Inflorescence	-0.12***	-0.96***	-1.31***	-0.23***	-0.25***	0.27***	0.24***	0.86***	1.21***	0.29***
Corolla Diameter (mm)	-0.52	2.32***	-1.34**	-1.42**	3.18***	4.45***	1.98***	-2.90***	1.18*	-6.92***

Fruit Pedicel Length (mm)	-10.65**	5.97***	-6.20***	6.20***	12.55***	0.97	0.87	-1.47***	2.42***	-10.67***
Fruit Pedicel Diameter (mm)	-2.52***	3.11***	0.64***	1.32***	2.72***	1.32***	-0.43*	-2.44***	-0.74***	-2.98***
Fruit Length (cm)	-2.88***	0.03	-1.81***	1.75***	0.15	1.02***	0.19	0.17	4.83***	-3.46***
Fruit Width (cm)	0.99***	0.81***	1.00***	0.48***	-0.02	0.56***	0.16	-1.20***	-1.22***	-1.55***
Fruit weight (g)	1.09	17.45***	17.37***	58.00***	31.66***	35.06***	8.92*	-55.45***	-3.62	-110.47***
Fruit Calyx Prickles	-0.02	-0.27***	-0.36***	0.06***	0.17***	1.81***	-0.02	-0.36***	-0.52***	-0.49***
Yield (kg/plant)	-0.31***	-0.22**	-0.54***	0.35***	-0.20**	0.43***	0.31***	0.18*	0.51***	-0.51***
Tomato Analyzer descriptors										
Perimeter (cm)	-2.76***	1.55**	1.67**	3.38***	1.62**	2.15***	-0.33	-3.76***	4.04***	-7.56***
Area (cm^2)	-7.56***	9.01***	10.66***	10.34***	6.88***	8.91***	-1.18	-15.26***	1.37	-23.17***
Width Mid-height (cm)	1.04***	1.00***	2.18***	0.01	0.29*	0.58***	-0.44***	-1.63***	-1.72***	-1.31***
Maximum Width (cm)	0.91***	0.93***	2.06***	0.13	0.31*	0.55***	-0.39***	-1.70***	-1.41***	-1.40***

Height Mid-width (cm)	-2.69***	0.21	-1.01***	1.88***	0.75***	0.80***	0.34	-0.57**	3.09***	-2.79***
Maximum Height (cm)	-2.61***	0.16	-0.97***	1.84***	0.72***	0.74***	0.33	-0.59**	3.23***	-2.86***
Curved Height (cm)	-2.36***	0.25	-0.69***	1.94***	0.72***	0.76***	0.16	-0.78***	3.05***	-3.05***
Fruit Shape Index External I	-0.60***	-0.22***	-0.52***	0.22***	0.00	-0.06	0.08*	0.36***	0.99***	-0.25***
Fruit Shape Index External II	-0.69***	-0.27***	-0.59***	0.23***	-0.02	-0.09*	0.06	0.34***	1.33***	-0.30***
Curved Fruit Shape Index	-0.65***	-0.28***	-0.57***	0.24***	-0.04	-0.11*	0.02	0.31***	1.42***	-0.33***
Proximal Fruit Blockiness	0.03	-0.05**	0.00	-0.03	0.01	0.00	-0.02	0.02	0.07***	-0.02
Distal Fruit Blockiness	-0.06***	-0.04***	-0.05***	0.03***	0.01	-0.01	0.03***	0.01	0.08***	-0.02
Fruit Shape Triangle	0.12***	-0.03	0.06*	-0.07*	-0.01	0.01	-0.07*	0.00	0.00	-0.02
Fruit Shape Index Internal	-0.69***	-0.27***	-0.60***	0.23***	-0.02	-0.09*	0.06	0.34***	1.34***	-0.31***

[a]***, **, * indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$ respectively.

The range of variation for SCA values with respect to means for individual descriptors is given in Table 5. The lowest SCA range among hybrids for SCA was for Leaf Blade Width (-9.98 % to 14.00 %), while the highest was found for Fruit Calyx Prickles (-139.00 % to 198.11 %). However, for most traits the SCA values ranged between -50 % and 50 % (Table 5). Other traits, apart from Fruit Calyx Prickles, with values outside these ranges were Yield, with values between -41.77 % and 87.20 %, and several Tomato Analyzer descriptors like Area, Height Mid-width, and Maximum Height with positive values above 50 %, and Fruit Shape Index External II, curved Fruit Shape Index, Proximal Fruit Blockiness, and Fruit Shape Triangle with negative values below -50 % (Table 5). In general, traits with high absolute value for the SCA range had low GCA/SCA ratios (Table 3).

Table 5. Range of specific combining ability (SCA) estimates. Values are expressed as percentage over the mean of the 45 hybrids obtained among 10 eggplant parents.

	SCA values (% over mean)	
Traits	Minimum	Maximum
Conventional descriptors		
Plant Height (cm)	-19.14	26.35
Stem Diameter (mm)	-22.74	23.13
Leaf Pedicel Length (cm)	-20.94	33.93
Leaf Blade Length (cm)	-10.50	20.01
Leaf Blade Width (cm)	-9.98	14.00
Number of Flowers per Inflorescence	-38.45	47.65
Corolla Diameter (mm)	-23.32	21.23
Fruit Pedicel Length (mm)	-47.78	41.31

Fruit Pedicel Diameter (mm)	-21.26	17.80
Fruit Length (cm)	-23.76	49.14
Fruit Width (cm)	-19.14	27.15
Fruit Weight (g)	-28.13	41.78
Fruit Calyx Prickles	-139.00	198.11
Yield (kg/plant)	-41.77	87.20
Tomato Analyzer descriptors		
Perimeter (cm)	-19.01	37.15
Area (cm^2)	-32.87	58.54
Width Mid-height (cm)	-19.61	25.03
Maximum Width (cm)	-18.56	24.42
Height Mid-width (cm)	-20.43	56.46
Maximum Height (cm)	-20.70	54.66
Curved Height (cm)	-21.98	52.46
Fruit Shape Index External I	-30.90	43.27
Fruit Shape Index External II	-49.00	36.30
Curved Fruit Shape Index	-58.61	31.86
Proximal Fruit Blockiness	-54.93	19.97
Distal Fruit Blockiness	-16.68	15.30
Fruit Shape Triangle	-70.90	24.82
Fruit Shape Index Internal	-49.50	27.76

Correlations among traits

One hundred twenty-two out of the 378 pair-wise correlations (32.3 %) among traits were significant according to the Bonferroni test ($p < 0.05$; $r \geq 0.4928$) (S2 Table). Only 16 out of the 122 significant correlations (13.1 %) had negative values. For 24 of the positive correlations, *r* values were higher than 0.8. In general, descriptors related to leaf size, Corolla Diameter, fruit size, elongated shapes and Yield were found to be significantly correlated, although Yield was not correlated with Fruit Weight (S2 Table). Many significant correlations were found among traits related to fruit shape, both for conventional and Tomato Analyzer descriptors. Also, a negative correlation was found between the Number of Flowers per Inflorescence and fruit width traits (S2 Table).

Genetic distances and correlation with hybrid performance and genetic parameters

The genetic distance (GD) based on 7,335 coding SNPs ranged from GD=0.0094 between DH621 and IVIA-371 to a maximum value of GD=0.0389 between MEL1 and INS2 (S3 Table). The highest values for GD (above 0.03) were found between the weedy *S. insanum* INS2 and all the cultivated accessions, and also between the cultivated A0416 and MEL1 accessions (S3 Table). The lowest values of GD were found among the three Spanish landraces (AN-S-26, H15, IVIA-371) and among the latter and the élite line DH621, which in all cases had GD values below 0.015 (S3 Table). These results are confirmed in the cluster analysis dendrogram, which shows that *S. insanum* INS2 is basal to the *S.*

melongena accessions, while the three Spanish and the élite line DH621 cluster together (Fig. 2).

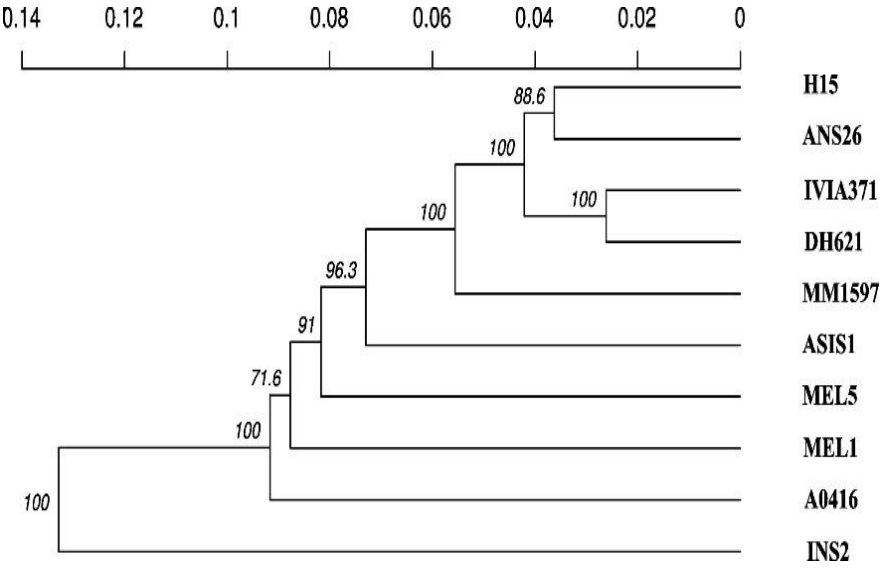

Fig. 2. UPGMA dendrogram displaying relationships of nine *S. melongena* (A0416, ANS26, ASIS1, DH621, H15, IVIA371, MEL1, MEL5, and MM1597) and one *S. insanum* (INS2) accessions based on 7,335 polymorphic SNPs. Phenetic relationships among accessions were derived from Hamming distance (bitwise distance). Bootstrap values (based on 1000 replications; expressed in percentage) are indicated at the corresponding nodes.

When considering the 45 hybrids among all parents, including the weedy INS2, GD was significantly correlated ($p < 0.05$) with hybrid's trait values for 14 traits out of a total of 28 (Table 6). However, significant *r*

values between GD and hybrid's trait values were generally low, with absolute values always below 0.5. Most of the significant correlations with GD were negative, including traits like leaf size descriptors, fruit size descriptors and Yield. The correlations between GD and trait heterosis (*Het*) generally were non-significant, and only significant positive correlations with GD were found for Leaf Blade Width and Fruit Pedicel Diameter, while negative correlations were found for Proximal Fruit Blockiness and Fruit Shape Triangle. Regarding the correlations between GD and SCA the only significant correlation (negative) was observed for Fruit Weight (Table 6). Given that the inclusion in these correlation analyses of the weedy INS2, which presents high GD values with the other accessions, might distort the results, we performed the same analysis using only the nine cultivated accessions and their respective 36 hybrids. The results obtained were similar to those obtained with all the accessions with some variations (Table 6). In this way, a negative correlation between GD and hybrid value for Plant Height, which was non-significant when considering all accessions, was found to be significant when excluding INS2. However, the negative correlations between GD and hybrid values for Corolla Diameter, Fruit Pedicel Length, Yield, and Curved Fruit Shape Index, which were significant in the analysis with all accessions, were not significant when only *S. melongena* accessions are considered. Regarding the relationship between GD and *Het* in the *S. melongena* accessions, the same significant correlations were detected than when all the materials are included in the analyses, except for a new significant positive correlation between GD and Stem Diameter. Finally, no significant correlations were detected between GD and SCA for the *S. melongena* materials (Table 6).

Table 6. Correlations between genetic distances among parents and hybrid trait values, heterosis (*Het*), and specific combining ability (SCA). Results are presented considering the 10 eggplant parents (nine cultivated *S. melongena* and one weedy *S. insanum*; n=45 hybrids), and only the nine *S. melongena* parents (n=36 hybrids).

	All parents			Only *S. melongena* parents		
Traits	Trait[a]	*Het*[a]	SCA[a]	Trait[a]	*Het*[a]	SCA[a]
Conventional descriptors						
Plant Height (cm)	-0.292	-0.016	0.126	-0.388*	0.005	0.127
Stem Diameter (mm)	0.021	0.143	0.309*	0.066	0.452**	0.278
Leaf Pedicel Length (cm)	-0.468***	0.131	-0.182	-0.408**	0.074	-0.210
Leaf Blade Length (cm)	-0.558***	0.200	-0.008	-0.491**	-0.124	-0.056
Leaf Blade Width (cm)	-0.377**	0.359	-0.085	-0.193	0.172	-0.219
Number of Flowers per Inflorescence	0.099	-0.084	0.021	0.164	-0.094	-0.037
Corolla Diameter (mm)	-0.318*	0.200	-0.072	-0.242	0.007	-0.099
Fruit Pedicel Length (mm)	-0.356*	0.257	-0.094	-0.304	0.196	-0.198
Fruit Pedicel Diameter (mm)	-0.397**	0.338*	-0.074	-0.352*	0.331*	-0.109
Fruit Length (cm)	-0.470***	0.060	-0.188	-0.341*	0.040	-0.113
Fruit Width (cm)	-0.231	0.094	-0.137	-0.111	0.044	-0.008
Fruit Weight (g)	-0.450***	0.034	-0.438**	-0.391*	-0.035	-0.321
Fruit Calyx Prickles	-0.057	0.016	-0.157	-0.092	0.035	-0.223
Yield (kg/plant)	-0.296*	0.190	0.152	-0.137	0.116	0.140
Tomato Analyzer descriptors						
Perimeter (cm)	-0.471***	0.078	-0.183	-0.372*	0.019	-0.121
Area (cm^2)	-0.404**	0.098	-0.315*	-0.383*	0.017	-0.276
Width Mid-height (cm)	-0.109	0.121	-0.248	-0.143	0.009	-0.246
Maximum Width (cm)	-0.131	0.166	-0.230	-0.160	0.038	-0.235
Height Mid-width (cm)	-0.453***	0.070	-0.186	-0.338*	0.049	-0.137
Maximum Height (cm)	-0.448***	0.074	-0.174	-0.335*	0.028	-0.128
Curved Height (cm)	-0.456***	0.093	-0.196	-0.354*	0.030	-0.153
Fruit Shape Index External I	-0.283	-0.063	0.058	-0.158	-0.009	0.104
Fruit Shape Index External II	-0.280	-0.052	0.086	-0.153	0.050	0.100

Curved Fruit Shape Index	-0.286*	-0.022	0.068	-0.164	0.031	0.064
Proximal Fruit Blockiness	0.010	-0.286*	-0.247	0.067	-0.375*	-0.173
Distal Fruit Blockiness	-0.106	0.171	0.041	0.048	0.193	0.012
Fruit Shape Triangle	0.060	-0.347*	-0.228	0.028	-0.444**	-0.151
Fruit Shape Index Internal	-0.281	-0.033	0.082	-0.154	0.049	0.100

[a]***, **, * indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively.

Discussion

F1 hybrids are often heterotic and generally present a better performance than non-hybrid varieties under sub-optimal conditions [11,57]. Therefore, F1 hybrid development is one of the most employed strategies for vegetable crops breeding. Selection of parents giving hybrids with improved performance is one of the challenges faced by breeders [23,24]. In this way, knowledge of values of genetic parameters for traits with agronomic relevance, including the contribution of additive and non-additive effects, heritability values, and correlations among them provides important information for identifying appropriate parental combinations [38]. In addition, given that, the number of potential hybrid combinations grows exponentially with increasing number of parents, tools that allow predicting hybrid performance facilitate the selection of parents [27].

In several crops, genetic distances among parents have been proved useful to predict the performance of hybrids, although the results depend on the crop, the diversity present in the parents, and the markers used [28,39,40,58,59]. In eggplant, to our knowledge, a single work studied the relationship between genetic distances, based on AFLP markers, of parents and yield and fruit weight of hybrids using Spanish local varieties as parents [39]. These authors found relatively high correlations ($r > 0.6$)

between parents genetic distance and yield or fruit weight of hybrids, although their results were based on just 10 hybrids. Our work tried to provide an integrated perspective for obtaining relevant information for the eggplant breeding. We used a half-diallel analysis, which gives information on the magnitude of general and specific combining abilities and trait heritabilities [25–27], using 10 parents from different genetic backgrounds, origins, and morphological characteristics. The parents and hybrids were characterized for a wide number of traits of agronomic interest and fruit shape, and the potential of genetic distances among parents for predicting hybrid performance, heterosis and SCA was evaluated. To our knowledge this is the most comprehensive work done so far for devising strategies for the selection of parents for hybrids development in eggplant.

We found that parents and hybrids displayed wide and overlapping ranges of variation, as compared with other works evaluating the diversity for morphological and agronomic traits of eggplant [43,45,60,61]. However, despite the wide diversity, on average the group of hybrids had significantly higher vigour (plant height and stem diameter) and yield than the group of parentals, supporting the claim that eggplant hybrids represent a productive advantage over non-hybrid varieties [5,11,62]. In fact, in our work, hybrids had an average yield over 1/3 higher than parents. This is in agreement with many other works that have found that eggplant hybrids frequently have a better agronomic performance than non-hybrid varieties

[34–37,39,63], and therefore are of great interest for improving eggplant production.

The high diversity in the parental and hybrid materials for the traits evaluated was matched by significant GCA and SCA values for all traits, revealing the presence of significant additive and non-additive effects in all traits [26,27]. This suggests that a wide genetic variation exists for the traits evaluated among the parents included in the study. Wide variation in the GCA/SCA ratio among traits indicates that considerable differences exist among them in the gene action. In this way, traits with higher GCA/SCA ratios, like most of the fruit shape traits have a mostly additive genetic control as occurs in other crops such as tomato [64] or melon [65]. Traits with low GCA/SCA values have a predominantly non-additive (i.e., dominant, additive × dominant, and dominant × dominant effects) genetic control [26,27]. These traits with a higher relative proportion of SCA included several related to vigour (plant height and stem diameter), number of flowers per inflorescence, prickliness, and yield. Vigour traits in eggplant are heterotic both in intraspecific and interspecific crosses [36,37,39,43], indicating that this is a general phenomenon in the eggplant genepool. Regarding the number of flowers per inflorescence and prickliness both traits have been found to display significant heterosis in interspecific crosses [43]. While little information exists on the inheritance of the number of flowers per inflorescence in eggplant [66], prickliness has been described as a monogenic or oligogenic trait, with a mostly dominant genetic control, although in some interspecific crosses it is recessive [43,49,66]. Yield displayed the lowest levels for the GCA/SCA ratio, which is in agreement with other works in eggplant [67] and in other solanaceous fruit crops like tomato [68] or pepper [69], indicating that non-

additive effects and their interactions play a major role in the genetic control of this trait. This further supports the development of hybrids as an appropriate strategy for enhancing eggplant yield [5,39]. For fruit size traits in general values revealed a similar effect of GCA and SCA, indicating that, as in other studies with eggplant [67], both additive and non-additive effects are important. This suggests that breeding for fruit size will require parents with good GCA values, but also specific hybrid combinations.

Broad-sense heritability (H^2) values were generally high, indicating that most of the variation observed is genetically determined and that selection among varieties or hybrids will be efficient [38]. Probably the fact that the materials included encompassed a wide diversity for the traits evaluated also contributed to high H^2 values. This is in agreement with Hurtado et al. [45], whom found high H^2 values for eggplant fruit shape traits. However, when considering narrow-sense heritability (h^2), which only takes into account additive variance, values were lower, especially for traits with lowest GCA/SCA ratios. In this way, h^2 values for important agronomic traits, like those related to vigour, prickliness or yield were relatively low, difficulting genetic advances in breeding programmes [38]. However, Rodríguez-Burruezo et al. [39] found high correlation values between parental means and hybrid values (i.e., h^2 values) in hybrids of Spanish local varieties. This may be an indication that heritability values in eggplant largely depend on the population evaluated. On the contrary,

fruit shape traits generally had high values for h^2, suggesting a high selection efficiency in breeding programmes.

High GCA values for traits of interest were scattered among different accessions, indicating that none of the accessions tested had the best combination of GCA values for traits of interest in breeding. For example, the Spanish local accession IVIA371 had high GCA values for Yield, which is a favourable trait, but also for the number of prickles, which is unfavourable [5]. Regarding other traits, like fruit shape, for which different shapes may be demanded by the markets [11], accessions A0416 and MM1597 had, respectively, low and high GCA values associated to elongated fruits, and may be parents of interest for specific markets demanding flattened or elongated fruits, respectively. As expected, the weedy *S. insanum* INS2 accession had low values of GCA for yield and fruit size traits. Although *S. insanum* is self-compatible with eggplant and hybrids are fully fertile [70], fruits are smaller, and yield is lower [43,48]. Amazingly, despite being a wild species from the "spiny" group of eggplant wild relatives [71], INS2 had a negative GCA value for the number of prickles. Although *S. insanum* is generally prickly, due to introgression and genetic flow between *S. melongena* and *S. insanum* [72], there is a continuum of *S. insanum* forms between highly prickly forms and non-prickly ones [48,73], and INS2 corresponds to the latter.

SCA values with respect to trait means were very variable, and generally higher in traits with low h^2 values, like those related to plant vigour and yield. This suggests that for obtaining hybrids with high yield, many hybrids will have to be tested to identify good combinations. Also, high SCA values were observed for prickles. These results indicate that for

these traits, obtaining good hybrids require specific combinations of parents, being the GCA values of the parents of lesser importance [25–27].

Positive correlations detected between traits related to leaf, flower and fruit size suggests that the size of these organs might have a common genetic or physiological basis, as has been found in tomato [74–76]. Yield was also positively correlated to leaf and flower size traits, but not to fruit size, indicating that high yields can be obtained even though fruits are not large, which would require higher fruit set ratios. Interestingly, the number of flowers per inflorescence was negatively correlated with wide fruits. In this respect, van der Knaap and Tanksley [77] found that in tomato the number of flowers per inflorescence and several fruit shape traits were correlated, which may suggest that a common hormonal control may affecting both traits. As expected and found in other eggplant works, most of the fruit shape traits were interrelated [45], suggesting that although a good characterization of eggplant fruit shape can be obtained with Tomato Analyzer software [43,45,78], good information on fruit shape in eggplant can be retrieved with a limited number of descriptors.

Genetic distances based on high-throughput SNP markers were largely in agreement with taxonomic relationships and origins [17,71]. In this way, *S. insanum*, which is the ancestor of *S. melongena* [48,73] was the genetically most distant accession compared to the others. This *S. insanum* accession was also basal to the *S. melongena* accessions in the cluster analysis dendrogram as found in the previous study of Acquadro et al. [17]. Interestingly, our study allowed clarifying relationships among *S.*

melongena materials that were unresolved in the general study with a large number of accessions from *S. melongena* relatives [17]. The fact that the three Spanish accessions, together with the élite background breeding line DH621, derived from the commercial hybrid Ecavi, which is used in the Mediterranean region [11], cluster together is in agreement with a general genetic differentiation of the Mediterranean eggplants group [79, 80]. The fact that accession A0416 is basal to the other *S. melongena* accessions, and has a unique flattened shape, which is quite unusual in *S. melongena* [43,45], might be an indication of introgression with some related species, like *S. aethiopicum* group Kumba, which has flattened fruits [78], although further genotyping studies should be performed to confirm this hypothesis.

Correlations between genetic distances and hybrid trait values or parameters like heterosis or SCA can be very useful for selecting hybrids [81]. In our case, few correlations were observed between genetic distances among parents and hybrid trait values, heterosis, or SCA, independently if the weedy *S. insanum* was excluded from the correlation analyses or not. In any case, the significant correlation values obtained had a relatively low absolute value, with absolute values for *r* always below 0.5, and therefore having a limited predictive value. Negative correlations between hybrid values and genetic distance for leaf and fruit size traits is probably resulting from the fact that the two accessions with highest genetic distances with respect to the others (INS2 and A0416) are the ones with smallest leaves and fruits. Also, a negative correlation of genetic distance with yield when hybrids with all parental accessions are included is probably a consequence of the low yield of INS2. In fact, when hybrids with this parent are excluded this correlation is not significant. Traits for which genetic distances may have some predicting value are stem

diameter, where a positive correlation is obtained with heterosis when all accessions are included, and with SCA when only *S. melongena* accessions are included. Overall, our results indicate that genetic distances based on coding SNP markers in the materials studied are of little predictive value. This is in contrast with a previous study of Rodríguez-Burruezo et al. [39], who found positive correlations between AFLP-based genetic distance and yield and fruit weight in 10 hybrids of Spanish local varieties. The fact that the materials used are very distinct, and much more diverse in our case, the markers used are different (AFLPs vs. SNPs) and the low number of hybrids evaluated in the study of Rodríguez-Burruezo et al. [39] might account for these differences. This suggests that differences in the predictive value of genetic distances for the performance, heteroris, or SCA of hybrids does not only depend on crops and markers used [28,31,32,40] but differences may also exist within a crop, as has been demonstrated for maize [58,81].

Overall our study provides relevant information for eggplant breeding, in particular for the development of improved F1 hybrids. The highly significant differences observed for GCA and SCA for all traits indicates that there is large genetic and gene action diversity in the set of parents and hybrids that can be exploited for breeding. The differences in GCA/SCA ratios and heritabilities, as well as correlations among traits also will condition the breeding strategy to be followed in order to maximize genetic gains in eggplant [38]. With our results we suggest that hybrids are a fast and appropriate strategy to develop improved eggplant

cultivars. The fact that genetic distances among parents are not good predictors of the performance of eggplant hybrids indicates that many hybrid combinations may have to be tested to identify superior hybrids. It also suggests that other molecular techniques, like the use of markers linked to genes or QTLs controlling traits of interest [66], may be a more appropriate strategy for preselecting parents in eggplant hybrid breeding programs than the use of genetic distances among parents.

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S1 Table. Values for each of the parents and hybrids for the 28 conventional and Tomato Analyzer descriptors evaluated.

Parent or hybrid	Yield (Kg)	Corrolla Diameter (mm)	Leaf Blade Length (cm)	Leaf Blade Width (cm)	Leaf Pedicel Length (cm)	Plant Height (cm)	Number of Flowers per Inflorescence	Stem Diameter (mm)	Fruit weight (g)	Fruit Length (cm)	Fruit Width (cm)	Fruit Pedicel Length (mm)	Fruit Pedicel Diameter (mm)	Fruit calyx prickles
MM1597	1.53	24.40	26.83	22.83	7.80	81.07	5.33	17.33	94.98	19.90	3.83	50.10	9.90	0.00
DH621	2.72	22.47	28.47	16.90	9.03	75.53	1.33	15.00	210.22	13.47	7.47	55.10	15.67	1.00
ANS26	3.32	43.30	24.56	15.37	8.65	85.63	1.11	15.33	240.54	9.83	9.83	67.83	21.33	1.00
HI5	2.04	40.03	24.10	17.50	9.68	77.83	3.22	15.50	190.86	8.13	4.93	93.43	20.17	1.00
A0416	1.48	27.83	19.63	12.05	5.55	47.70	3.00	11.77	128.92	4.70	8.27	26.77	8.00	0.00
IVIA371	4.55	43.00	25.53	18.18	12.04	73.47	5.00	18.00	245.70	10.67	8.13	46.60	18.37	5.00
ASIS1	1.94	33.90	25.59	16.91	7.11	82.97	1.00	11.17	154.07	6.00	7.83	50.13	12.33	0.00
MEL1	3.30	36.53	27.55	19.22	8.12	65.80	4.78	16.67	185.77	10.30	6.57	51.83	13.37	1.00
MEL5	1.96	27.07	21.93	14.21	8.45	66.20	5.00	11.43	95.16	9.00	4.53	43.97	10.33	1.00
INS2	0.93	19.37	14.96	10.79	4.83	70.87	3.00	17.33	55.20	4.40	4.68	23.40	7.50	0.00
MM1597 × DH621	4.70	39.75	30.29	20.73	8.01	103.27	5.11	22.03	268.41	15.13	5.70	42.23	13.30	1.00
MM1597 × ANS26	3.20	41.73	29.81	22.45	12.24	112.30	5.00	18.67	212.10	13.90	5.20	59.90	16.90	1.00
MM1597 × H15	5.78	47.17	27.82	19.61	8.12	121.50	5.22	17.67	278.87	18.87	4.90	62.50	17.43	1.00
MM1597 × A0416	3.06	38.97	25.53	18.77	7.45	84.63	7.00	17.67	240.15	9.10	7.97	40.10	10.83	1.00
MM1597 × IVIA371	6.91	46.10	28.46	18.86	9.51	95.53	5.00	17.00	254.55	16.67	6.10	35.83	13.77	1.00
MM1597 × ASIS1	2.54	34.00	30.79	23.22	10.65	91.53	4.00	17.83	249.55	12.33	7.33	51.07	15.00	1.00
MM1597×MEL1	2.80	37.40	24.74	19.25	6.23	90.70	5.00	18.17	169.76	11.83	5.37	45.57	14.10	1.00
MM1597 × MEL5	4.85	36.57	25.10	18.41	8.73	91.23	7.00	20.00	178.25	14.47	4.73	39.67	11.17	0.00
MM1597 × INS2	2.84	43.63	24.77	18.86	11.14	77.27	5.00	15.17	317.27	12.00	7.70	70.10	17.20	0.00
DH621 × ANS26	3.88	30.40	30.12	15.76	10.02	104.53	3.00	17.83	332.66	10.63	8.43	59.67	19.40	3.00
DH621 × H15	2.94	40.20	25.16	18.02	7.98	78.97	3.22	18.83	266.94	7.93	7.13	40.10	11.07	1.00
DH621 × A0416	3.54	44.27	26.09	17.52	10.61	70.90	5.00	12.83	353.31	14.87	8.03	69.13	17.37	3.00

DH621 × IVIA371	1.95	33.70	30.14	17.49	9.21	108.23	3.00	18.00	293.83	7.73	8.17	34.70	17.60	1.00
DH621 × ASIS1	4.70	33.80	26.64	17.85	7.46	104.70	5.00	15.50	350.43	11.23	8.20	53.83	15.23	3.00
DH621 × MEL1	4.12	30.60	26.43	16.51	9.41	102.43	5.00	16.00	142.18	10.27	5.97	38.47	12.23	0.00
DH621 × MEL5	2.21	32.30	25.41	16.62	8.16	92.87	3.00	17.83	266.97	8.20	8.37	56.67	20.37	1.00
DH621 × INS2	2.32	40.27	24.48	16.94	7.95	82.63	5.00	14.00	196.53	5.93	7.23	22.83	13.33	1.00
ANS26 × H15	1.95	40.67	25.74	18.35	11.16	67.27	3.00	15.00	192.06	10.24	7.20	54.30	17.83	1.00
ANS26 × A0416	2.74	38.47	26.06	16.50	8.35	102.93	1.00	15.50	246.61	7.61	8.57	33.57	20.07	0.00
ANS26 × IVIA371	3.59	41.17	25.48	17.59	8.53	82.40	3.00	20.33	224.71	9.73	7.50	45.53	13.57	1.00
ANS26 × ASIS1	2.97	30.57	24.80	17.95	11.68	84.27	3.00	19.50	135.60	10.97	5.47	53.10	12.03	0.00
ANS26 × MEL1	2.76	40.53	25.44	16.79	8.99	78.33	3.00	15.57	235.11	6.47	7.93	41.67	14.83	1.00
ANS26 × MEL5	2.13	45.20	23.57	19.10	9.33	83.90	3.00	15.50	294.51	9.37	7.80	45.73	15.50	5.00
ANS26 × INS2	1.90	33.70	29.31	18.30	8.43	90.20	3.00	15.00	314.14	7.17	8.77	30.17	17.07	1.00
H15 × A0416	3.84	40.87	25.63	16.07	8.22	91.07	3.00	20.50	239.03	10.20	7.23	51.17	16.17	3.00
H15 × IVIA371	3.52	32.53	24.19	15.53	9.25	83.60	5.00	17.50	141.21	10.40	5.83	52.33	12.27	1.00
H15 × ASIS1	4.07	40.10	23.67	15.48	7.37	66.43	5.00	17.27	310.73	7.43	10.10	34.33	13.50	3.00
H15 × MEL1	3.49	30.63	24.68	15.60	7.05	77.80	1.00	15.00	259.49	5.77	9.20	25.33	12.60	1.00
H15 × MEL5	3.50	36.97	25.19	17.34	8.08	61.83	3.00	14.00	187.46	6.40	7.63	37.00	11.77	1.00
H15 × INS2	3.46	33.90	23.85	15.78	6.70	70.57	5.00	15.00	167.91	5.93	6.43	37.00	9.90	3.00
A0416 × IVIA371	3.36	31.47	28.34	18.83	8.27	75.53	3.00	19.27	216.18	6.83	7.50	34.00	14.50	5.00
A0416 × ASIS1	2.92	40.53	23.53	16.64	7.41	61.57	5.00	18.00	266.42	11.43	7.47	45.00	14.83	1.00
A0416 × MEL1	3.73	35.10	26.46	17.68	9.88	59.80	5.00	20.67	136.90	10.00	5.23	47.17	12.00	1.00
A0416 × MEL5	3.27	40.07	25.73	18.63	7.97	73.47	3.00	17.43	239.22	8.70	8.40	42.17	13.13	0.00

A0416 × INS2	2.12	31.43	24.57	16.85	8.06	89.17	4.00	15.33	182.89	8.40	6.68	39.70	13.60	0.00
IVIA371 × ASIS1	3.66	36.90	24.58	16.86	7.02	86.50	7.00	16.60	163.72	10.23	5.97	42.00	11.33	1.00
IVIA371 × MEL1	3.49	30.47	23.30	18.49	6.72	99.33	5.22	23.33	87.68	8.53	4.23	39.00	11.10	0.00
IVIA371 × MEL5	4.34	28.50	28.57	17.80	8.04	90.87	5.00	21.50	123.32	6.87	5.33	43.00	13.33	0.00
IVIA371 × INS2	2.17	32.63	21.48	15.99	7.19	88.00	5.00	22.50	93.88	6.07	6.03	37.33	13.43	0.00
ASIS1 × MEL1	2.86	29.17	20.52	14.45	7.99	96.63	5.00	19.50	83.83	5.50	5.20	40.33	13.70	0.33
ASIS1 × MEL5	2.00	23.50	18.85	14.28	5.56	65.97	5.00	18.57	63.63	3.73	5.60	27.27	8.17	1.00
ASIS1 × INS2	1.69	30.93	22.31	15.43	6.27	76.90	4.00	17.13	77.41	6.70	4.90	39.97	12.03	5.00
MEL1 × MEL5	2.50	29.60	23.09	17.09	6.05	84.50	5.00	21.00	71.17	4.38	5.55	21.67	11.73	0.00
MEL1 × INS2	3.14	29.97	19.65	14.27	5.45	78.33	4.00	23.67	67.50	4.50	4.93	31.50	10.17	0.00
MEL5 × INS2	4.08	30.50	20.07	14.22	6.04	101.23	4.00	19.67	68.34	6.03	4.13	32.03	7.83	1.00

Parent or hybrid	Perimeter	Area	Width Mid-height	Maximum Width	Height Mid-width	Maximum Height	Curved Height	Fruit Shape Index External I	Fruit Shape Index External II	Curved Fruit Shape Index	Proximal Fruit Blockiness	Distal Fruit Blockiness	Fruit Shape Triangle	Fruit Shape Index Internal
MM1597	28.64	24.88	2.27	3.65	11.16	12.05	12.53	3.33	5.01	5.69	0.76	0.97	0.78	5.05
DH621	37.63	78.88	7.08	7.77	13.66	13.90	14.76	1.80	1.93	2.06	0.47	0.80	0.59	1.93
ANS26	25.42	37.46	6.85	6.93	6.91	7.03	7.63	1.01	1.01	1.12	0.46	0.67	0.69	1.01
HI5	30.41	57.85	7.24	7.45	10.08	10.17	10.36	1.37	1.40	1.44	0.49	0.73	0.68	1.40
A0416	21.46	27.61	7.05	7.09	4.32	4.88	5.52	0.69	0.61	0.84	0.75	0.64	1.19	0.61
IVIA371	29.45	57.69	7.33	7.50	9.65	9.80	10.15	1.31	1.32	1.38	0.57	0.72	0.79	1.32
ASIS1	31.31	65.08	10.56	10.59	7.03	7.53	7.95	0.71	0.67	0.75	0.74	0.67	1.10	0.66
MEL1	26.67	43.07	5.47	5.81	9.44	9.61	9.75	1.66	1.73	1.78	0.55	0.80	0.68	1.73

MEL5	20.14	22.60	3.52	3.53	7.85	7.91	8.00	2.25	2.24	2.29	0.61	0.73	0.84	2.25
INS2	16.43	17.48	4.64	4.65	4.58	4.68	4.92	1.01	0.99	1.07	0.63	0.66	0.95	0.99
MM1597 × DH621	36.90	58.90	4.40	4.90	15.13	15.55	16.17	3.17	3.42	3.68	0.63	0.84	0.76	3.43
MM1597 × ANS26	40.47	86.27	6.66	6.97	15.37	15.53	16.16	2.27	2.33	2.47	0.66	0.71	0.95	2.34
MM1597 × H15	47.26	97.69	5.53	6.10	19.66	19.93	20.28	3.29	3.56	3.67	0.75	0.88	0.86	3.60
MM1597 × A0416	28.93	54.64	6.78	6.83	10.14	10.24	10.32	1.50	1.49	1.52	0.56	0.63	0.89	1.49
MM1597 × IVIA371	38.79	65.08	4.94	5.29	15.46	15.53	16.16	2.94	3.13	3.29	0.67	0.79	0.85	3.16
MM1597 × ASIS1	32.39	67.79	7.00	7.07	11.66	11.74	12.14	1.67	1.67	1.75	0.69	0.66	1.05	1.68
MM1597×MEL1	33.18	53.00	4.56	5.04	12.96	13.26	13.16	2.64	2.86	2.90	0.67	0.90	0.74	2.86
MM1597 × MEL5	37.38	52.52	4.03	4.60	14.48	15.24	14.64	3.40	3.74	3.79	0.66	0.83	0.80	3.74
MM1597 × INS2	37.72	82.77	6.68	7.14	14.23	14.32	14.85	2.00	2.13	2.23	0.67	0.83	0.81	2.15
DH621 × ANS26	33.12	64.24	7.35	7.46	11.16	11.31	11.58	1.53	1.53	1.59	0.47	0.68	0.70	1.53
DH621 × H15	28.94	53.99	8.28	8.32	7.92	8.03	8.61	0.97	0.96	1.05	0.67	0.62	1.10	0.96
DH621 × A0416	38.33	86.40	7.34	7.58	14.19	14.31	14.88	1.91	1.96	2.03	0.65	0.75	0.87	1.96
DH621 × IVIA371	35.46	80.67	9.57	9.75	10.39	10.63	11.71	1.10	1.10	1.25	0.50	0.69	0.75	1.09
DH621 × ASIS1	34.41	71.23	6.88	7.19	12.44	12.60	13.15	1.77	1.82	1.91	0.58	0.78	0.74	1.83
DH621 × MEL1	23.88	31.40	4.16	4.22	9.18	9.29	9.43	2.21	2.23	2.29	0.59	0.73	0.81	2.23
DH621 × MEL5	28.96	58.61	7.83	7.86	9.31	9.40	10.14	1.20	1.20	1.30	0.56	0.64	0.87	1.20

DH621 × INS2	31.88	70.16	9.81	9.87	8.60	8.92	9.80	0.91	0.88	1.01	0.63	0.65	0.98	0.88
ANS26 × H15	38.47	98.38	10.01	10.05	12.20	12.52	13.42	1.24	1.22	1.35	0.56	0.66	0.85	1.21
ANS26 × A0416	39.40	100.91	11.59	11.61	11.21	11.38	12.36	0.98	0.97	1.12	0.50	0.61	0.82	0.97
ANS26 × IVIA371	34.46	73.64	7.47	7.76	12.22	12.40	12.80	1.62	1.66	1.72	0.54	0.73	0.74	1.68
ANS26 × ASIS1	31.27	60.24	6.22	6.36	11.53	11.66	12.09	1.87	1.89	1.99	0.64	0.75	0.86	1.90
ANS26 × MEL1	28.82	53.83	8.19	8.37	7.34	7.81	8.79	0.93	0.90	1.09	0.68	0.73	0.95	0.90
ANS26 × MEL5	29.70	57.66	7.36	7.50	9.66	9.72	10.05	1.30	1.32	1.35	0.61	0.70	0.90	1.32
ANS26 × INS2	35.90	89.24	10.69	10.77	9.88	10.26	11.36	0.95	0.92	1.07	0.70	0.65	1.07	0.92
H15 × A0416	35.83	74.47	6.87	7.26	12.82	13.28	13.90	1.83	1.87	1.96	0.56	0.80	0.70	1.88
H15 × IVIA371	22.18	28.23	4.16	4.22	8.21	8.34	8.34	1.97	1.97	2.01	0.62	0.71	0.86	1.98
H15 × ASIS1	32.11	62.11	8.85	8.88	8.92	9.15	9.54	1.04	1.02	1.09	0.49	0.62	0.80	1.02
H15 × MEL1	35.57	63.82	10.37	10.42	7.75	8.13	10.29	0.78	0.75	1.12	0.30	0.72	0.42	0.75
H15 × MEL5	26.86	46.98	7.61	7.68	7.34	7.55	8.04	0.98	0.96	1.06	0.66	0.69	0.95	0.96
H15 × INS2	22.70	33.91	6.48	6.49	6.02	6.42	7.83	0.99	0.94	1.21	0.74	0.67	1.12	0.94
A0416 × IVIA371	31.27	68.81	8.81	8.84	9.19	9.42	9.91	1.06	1.04	1.12	0.73	0.66	1.11	1.03
A0416 × ASIS1	34.18	73.64	7.35	7.60	12.05	12.17	12.78	1.61	1.65	1.69	0.63	0.73	0.87	1.66
A0416 × MEL1	32.21	54.67	5.56	5.80	11.61	11.72	12.28	2.03	2.10	2.21	0.59	0.83	0.72	2.10
A0416 × MEL5	27.73	50.25	7.28	7.37	8.37	8.51	8.98	1.16	1.15	1.24	0.63	0.68	0.91	1.15
A0416 × INS2	28.68	54.71	7.39	7.42	9.00	9.23	10.02	1.25	1.23	1.34	0.62	0.66	0.93	1.23
IVIA371 × ASIS1	26.25	37.55	4.56	4.61	9.90	10.06	10.18	2.18	2.18	2.23	0.60	0.75	0.80	2.18
IVIA371 × MEL1	27.26	40.33	4.86	5.05	9.83	10.01	10.31	1.98	2.02	2.13	0.61	0.77	0.80	2.02

IVIA371 × MEL5	27.98	46.23	5.18	5.53	10.46	10.64	11.15	1.94	2.04	2.20	0.58	0.84	0.70	2.05
IVIA371 × INS2	21.15	30.25	5.30	5.39	6.95	7.05	7.19	1.31	1.31	1.35	0.55	0.72	0.77	1.31
ASIS1 × MEL1	23.63	33.89	5.59	5.64	7.56	7.66	8.01	1.36	1.36	1.43	0.56	0.69	0.81	1.36
ASIS1 × MEL5	19.51	24.13	5.87	5.91	4.95	5.04	5.28	0.85	0.84	0.90	0.73	0.64	1.13	0.84
ASIS1 × INS2	23.26	34.27	5.99	6.04	6.93	7.14	7.66	1.18	1.15	1.27	0.56	0.69	0.82	1.15
MEL1 × MEL5	22.37	31.07	6.25	6.30	6.18	6.38	6.88	1.02	0.99	1.10	0.48	0.65	0.75	0.99
MEL1 × INS2	21.17	28.38	5.09	5.23	6.93	7.24	7.26	1.39	1.36	1.42	0.45	0.70	0.64	1.36
MEL5 × INS2	21.45	27.93	4.76	4.88	7.41	7.48	7.64	1.53	1.56	1.61	0.52	0.69	0.75	1.56

S2 Table. Pearson linear correlation coefficients between descriptors. Only those traits for which at least one correlation was significant (values in bold) according to the Bonferroni test ($p < 0.05$; $r \geq 0.4928$) are included.

	Leaf Blade Width	Fruit Pedicel Length	Fruit Pedicel Diameter	Fruit Length (cm)	Fruit Width	Fruit weight	Perimeter	Area	Width Mid-height	Maximum Width	Height Mid-width	Maximum Height	Curved Height	Fruit Shape Index External I	Fruit Shape Index External II	Curved Fruit Shape Index	Distal Blockiness	Fruit Shape Triangle	Fruit Shape Index Internal
Leaf Pedicel Length (cm)	**0.527**	**0.591**	**0.605**	**0.502**	0.191	0.470	**0.540**	**0.551**	0.171	0.196	**0.545**	**0.532**	**0.540**	0.238	0.199	0.182	0.141	-0.076	0.199
Leaf Blade Length	**0.752**	0.348	**0.542**	**0.568**	0.284	**0.618**	**0.705**	**0.632**	0.237	0.289	**0.648**	**0.651**	**0.681**	0.341	0.328	0.335	0.280	-0.112	0.328
Leaf Blade Width		0.358	0.358	0.309	-0.045	0.374	**0.589**	0.457	-0.023	0.041	**0.652**	**0.658**	**0.655**	**0.504**	**0.536**	**0.540**	0.436	0.000	**0.536**
Number of Flowers per Inflorescence		-0.051	-0.051	-0.333	-0.486	-0.170	-0.093	-0.261	**-0.624**	**-0.625**	0.239	0.237	0.169	**0.559**	**0.519**	0.484	0.354	0.027	**0.518**
Corrolla Diameter		0.391	0.391	**0.526**	0.354	**0.639**	**0.585**	**0.567**	0.276	0.293	**0.514**	**0.505**	**0.510**	0.161	0.099	0.070	0.065	0.004	0.099
Fruit Pedicel Length			**0.621**	**0.621**	**0.621**	0.365	0.384	0.341	-0.070	-0.034	**0.496**	0.484	0.462	0.306	0.290	0.263	0.341	-0.236	0.290
Fruit Pedicel Diameter				0.294	0.492	**0.668**	**0.599**	**0.688**	0.469	**0.496**	0.458	0.444	0.475	-0.029	-0.054	-0.067	-0.016	-0.247	-0.054
Fruit Length					0.148	0.380	**0.637**	0.383	-0.348	-0.271	**0.851**	**0.857**	**0.834**	**0.842**	**0.853**	**0.844**	**0.693**	-0.211	**0.853**
Fruit Width						**0.726**	0.287	0.463	**0.753**	**0.748**	-0.071	-0.074	-0.011	**-0.538**	**-0.532**	**-0.516**	-0.456	0.084	**-0.532**
Fruit weight							**0.711**	**0.747**	**0.518**	**0.551**	**0.521**	**0.514**	**0.558**	0.014	-0.015	-0.020	-0.025	-0.029	-0.014
Yield							0.408	0.203	-0.232	-0.217	**0.547**	**0.540**	**0.523**	**0.523**	0.428	0.397	0.373	-0.291	0.428
Perimeter								**0.919**	0.386	0.455	**0.870**	**0.876**	**0.912**	0.384	0.355	0.351	0.332	-0.170	0.355
Area									**0.644**	**0.690**	**0.696**	**0.692**	**0.743**	**0.059**	0.023	0.016	0.034	-0.030	0.023
Width Mid-height										**0.994**	-0.085	-0.085	-0.003	**-0.678**	**-0.668**	**-0.644**	**-0.579**	0.205	**-0.668**

Maximum Width	-0.016	-0.012	0.072	**-0.619**	**-0.596**	**-0.567**	**-0.500**	0.173	**-0.595**
Height Mid-width		**0.999**	**0.989**	**0.738**	**0.685**	**0.657**	**0.603**	-0.269	**0.685**
Maximum Height			**0.991**	**0.747**	**0.700**	**0.676**	**0.619**	-0.265	**0.700**
Curved Height				**0.691**	**0.650**	**0.636**	**0.589**	-0.265	**0.651**
Fruit Shape Index External I					**0.976**	**0.953**	**0.820**	-0.296	**0.975**
Fruit Shape Index External II						**0.994**	**0.846**	-0.284	1.000
Curved Fruit Shape Index							**0.847**	-0.279	**0.995**
Distal Fruit Blockiness								**0.796**	0.273
Fruit Shape Triangle									**0.846**

S3 Table. Genetic distance (GD) matrix among the 10 eggplant parents. GD values are based on identity by state (IBS) and calculated as GD=1-IBS.

	ANS26	ASIS1	DH621	HI5	IVIA371	MEL 1	MEL5	MM1597	INS2
A0416	0.0230	0.0232	0.0235	0.0235	0.0240	0.0309	0.0299	0.0245	0.0386
ANS26		0.0197	0.0118	0.0118	0.0122	0.0222	0.0206	0.0158	0.0321
ASIS1			0.0200	0.0204	0.0206	0.0278	0.0262	0.0205	0.0354
DH621				0.0136	0.0094	0.0214	0.0203	0.0152	0.0321
HI5					0.0146	0.0231	0.0223	0.0174	0.0335
IVIA371						0.0218	0.0208	0.0150	0.0323
MEL 1							0.0284	0.0250	0.0389
MEL5								0.0226	0.0375
MM1597									0.0305

Chapter 5: Diallel Analysis for Fruit Phenolics Content, Flesh Color, and Browning Related Traits in Eggplant

1. Introduction

Eggplant (*Solanum melongena* L.) is the third most consumed vegetable of family Solanaceae [1,2]. Eggplants have high human-health beneficial effects related to its high content phenolic acids [3–5]. These phenolic acids are important for their various health promoting effects such as the protection against chronic diseases like cancer and arthritis[6]. Among the different types of phenolic acids identified in the eggplant, the chlorogenic acid is most frequent which makes up to 90% of total phenolic acids [5,7]. The content of phenolic acids in the eggplant flesh varies among cultivars; also, the wild relatives of eggplant generally have a higher diversity and concentrations of phenolic acids content than the modern cultivated varieties [8,9].

Various reports suggest that increasing the phenolics content in the fruit flesh might also increase the susceptibility of eggplant flesh to browning [10,11]. In this way, previous studies have pointed out that chlorogenic acid content moderately influences the fruit flesh browning in eggplant [12]. In order to develop modern eggplants cultivars with a higher content of phenolics several kinds of genetic

materials have been screened and a significant amount of variation has been observed for the content of phenolics in the cultivated varieties, wild species and also interspecific hybrids [8,9,13]. Recently we have studied the diversity for the phenolic content in the cultivated eggplant and its wild relatives from all the primary, secondary and tertiary genepools [8,14].

Diallel based genetic studies are informative to determine the variation for the trait in question and to identify parents and cross combinations likely to produce better hybrids [15,16]. The half-diallel mating design, which includes one-way direct crosses and their parents [17,18], provides valuable information regarding the combining abilities of parents, which are the important predictors of the breeding value of hybrids. In this way, general combining ability (GCA) indicates additive gene action, while the specific combining ability (SCA) points towards the non-additive gene action, which can be caused by dominance, epistasis, and overdominance effect in controlling the trait in question[19].

The genome eggplant sequence is already available [20] and several studies have been carried using molecular markers from RAPDs to more recent ones with SNPs [20,21]. Several studies have used these molecular markers to estimate the genetic distances among parents and evaluated its value to predict the performance of hybrids [22–25]. However, in eggplant there is a limited knowledge of the use of molecular markers for predicting hybrid performance [23], and to

our knowledge no studies of their potential interest for predicting the fruit phenolics content, fruit colour and browning of hybrids.

Therefore, the present investigation was undertaken to provide information of the genetics and inheritance of the content in phenolics, fruit flesh colour, and browning in eggplant. In our study we estimate combining abilities (GCA and SCA), heritabilities, and determine the usefulness of SNPs based genetic distances for predicting the performance of hybrids for these traits.

2. Material and Methods

2.1.Plant material and growing conditions

Nine eggplant cultivars and one accession of the eggplant primary genepool wild species *S. insanum* (INS2) were used for this study. The eggplant cultivars were previously found to be morphologically diverse and their main characteristics are described in Kaushik et al. (2018). These 10 genotypes were crossed in the diallel mating design without reciprocals to produce 45 F_1 hybrids. All the parental plants and hybrids were grown under the open field situation in a plot located at the Universitat Politècnica de València (Coordinates at: 39° 28′ 55″ N, 0° 22′ 11″ W; altitude 7 m a.s.l.). Three replications consisting of three

plants were distributed according to a randomised complete block design. Plants were watered by means of drip irrigation and fertigation was provided by distributing 80 g·plant^{-1} of a 10N–2.2P–24.9K plus micronutrients fertiliser (Hakaphos Naranja; Compo Agricultura, Barcelona, Spain) throughout the cultivation period through the irrigation system. At appropriate age, plants were trained on bamboo canes. Weeds were manually removed and no phytosanitary measure needed.

Sample preparation

Samples from each replication consisted of five fruits, which were picked at commercially ripe stage (physiologically immature) for the characterisation of phenolics, fruit colour and browning. Fruits were opened transversally, and one half of the fruit was snap frozen with liquid nitrogen that was further kept at -80ºC till further use. Whilethe other half was used for measuring the flesh browning.

2.2.Characterisation of fruit

Fruit flesh browning was measured using a CR-300 chromameter (Minolta, Osaka, Japan) at the midpoint position of the centre of the fruit) in each of the five fruits that constitute one sample The values for CIELAB colour parameters L*, a*, b* were measured

immediately after the fruit was cut (L^*_0, a^*_0, b^*_0); also, the fruit flesh colour measured as distance to pure white (DW; Prohens et al., 2007) was calculated (DW_0). New measurements of L*0, a*0, and b*0parameters were taken after 5-10 (XX) min (L^*_{XX}, a^*_{XX}, b^*_{XX}). These values were processed to estimate the degree of fruit flesh browning (DB) and colour difference (CD) using the formulas defined in Prohens et al. (2007).

The percentage of change in weight before and after lyophilisation process was used as the measure of dry matter content. The Folin-Ciocalteu spectrophotometric method was used to measure the total phenolics (mg/g dw) of the eggplant flesh as defined in detail elsewhere [26]. The total phenolics content was quantified using chlorogenic acid as standard for comparing the spectra at 750 nm using the spectrophotometer (Jenway, Essex, UK). The determination of chlorogenic acid (CGA) content was done with the help of high-performance liquid chromatography (HPLC) on a 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA). The calculations were performed by the OpenLAB CDS ChemStation Edition software package (Agilent Technologies) according to the manufacturer instructions [27]. The percentage of peak area for chlorogenic acid was determined using the chlorogenic acid peak area and total peak area of other phenolic acis (mainly hydroxycinnamic acid conjugates).The polyphenol oxidase activity was determined

based on the protocol defined elsewhere[6]. The reaction activity was determined as the increase in the absorbance at 420 nm with the help of Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Montchanin, DE, USA). Further, the unit change in enzyme activity was calculated as the increase in 0.1 absorbance unit per minute per milligram of dry weight.

2.3.Data analysis

For each trait measured, the mean and range were calculated for the parental (n = 10) and hybrid (n = 45) groups. Mean values of parents and hybrids were compared with *t*-tests to detect differences among the two groups. The significance of differences among group means was evaluated at $p < 0.05$ using the Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). The diallel analysis were performed based on the Griffing´s Method 2 (parents and F1 hybrids) and Model 1 (fixed effects) [17]. These calculations were done using the using AGD-R (Analysis of Genetic Designs with R) software package [28]. The Baker ratio was estimated as GCA/SCA = 2 × s2GCA / ((2 × s2GCA) + s2SGA) [18]. Relative SCA values of individual hybrids were expressed in percentage (%) over the average of the trait. Pair-wise Pearson linear coefficients of correlation (r) were determined by the Statgraphics Centurion XVI software. The mid-parent heterosis of F1 (Het; %) was calculated using formula Het = 100

× ((F1 − MP)/MP), where F1 = hybrid mean, and MP = mean of the parents.

2.4.Genetic distance and its correlation

Genotypic data obtained for the ten accessions used here through the RAD sequencing approach in a previous study[23,29] were used here. In total 7,335 polymorphic SNPs were used for determining genetic distances between the 10 parents used in our study. The TASSEL software version 5.0 Standalone was used to determine the genetic distances based on the identity-by-state (IBS) genetic distance (GD) as GD = 1-IBS [30]. Genetic distance of parents of individual hybrids was further used to determine Pearson linear correlations between GD and hybrid trait values, heterosis, and SCA.

3. Results

3.1.Variation in parents and hybrids

The average values of the parental genotypes and hybrids were similar in the means for most of the traits studied (Table 1). Interestingly, the coefficient of variation was higher in the parental genotypes as compared to the hybrids (Table 1). Furthermore, the

coefficient of variation were larger in values in the parents than their hybrids (Table 1) .

Table 1. Mean and range of variation for all the biochemical traits studied

Descriptors	**Parents**	**Hybrids**	**Probability**
Dry Matter	10.07	9.41	0.2613
Range	(7.43 to 14.10)	(6.95 to 12.82)	
CV	106.44	15.81	
Total Phenolics	11.02	10.95	0.9458
Range	(7.58 to 15.97)	(5.81 to 17.47)	
CV	109.36	27.65	
Chlorogenic Acid (mg/g)	2.88	2.81	0.5249
Range	(2.08 to 4.26)	(1.82 to 3.39)	
CV	112.57	15.21	
Area %	69.78	72.86	0.2870
Range	(45.25 to 83.14)	(60.15 to 88.14)	
CV	64.84	9.87	
L^*_0	81.02	81.57	0.6430
Range	(72.77 to 88.55)	(73.27 to 86.25)	
CV	91.88	3.50	
a^*_0	2.33	3.04	0.1717
Range	(-4.31 to 0.08)	(-6.17 to 0.33)	
CV	134.80	48.94	
b^*_0	17.40	18.25	0.5634
Range	(10.21 to 23.01)	(8.97 to 25.54)	
CV	132.27	22.55	
DW_0	26.12	26.39	0.8761
Range	(16.06 to 34.86)	(19.51 to 34.85)	
CV	133.46	16.80	
PPO	2.75	2.03	0.1151
Range	(1.20 to 8.13)	(0.66 to 4.33)	
CV	75.70	50.96	
DB	3.72	3.41	0.7554
Range	(1.48 to 9.35)	(0.76 to 16.45)	

CV	58.68	82.80	
CD	6.14	5.38	0.5624
Range	(1.82 to 16.80)	(1.88 to 20.90)	
CV	50.02	63.53	

The estimates of mean sum of squares (ANOVA) for general combining ability (GCA) of parents, and specific combining ability (SCA) of the hybrids were highly significant ($P \leq 0.01$) (Table 2). In general, the values of GCA effects were higher as compared to SCA effects (Table 2). The predominance of additive gene action was noticed based on the Baker ratio (>0.75) for all of the traits studied (Table2). The estimates of broad sense heritability (≥0.50) were larger as compared to those for narrow sense heritability (≤0.50) (Table 2). The CGA content was determined with the lowest values for both narrow sense (0.02) and broad sense heritability (0.23) (Table 2). Dry Matter, Phenolics, CGA, Area%, L*0, a*0, b*0, DW_0, PPO and DB showed low (≤ 0.30) narrow sense heritability. Interestingly, all traits except CGA (0.23) exhibited broad sense heritability value above 0.5 (Table 2).

Table 2. Mean squares for block, genotypes, GCA, SCA, Baker ratio, and narrow sense and broad sense heritabilities for the ANOVA for the fruit traits evaluated.

Descriptors[a]	Block	Genotypes	GCA	SCA	Error	Baker Ratio	Narrow Heritability	Broad Heritability
d.f.	2	54	9	45	108			
Dry Matter	2.21^{ns}	8.17***	15.77***	6.65***	1.81	0.83	0.18	0.57
Phenolics	23.83*	26.48***	67.13***	18.35***	7.31	0.88	0.23	0.50
CGA	0.29^{ns}	0.63***	0.94***	0.57***	0.30	0.77	0.09	0.23
Area%	25.32^{ns}	201.58***	527.42***	136.41***	30.48	0.89	0.30	0.67
L^*_0	41.77***	33.40***	116.03***	16.88***	5.63	0.93	0.40	0.64
a^*_0	4.01^{ns}	6.54***	20.45***	3.76***	1.60	0.92	0.31	0.53
b^*_0	32.89***	52.00***	205.52***	21.30***	4.56	0.95	0.52	0.79
DW_0	69.38***	69.81***	268.83***	30.01***	8.08	0.95	0.48	0.73
PPO	0.09^{ns}	5.09***	11.32***	3.85***	0.81	0.85	0.24	0.66
DB	0.60^{ns}	23.08***	52.35***	17.22***	2.60	0.86	0.27	0.75
CD	3.23^{ns}	40.55***	106.22***	27.41***	4.61	0.89	0.32	0.74

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively.

3.2.GCA and SCA effects

The GCA effects for Dry Matter ranged from -0.87 (MEL1) to 1.29 (INS2) and for phenolics from -2.23 (A0416) to 2.37 (INS2) (Table 3). For CGA, the general combining ability estimates was non-significant for all the parents except for ASI-S-1 (-0.27) and INS 2 (0.39) (Table 3). The general combining ability effects for Area% CGA ranged from -8.60 (MM1597) to 3.60 (IVIA-371) (Table 3). The general combining ability estimates for L* ranged from -3.14 (INS 2) to 3.00 (MEL 5), while they ranged from -0.91 (DH 621) to 1.22 (A0416) and from -2.90 (MEL 1) to 3.30 (MM1597) for a^*_0 and b^*_0 respectively (Table 3). The GCA effect for PPO activity ranged from -0.50 (IVIA-371) to 1.30 (INS 2). The GCA effect for DB and CD ranged from -0.85 (MEL 5) to 3.25 (INS 2) and -1.50 (MEL 5) to 4.35 (INS 2) respectively (Table 3).

The SCA effects are presented in the Table 4. For dry matter content highest positive SCA values of 2.64, 2.47 and 2.28 were observed in crosses IVIA-371 × MEL5, MM 1597 × H15 and DH 621 × IVIA-371 respectively (Table 4). For phenolics the significant SCA effects were recorded for the crossesH15 × IVIA-371 (5.97), AN-S-26 × ASI-S-1 (4.90) and DH 621 × MEL 1 (3.37) respectively (Table 4). The highly significant positive SCA effects for CGA were recorded

for cross combinations H15 × IVIA-371 (1.08) and IVIA-371 × INS2 (0.65) (Table 4). The following crosses:IVIA-371 × INS2 (13.44), IVIA-371 × MEL 5 (12.25) and DH 621 × ASI-S-1 (11.36) exhibited the significant SCA effect for the Area % under the curve (Table 4).

The positive and high SCA effects of 4.50, 4.30 and 3.40 for the L^*_0 in the cross combinations IVIA-371 × INS2, AN-S-26 × H15 and DH 621 × INS2 respectively (Table 4). The significant and positive SCA effects for b^*_0 were recorded in the crosses DH 621 × MEL 5 (7.42), A0416 × MEL 5 (4.82) and DH 621 × IVIA-371 (3.97) respectively (Table 4). Likewise, the cross combinations positively significant SCA effects were A0416 × INS2 (7.52) DH 621 × MEL 5 (5.62) and ASI-S-1 × INS2 (5.54) respectively (Table 4). While in order to select the varieties with low PPO activity, DB, and CD the negative effects (SCA) are desirable direction for selection they were identified MM 1597 × INS2 (-2.81), IVIA-371 × INS2 (-1.81) and A0416 × INS2 (-1.70) respectively (Table 4). Whereas for DB and CD the crosses identified with highly significant negative SCA effects were IVIA-371 × INS2 (-4.76, -6.82), DH 621 × INS2 (-3.13, -5.16) and ASI-S-1 × INS2 (-2.78, -2.60) respectively (Table 4).

Table 3. General combining ability (GCA) estimates of the parents (n=10).

Descriptors[a]	MM 1597	DH 621	AN-S-26	HI5	A0416	IVIA-371	ASI-S-1	MEL 1	MEL 5	INS2
Dry Matter	-0.26^{ns}	-0.40 ^{ns}	0.37 ^{ns}	0.88***	-0.26 ^{ns}	-0.49*	0.01^{ns}	-0.87***	-0.27^{ns}	1.29***
Phenolics	0.69 ^{ns}	-1.10*	-0.81 ^{ns}	0.55 ^{ns}	-2.23***	1.07*	1.05*	-0.60^{ns}	-1.00*	2.37***
CGA	-0.02^{ns}	-0.02^{ns}	-0.06^{ns}	-0.02^{ns}	-0.03^{ns}	-0.02^{ns}	-0.28**	0.03^{ns}	0.04^{ns}	0.39***
Area %	-8.60***	1.23 ^{ns}	-1.11 ^{ns}	1.55 ^{ns}	1.98*	3.59*	1.64^{ns}	3.60***	0.62^{ns}	-4.48***
L^*_0	-2.06***	-0.92*	0.62 ^{ns}	-0.33 ^{ns}	-0.42 ^{ns}	1.10*	0.42^{ns}	1.73***	3.00***	-3.14***
a^*_0	-0.83***	-0.91***	-0.43 ^{ns}	-0.78***	1.22***	0.78***	-0.11^{ns}	0.65**	-0.10^{ns}	0.51*
b^*_0	3.30***	2.97***	0.17 ^{ns}	1.78***	-0.82*	-2.65***	1.56***	-2.89***	-3.15***	-0.27^{ns}
DW_0	3.86***	2.76***	-0.33 ^{ns}	1.50**	-0.32	-2.69***	0.76^{ns}	-3.31***	-4.35***	2.13***
PPO	-0.23 ^{ns}	-0.32*	0.35*	0.01 ^{ns}	0.47**	-0.50**	-0.44**	-0.22^{ns}	-0.42*	1.30***
DB	-0.40 ^{ns}	-0.18 ^{ns}	-0.57*	0.18 ^{ns}	0.38 ^{ns}	-0.61*	-0.74*	-0.46^{ns}	-0.85**	3.25***
CD	-0.50 ^{ns}	0.56 ^{ns}	-0.66 ^{ns}	0.75*	0.33	-1.16**	-1.00*	-1.18**	-1.50***	4.35***

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively.

Table 4. Specific combining ability (SCA) estimates of the hybrids (n=45).

Hybrids[a]	Dry Matter	Phenolics	CGA	Area %	L^*_0	a^*_0	b^*_0	DW_0	PP0	DB	CD
MM 1597 × DH 621	-1.41*	-0.38 ns	0.47ns	2.11 ns	-0.84ns	0.02ns	1.17ns	1.40 ns	1.31**	-1.20 ns	-1.75 ns
MM 1597 × AN-S-26	-2.14***	1.05 ns	0.36ns	7.89**	-0.22ns	0.90ns	2.51*	1.83 ns	-0.15ns	0.50 ns	2.30*
MM 1597 × H15	2.47***	-1.18 ns	-0.58ns	-5.56 ns	0.90ns	-0.42ns	0.70ns	0.02 ns	0.90ns	-0.41 ns	-0.56 ns
MM 1597 × A0416	0.76ns	0.85 ns	-0.23ns	5.17 ns	2.22ns	-1.65*	-3.66***	-3.93**	-0.40ns	0.12 ns	-0.20 ns
MM 1597 × IVIA-371	0.18ns	1.12 ns	0.06ns	3.11 ns	1.51ns	-1.16ns	-2.07ns	-2.30 ns	-0.50ns	-1.28 ns	-1.38 ns
MM 1597 × ASI-S-1	-0.55ns	-2.15 ns	-0.04ns	-3.61 ns	1.14ns	0.50ns	-1.14ns	-1.73 ns	-0.20ns	-1.67 ns	-2.61*
MM 1597×MEL 1	-1.04ns	-0.74 ns	-0.11ns	8.44**	2.54*	-0.60ns	-0.42ns	-2.26 ns	-0.04ns	-1.44 ns	-2.38*
MM 1597 × MEL 5	-1.66*	2.48 ns	-0.08ns	3.35 ns	1.53ns	-0.16ns	-1.90ns	-2.52 ns	-0.65ns	-0.85 ns	-2.12 ns
MM 1597 × INS2	0.72ns	2.68 ns	-0.81**	5.07 ns	1.37ns	-0.90ns	-1.17ns	-2.00 ns	-2.81***	-1.05 ns	-1.40 ns
DH 621 × AN-S-26	-1.52*	0.04 ns	0.02ns	-1.80 ns	2.20ns	-1.14ns	-0.43ns	-1.83 ns	0.95*	-1.20 ns	-1.57 ns
DH 621 × H15	0.58ns	-2.43 ns	-0.21ns	-3.81 ns	0.51ns	0.56ns	0.70ns	-0.02 ns	0.17ns	0.42 ns	0.60 ns
DH 621 × A0416	-1.83**	-1.11 ns	-0.42 ns	-7.14**	-3.53**	3.30***	3.97***	5.14***	0.60ns	1.66 ns	2.58*
DH 621 × IVIA-371	2.28***	-1.97 ns	0.02ns	-2.21 ns	0.34ns	-0.26ns	1.51ns	0.77 ns	1.33**	0.60 ns	1.43 ns
DH 621 × ASI-S-1	-0.51ns	-2.48 ns	0.13ns	11.36***	-0.21ns	-1.00ns	-2.02ns	-1.20 ns	-0.90 ns	0.85 ns	0.54 ns
DH 621 × MEL 1	-1.16ns	3.37*	1.09***	5.43 ns	1.46ns	-0.80ns	-0.97ns	-1.80 ns	-0.37 ns	0.27 ns	-0.53 ns

DH 621 × MEL 5	1.07^{ns}	-3.17*	-0.43^{ns}	-13.99***	-3.50^{ns}	-0.28^{ns}	7.42***	7.52***	0.30 ^{ns}	3.52***	6.26***
DH 621 × INS2	-1.39^{ns}	0.90 ^{ns}	-0.36 ^{ns}	7.16*	3.40**	-1.08^{ns}	-2.88***	-4.52***	-0.56 ^{ns}	-3.13***	-5.16***
AN-S-26 × H15	1.26^{ns}	0.08 ^{ns}	0.24^{ns}	0.03 ^{ns}	4.30***	-0.15^{ns}	-2.10^{ns}	-4.29**	0.65 ^{ns}	0.14 ^{ns}	0.02 ^{ns}
AN-S-26 × A0416	1.93***	-3.90**	0.22^{ns}	0.60 ^{ns}	-1.35^{ns}	1.00^{ns}	1.12^{ns}	1.75 ^{ns}	-0.42 ^{ns}	-1.27 ^{ns}	-1.68 ^{ns}
AN-S-26 × IVIA-371	0.94^{ns}	-1.13 ^{ns}	-0.92***	-7.23*	1.26^{ns}	0.07^{ns}	-0.84^{ns}	-1.50 ^{ns}	1.10*	-0.82 ^{ns}	-0.75 ^{ns}
AN-S-26 × ASI-S-1	1.01^{ns}	4.90***	0.56*	3.14 ^{ns}	-3.64**	2.31***	2.10^{ns}	3.81*	0.20 ^{ns}	-0.05 ^{ns}	-0.84 ^{ns}
AN-S-26 × MEL 1	1.70*	-0.82 ^{ns}	0.17^{ns}	-3.77 ^{ns}	1.54^{ns}	-1.82**	0.00^{ns}	-0.98 ^{ns}	-0.53 ^{ns}	1.71*	3.58***
AN-S-26 × MEL 5	-1.05	0.80 ^{ns}	0.44^{ns}	1.35 ^{ns}	-0.05^{ns}	-0.84^{ns}	0.27^{ns}	0.18 ^{ns}	-0.92 ^{ns}	-0.40 ^{ns}	-0.70 ^{ns}
AN-S-26 × INS2	-1.62*	-2.76 ^{ns}	-0.22^{ns}	-0.70 ^{ns}	2.54*	-1.37*	3.08***	0.37 ^{ns}	0.52 ^{ns}	-1.60 ^{ns}	-2.20 ^{ns}
H15 × A0416	1.06^{ns}	-2.60 ^{ns}	0.02^{ns}	1.54 ^{ns}	0.07^{ns}	-1.58*	1.55^{ns}	1.08 ^{ns}	-0.03 ^{ns}	1.95*	3.03**
H15 × IVIA-371	-0.79^{ns}	5.97***	-0.55^{ns}	-9.62***	-0.51^{ns}	0.57^{ns}	-0.02^{ns}	0.23 ^{ns}	-0.96*	-0.66 ^{ns}	-0.91 ^{ns}
H15 × ASI-S-1	-1.08^{ns}	2.73 ^{ns}	0.11 ^{ns}	-4.72 ^{ns}	-0.86^{ns}	-0.43^{ns}	0.25^{ns}	0.70 ^{ns}	0.10 ^{ns}	-0.35 ^{ns}	-0.82 ^{ns}
H15 × MEL 1	-1.50*	1.80 ^{ns}	0.48^{ns}	-0.85 ^{ns}	-0.26^{ns}	0.66^{ns}	3.27**	2.56 ^{ns}	-0.02 ^{ns}	-1.55 ^{ns}	-0.81 ^{ns}
H15 × MEL 5	-0.86^{ns}	1.28 ^{ns}	0.20^{ns}	0.15 ^{ns}	-0.96^{ns}	-0.31^{ns}	-1.28^{ns}	-0.12 ^{ns}	-0.07 ^{ns}	-1.70*	-2.58*
H15 × INS2	1.12^{ns}	-3.66**	-0.46^{ns}	-5.44 ^{ns}	-1.61^{ns}	-0.70^{ns}	-1.44^{ns}	0.20 ^{ns}	0.76 ^{ns}	9.56***	10.28***
A0416 × IVIA-371	1.87**	-0.82 ^{ns}	0.55^{ns}	8.61**	-1.33^{ns}	-0.20^{ns}	-3.37***	-0.83 ^{ns}	-1.01*	-0.70 ^{ns}	-0.90 ^{ns}
A0416 × ASI-S-1	-0.66^{ns}	-2.26 ^{ns}	0.27^{ns}	-2.15 ^{ns}	0.20^{ns}	-0.50^{ns}	2.78*	1.79 ^{ns}	0.50 ^{ns}	-0.85 ^{ns}	-0.57 ^{ns}

A0416 × MEL 1	-0.81^{ns}	0.73 ^{ns}	0.07^{ns}	-3.38 ^{ns}	-0.08^{ns}	-2.18***	1.51 ^{ns}	1.41 ^{ns}	0.13 ^{ns}	0.32 ^{ns}	0.70 ^{ns}
A0416 × MEL 5	0.21^{ns}	-1.46 ^{ns}	-0.23 ^{ns}	3.11 ^{ns}	-2.97*	-0.66^{ns}	4.82***	5.54***	0.21 ^{ns}	0.11 ^{ns}	1.00 ^{ns}
A0416 × INS2	-1.82**	-2.35 ^{ns}	-0.17^{ns}	-1.46 ^{ns}	-4.64***	1.38*	2.41*	5.08***	-1.70***	0.26 ^{ns}	-0.42 ^{ns}
IVIA-371 × ASI-S-1	-0.77^{ns}	1.86 ^{ns}	-0.25^{ns}	-4.98 ^{ns}	-0.52^{ns}	-0.32^{ns}	-2.87*	-1.27 ^{ns}	0.94 ^{ns}	-0.27 ^{ns}	-0.44 ^{ns}
IVIA-371 × MEL 1	-1.76*	-4.61***	-0.03^{ns}	-6.58*	-1.94^{ns}	-0.03^{ns}	3.96***	4.15**	0.92 ^{ns}	-2.03*	-1.28 ^{ns}
IVIA-371 × MEL 5	2.64***	3.54*	0.55^{ns}	12.25***	3.00*	1.25^{ns}	-5.16***	-5.86***	-1.13*	0.22 ^{ns}	-0.57 ^{ns}
IVIA-371 × INS2	0.47^{ns}	2.80 ^{ns}	0.51^{ns}	13.44***	4.50***	-0.20^{ns}	-4.34***	-6.12***	-1.81***	-4.76***	-6.82***
ASI-S-1 × MEL 1	1.12^{ns}	1.83 ^{ns}	-0.79**	7.80**	0.68^{ns}	0.17^{ns}	-2.20 ^{ns}	-2.05 ^{ns}	-0.92 ^{ns}	-0.64 ^{ns}	-0.98 ^{ns}
ASI-S-1 × MEL 5	-0.28^{ns}	2.27 ^{ns}	0.09^{ns}	2.13 ^{ns}	0.11^{ns}	-0.48^{ns}	-1.30 ^{ns}	-0.87 ^{ns}	-0.95*	3.13***	3.08**
ASI-S-1 × INS2	-1.25^{ns}	0.71 ^{ns}	0.31^{ns}	-3.70 ^{ns}	-4.48***	1.05^{ns}	3.60***	5.62***	-1.27**	-2.78***	-2.60*
MEL 1 × MEL 5	-1.08^{ns}	-3.56*	-0.22^{ns}	3.14 ^{ns}	-2.97*	0.37^{ns}	1.70^{ns}	3.20*	0.70 ^{ns}	0.08 ^{ns}	0.77 ^{ns}
MEL 1 × INS2	0.78^{ns}	-1.02 ^{ns}	-0.05^{ns}	-3.40 ^{ns}	1.10^{ns}	0.74^{ns}	-0.66^{ns}	-1.28 ^{ns}	1.16*	5.93***	5.45***
MEL 5 × INS2	-0.98^{ns}	2.16 ^{ns}	-0.10^{ns}	4.94 ^{ns}	2.65*	0.55^{ns}	-1.20 ^{ns}	-2.92 ^{ns}	-1.00*	-1.22 ^{ns}	-2.30*

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively

3.3.Heterosis

Highly significant heterosis was measured for all the characters studied (Figure 1). The lowest fluctuation for the heterosis range was noticed for the L^*_0 (6.97) while the highest fluctuation was present for the a^*_0 (211.28) (Figure 1). The highly significant positive heterosis measured for the Dry Mater, Phenolics CGA, and Area was 43.30, 79.48, 50.77 and 38.47 respectively. Whereas, the desired highly significant negative heterosis was noticed for PPO (91.67), DB (-63.70) and CD (-80.66) respectively (Figure 1).

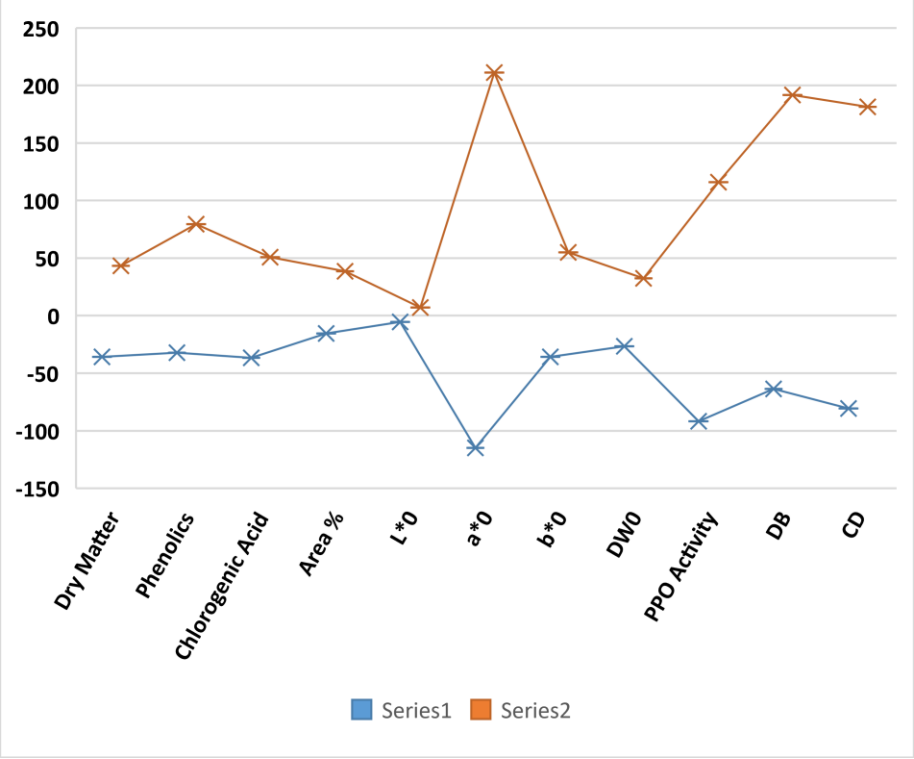

Figure 1. The range and average value of mid-parent heterosis for the biochemical traits studied. Where the corresponding series 1 (blue) represent the lowest value and series 2 (red) represent the highest value for the specific trait.

3.4.Correlations

Twenty-one out of total fifty-five correlations were significant at $p<0.05$. Three of these correlations presented high absolute values (~0.90); two of these were positive correlations (between DB and CD, and between b^*_0 and DW_0) while the other one was negative (between L^*_0 and DW_0) (Table 5). Dry Matter was positively correlated with DB and CD (Table 5). Total phenolics were negatively correlated with the PPO activity. GCA was found to be correlated with a^*_0. However, when considering the area percentage of chlorogenic acid chromatogram it is found to be positively correlated to L^*_0 and negatively correlated to b^*_0, DW_0, PPO activity and CD (Table 5). Moderately positive correlation of PPO activity was noticed with DB and CD (Table 5).

Table 5. Pearson linear correlations for all the 13 biochemical traits studied.

	Phenolics	Chlorogenic Acid (mg/g)	Area %	L^*_0	a^*_0	b^*_0	DW_0	PPO Activity	DB	CD
Dry Matter	0.17^{ns}	0.16^{ns}	-0.15^{ns}	-0.22^{ns}	-0.05^{ns}	0.03^{ns}	0.13^{ns}	0.26*	0.39***	0.44***
Phenolics		0.24^{ns}	0.00^{ns}	-0.12^{ns}	0.02^{ns}	-0.09^{ns}	0.01^{ns}	-0.12^{ns}	0.04^{ns}	0.01^{ns}
Chlorogenic Acid (mg/g)			-0.39***	-0.25^{ns}	0.25^{ns}	-0.02^{ns}	0.11^{ns}	0.40^{ns}	0.10^{ns}	0.18^{ns}
Area %				0.52***	0.12^{ns}	-0.49***	-0.56***	-0.47***	-0.30*	-0.36**
L^*_0					-0.12^{ns}	-0.64***	-0.89***	-0.34**	-0.47***	-0.58***
a^*_0						-0.36**	-0.17^{ns}	0.13^{ns}	0.14^{ns}	0.06^{ns}
b^*_0							0.92***	0.13^{ns}	0.07^{ns}	0.26^{ns}
DW_0								0.25^{ns}	0.28*	0.45***
PPO Activity									0.43^{ns}	0.50^{ns}
DB										0.96***

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively

Genetic distances and correlation with hybrid performance and genetic parameters

Among the cultivated accessions maximum genetic distance was noticed between the A0416 and MEL1 (Table 6). Whereas, genotype DH621 was determined to be very similar to genotypes AN-S-26, H15, and IVIA-371. For all the 45 hybrids the genetic distance was found to be significant for 4 traits out of total 10. The traits found significantly correlated with the genetic distance were a^*_0, b^*_0 and CD (Table 6). Interestingly, for the heterosis and SCA effects only PPO activity was found to be negatively correlated with the genetic distance (Table 6). When excluding the hybrids with *S. insanum* the significant r values were determined for all of the four flesh colour related parameters L^*_0, a^*_0, b^*_0, and DW_0Table 7). Whereas, trait heterosis was not significantly correlated with the genetic distance for any of the trait. Interesting, the SCA effects were found correlated with the genetic distance for L^*_0 (Table 6)

Table 6. Correlations between genetic distances among parents and hybrid trait values, heterosis (Het), and specific combining ability (SCA).

	All parents			Only *S. melongena* parents		
Traits	Trait[a]	*Het*[a]	SCA[a]	Trait[a]	*Het*[a]	SCA[a]
Dry Matter	0.125[ns]	-0.176[ns]	-0.143[ns]	-0.125[ns]	-0.043[ns]	-0.120[ns]
Phenolics	0.186[ns]	0.143[ns]	-0.048[ns]	-0.093[ns]	0.235[ns]	-0.068[ns]
Chlorogenic Acid	0.181[ns]	-0.137[ns]	-0.112[ns]	-0.003[ns]	0.100[ns]	-0.013[ns]
Area %	0.001[ns]	0.147[ns]	0.203[ns]	0.224[ns]	0.007[ns]	0.251[ns]
L^*_0	-0.075[ns]	0.050[ns]	-0.120[ns]	0.371*	-0.171[ns]	-0.366*
a^*_0	0.388**	-0.113[ns]	-0.026[ns]	0.404*	0.039[ns]	-0.115[ns]
b^*_0	-0.359*	-0.171[ns]	0.043[ns]	-0.433**	-0.030[ns]	0.167[ns]
DW_0	-0.203[ns]	-0.138[ns]	0.085[ns]	-0.446**	0.080[ns]	0.286[ns]
PPO Activity	0.147[ns]	-0.421**	-0.337*	-0.139[ns]	-0.327[ns]	-0.324[ns]
DB	0.443**	0.212[ns]	0.129[ns]	0.073[ns]	0.213[ns]	0.113[ns]
CD	0.336*	0.047[ns]	0.045[ns]	-0.136[ns]	0.193[ns]	0.105[ns]

*** ,** ,* indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively.

Discussion

Eggplant is among the vegetables with highest contents in phenolic compounds [31]. However, the fact that oxidation of phenolic acids produces brown compounds may be an impediment for the development of commercially successful eggplant varieties [12]. However, the knowledge of association among the descriptors is helpful for efficient breeding. Generally, the identification of suitable donor parent, evaluating the genetic variation and diversity is important for successful breeding [32–34].

Generally, in case of self-pollinated crops like eggplant, the alleles are largely fixed and genetic variation is limited among the popularly cultivated varieties [35,36]. Under such circumstances, the underexploited variability present in the different genepools in the farm of landraces and crop wild relatives is highly useful which can be donate useful genes for the improvement of the cultivated varieties [6,31]. In our study, we have used the 9 accessions differening in shape and sizes alongwith one accession of *S. insanum*. Overall, the mean sum of squares due to GCA were higher than otherwise due to SCA this generally favours selection breeding methods. Previously, the selection breeding methods were extensively used for the improvement of biochemical traits [37,38].

The diallel mating design excluding reciprocals is a powerful and manageable design to have a better understanding of combining abilities and gene actions of the genes governing the important traits of eggplant

[23,39]. This information on combining abilities and gene actions are of interest to breeders in order to devise a proper breeding strategy by involving suitable parents [40]. Here we have found that the only wild accessions used i.e. INS2 was having highly significant GCA effects for the traits except for the fruit colour related. Moreover, INS2 was even positively significant for the flesh browning related traits in which the direction of acceptability and selection was negative. INS2 was determined to be highly significant for the total phenolics and CGA content. *S. insanum* has an immense potential to contribute several favourable genes to modern day eggplant cultivars[41].

However, in the past wild relatives has contributed in the improvement of several traits in other solanaceous vegetable like tomato and potato[42]. Also, recently we have found that the wild relatives are sometimes three times higher in value for the important total phenolics and GCA content [8,27]. The significant SCA effects were scattered among the several cross combinations. For phenolics, the significant SCA effects were recorded in the crosses AN-S-26 × ASI-S-1, and DH 621 × MEL 1. Surprisingly, significant positive SCA effects for CGA were recorded for the different cross combinations H15 × IVIA-371 and IVIA-371 × INS2. This points out the presence of several kind of phenolic acids in eggplant flesh that might also express more with diverse crosses using wild relatives [7]. Interestingly, phenolics and chlorogenic acid content were not correlated with each other and also were not correlated with any other trait studied i.e., with DW_0, PPO Activity and DB. However, the area percentage of GCA was negatively correlated with all browning and colour related traits (except L^*_0). These results are in agreement with our previous

results. Earlier it was also shown that higher phenolics are not associated with the fruit browning [6].

Crossing a line into different cross combination gives the information about that line in all its cross combinations. The cross with its specific value is a result of sum of GCA of two lines is used in that particular cross combination. The SCA estimates are useful for finding the specific cross combinations that in the farm of heterosis for the highest expression of a trait. However, the preferred parents are those in which one parent is with high GCA while the overall cross combination is with high SCA value. Additive gene action for that traits demonstrates that it is better to use it and perform an efficient selection. This information on the quantitative genetics of eggplant can be used to inference decisions on parental choice for breeding for various morphological traits. Therefore, the present studies were carried out to understand the nature of gene action governing the inheritance of important morphological traits of eggplant as well as to know the combining abilities of parental and their hybrids, respectively, to develop a deep understanding and to correlate this information with their genetic distance obtained by using SNPs.

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Chapter 6: Standardisation of an Agroinfiltration Protocol for Eggplant Fruits and Proving its Usefulness by Over-expressing the SmHQT Gene

Introduction

Phenolic acids are among the most common phenolic compounds produced by different plant species [1,2]. Eggplant (*Solanum melongena* L.) is a member of family Solanaceae and contains high concentrations of phenolic acids, which are beneficial for human health and development. Phenolics of eggplant flesh have proved effective for the protection against several diseases like diabetes, cancer and arthritis [3–5]. Increasing the content of these phenolic compounds especially chlorogenic acid is among the major breeding objectives for eggplant. In the eggplant flesh, the chlorogenic acid forms an ester as 5-caffeoylquinic acid and it makes up to 90 percent of total phenolics found in the eggplant flesh. [6]. Moreover, it also forms more and lesser found esters such as 3-caffeoylquinic acid and 4-caffeoylquinic acid [7,8]. Increases in the concentrations of these phenolic compounds take place in response to environmental stress and under insect pest and pathogen infestations [9,10].

Nevertheless, cultivated eggplant has far less phenolic acids than its several wild relatives [3]. Therefore, several breeding approaches were undertaken in the past and are still ongoing to improve the phenolic acid content of cultivated eggplant e.g. introgression breeding and backcrossing [11]. Unfortunately, all these techniques can not completely get rid of unwanted genes such as those are associated with the wild species, e.g. prickles, bitterness, alkaloids, etc [12]. These genes are not uncommon to the wild relatives and landraces, which use these defence mechanisms against predators and diseases [13]. Therefore, the use of highly precise genome editing approaches and the use of transgenics technology cannot be overlooked, as they restrict the introduction of external DNA to a specific gene sequence or fragment of interest. With transgenic methods, only the specific sequence will be delivered in the genome so that it precisely expresses only the phenotypes associated with that gene [14–16].

Transgenic plants are routinely used to understand the molecular genetic function of a gene [17]. The plant transformation method based on *Agrobacterium tumefaciens* is amongst the most popular techniques of plant transformations and is widely applied to several commercially important crop plants, like maize and tomatoes [18,19]. Even with lots of new techniques adding, agrobacterium-based methods are more popular than any other technique used for the transgene delivery in plants. This is because this method is comparatively cheap and has a high transformation efficiency [20]. Further, agroinfiltration is a method of injecting agrobacterium with a gene of interest along with a T-DNA vector in the plant tissues, which allows the transient expression of a gene for the various purposes. Compared to large time investment in stable

transformation, agroinfiltration provides a rapid assay and can further help to elucidate its functions via studies on its function or involvement in the different pathways [21].

In plants there is an immense potential to mass produce recombinant proteins (e.g. enzymes) via their different organs, like leaves, roots, fruits, etc. [22,23]. Plants provide a low-cost system for the protein production because they are easily transformed and moreover the transformed protein can maintain an appropriate structure via post-translational modifications [24]. Also, the plant with a transient protein is ready for the evaluation within a few days' time as compared to the months reserved by the stable transformation method. Also, with stable transformation method, there can even be a high probability of bias because of chromosomal positions and epigenetic mechanisms by the constructs [25]. Overall, agroinfiltration has become a method of popular choice for assigning a gene function. This method is well established in several fruit-bearing plants like tomato, strawberry, melon and cucumber [26–29].

The chlorogenic acid synthesis pathway is known in eggplant and the enzymes are also mapped on eggplant genetic map [30]. The hydroxycinnamoyl CoA-quinate (HQT) is the central enzyme studied to increase the chlorogenic acid content in Solanaceae and in other families. The function of eggplant transferase (SmHQT) enzyme is the esterification of 4-coumaroyl CoA and quinic acid to form CGA, and further to provide

the entry molecule of the flavonoid pathway [31,32]. Furthermore, HQT is well studied in other solanaceous vegetables; in the case of tomato, over-expression of HQT resulted in the overproduction of chlorogenic acid by around two-fold [33]. In contrast, the suppression of HQT gene resulted in the reduction of chlorogenic acid content by 90% [34].

In eggplants, there is no established protocol to follow for the agroinfiltration assays. Therefore, our objectives with this study were to establish and standardise an effective agroinfiltration protocol for the eggplant fruit and thereafter by applying that protocol to the study the expression pattern in the eggplant genome transiently over-expressing of SmHQT gene. In our cassette we also co-expressed the P19 protein of tomato bushy stunt virus, which is well used for the characterisation and overexpression desired protein in the plant tissues as it prevents the post-transcriptional gene silencing (PTGS) of the infiltrated leaves which could result from plant response to the pathogenic, and in consequence the agroinfiltrated tissue can keep on expressing the desired protein product [35,36].

Methodology and Outcomes

1. Development of Eggplant HQT gene construct with specific promoter in a plant transformation vector

Genomic DNA was isolated from eggplant sample and used for amplification of the HQT gene along with its specific native promoter. Primers were designed and synthesized for specific amplification of the SmHQT fragments. The gene was amplified as 2 fragments; fragment-1 contained the promoter region and exon1 while fragment-2 contained the

second exon. Both fragments were combined in cloning protocol. The complete gene was then cloned in a pUC based cloning vector (pBlueScript; at *Hind*III and *Bam*HI restriction sites) and sequence confirmed.

PCR Standardization:

The fragments (FI-593bp and FII-874bp) were PCR amplified separately and assembled to obtain a full-length SmHQT gene (1467bp). The full-length PCR product was then gel purified before being used for the restriction digestion and ligation.

PCR Conditions

Component	**Amount**
Template (gDNA; 50ng)	1.0 µl
Forward Primer (100ng/µl)	2.0 µl
Reverse primer (100ng/µl)	2.0 µl
10X Assay Buffer	5.0 µl
dNTPs (10mM)	2.0 µl
ChromTaq (3U/µl)	0.5 µl
Water	37.5 µl
Total reaction volume	50.0 µl

PCR Cycle condition:

94ºC	94ºC	TaºC*	72ºC	72ºC
5 min	30 sec	40 sec	1min	10 min
	35 cycles			

*TaºC – Fragment I (32% GC): 50ºCTaºC – Fragment II (49% GC): 52ºC

Cloning & Sequencing:

The optimized gene was then cloned in cloning vector (pBlueScript vector) at *Hind*III/*Bam*HI sites. Probable clones were screened by restriction digestion and was further confirmed by sequencing.

Digestion Conditions for PCR product & Vector:

Component	**Amount**
DNA	4 µg
HindIII	36 units
BamHI	36 units
10X Assay buffer	40 µl
Water	X µl
Total reaction volume	400 µl

*Reaction was incubated at 37ºC for 2 hrs.

Component	Amount
Vector	100 ng
Insert	100 ng – 150 ng
Chromous Quick Ligase	1 µl
2X Chromous Quick Ligase assay buffer	10 µl
Water	X µl
Total reaction volume	20 µl

*Reaction was incubated at room temperature for 30 min.

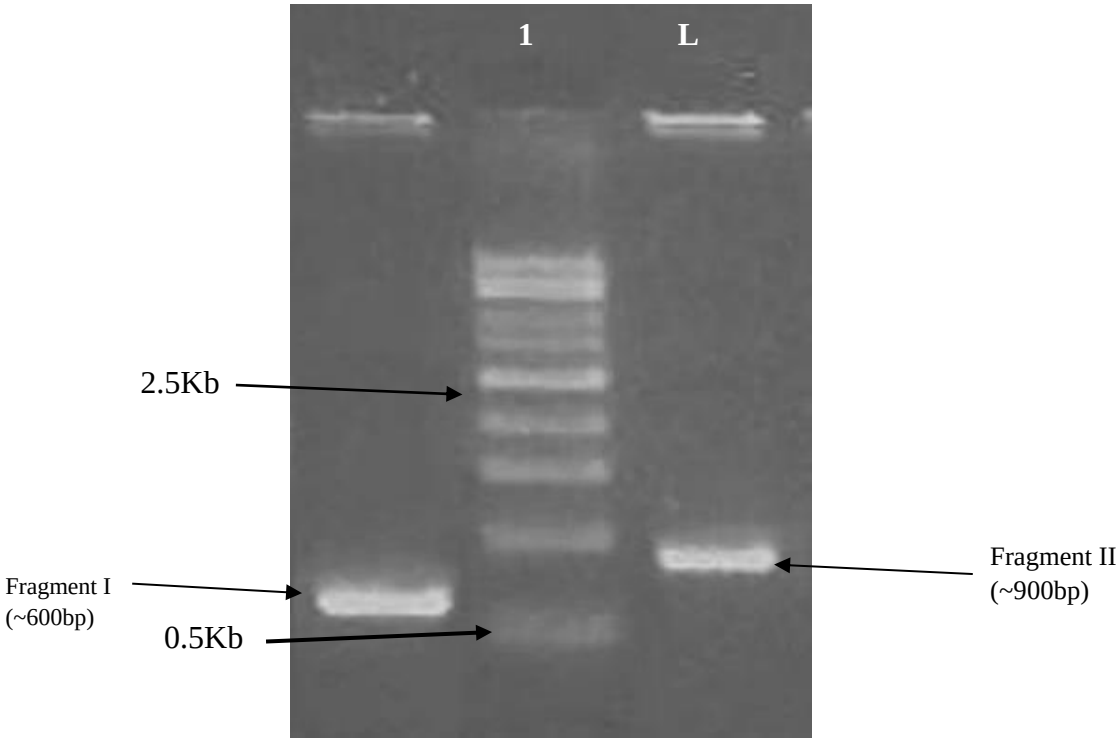

Figure 1: PCR fragment I & II loaded on a 1% Agarose gel

Transformation in competent cells:

The 10 µl of ligation reaction mix was transformed into 100 µl of DH5α competent cells. Incubated at 4ºC (ice) for 30 min and heat shock was given at 42ºC for 45 sec. Quick chilled on ice for 2 min and volume made-up to 1ml with LB broth. Incubated at 37 ºC for 1 hr in shaking incubator for recovery. The cells were then pelleted at 3000 rpm for 3min; Supernatant discarded and cells plated on AXI plate. Plates incubated overnight at 37ºC.

AXI Plate: To 100ml of LB add 100 µl ampicillin (Initial concentration: 100mg/ml), 120 µl X-gal (Initial concentration: 100mg/ml), 24 µl IPTG (Initial concentration: 1M).

Screening for clones:

Probable clones were screened by restriction digestion and checked for release of the gene. Positive clones were sequence confirmed and processed further for Sub-Cloning in expression vector (pBIN19).

Highlights:

- Yellow Highlight – Restriction enzyme sites
- Blue Highlight – SmHQT gene sequences
- Black Highlight – Vector Backbone

GTTCGGGCAGTGAAGCTTCACACTTTCCTTCCTTGTACCACACACTTT
AGCTCCTCCATCCTCCTTCTTTCTTTTTCTTTTTTCCCCAAGGAAATTA
CATTTTACACATCAAGAAAATTCCAAGAACATCAAGAAAATTATATT
TTCAAACACCCTTTTCTCTCCTTAACCTGTTTTGAAAAAGAAAAGTA
AAAATAATCATGAAAATTAGTATCAAAGAATCAACACTAGTGAAAC
CATCAAAGCCAACACCAACCAAGAGGATTTGGAGTTCTAATTTGGAT
TTAATTGTTGGAAGAATCCATCTTTTGACTGTTTATTTTTATAAACCA
AATGGATCTCCAAATTTCTTTGATAGTAATGTGATGAAAGAAGCATT
AAGCAATGTTTTAGTTTCATTTTATCCAATGGCTGGGAGATTATCTA

GGGATGATCAAGGAAGAATTGAAATAAATTGTAATAATGAAGGTGT
TTTGTTTGTTGAGGCCCAAAGTGATTCATGTGTTGATGATTTTGGTGA
TTTTACACCAAGTTTGGAACTTAGGAAACTTATTCCTAGTGTTCAAA
CTAATGGAGATATCTCAACCTTCCCACTCGTAATATTCCAGGTTACT
CGTTTTAGCTGTGGCGGAGTCGCTCTTGGCGGGGGAGTGTTCCACAC
GTTATCTGATGGTCTTTCATCCATCCACTTTATCAACACGTGGTCGGA
CATCACTCGTGGCCTATCCGTCGCGATCCCACCATTCATCGATCGGA
CCCTCCTTCGTGCACGGGACCCGCCAACGCCTTCTTTTGAGCATGTC
GAGTATCATCCTCCACCTACCCTCAACTCATCGAAAAACCATGAGTC
CACGGGCCCAAAGCCCAATACCACGGCCATGTTGAAATTCTCGACTG
AACAACTCGCGCTCCTTAAGTCCAAGTACGAGGGTAGCACTTATGAA
ATCCTTGCGGCCCACATCTGGCGTTGCGCGTGCAAGGCACGTGGATT
GCCAGACGATCAATTGTCCAAATTACACGTGGCCACTGATGGTAGGT
CGAGGCTTTGCCCTCCCTTGCCACCGGGCTACCTAGGAAATGTCGTG
TTCACGGCTACACCAATGGCTACATCATCCGAGCTTCAATCAGAACC
GTTGTCGAGTTCCGCTAAGAGAATTCACGATGTGTTATTGAAAATGG
ACGATAACTACCTAAGATCAGCTCTCGATTACCTCGAGTTACAGCCT
GACCTATCAACCCTGATTCGGGGGCCGACTTACTTTGCTAGCCCTAA
TCTTAACATAAATAGTTGGACTAGGCTGCCTGTCCATGAGTGTGACT
TTGGGTGGGGTAGGCCAATTCATATGGGACCAGCTTCCATCTTATAT
GAAGGGACAATTTATATTATACCAAGTCCAAATTCCAAGGATAGAA
ACTTGCGTTTGGCTGTTTGTCTAGATGCTGAACACATGCCACTATTCG
AAAAGTATTTGTATGACCTTTGAGGATCCCCGGGTACC
GAGCTCGAATACGTAGATGCTTCCGGC

Sub-Cloning of Sequence Confirmed SmHQT in pBIN19:

The pBS+SmHQT clone was restriction digested (*Hind*III/*Bam*HI); released gene was then cloned into pBIN19 at (*Hind*III/*Bam*HI). Further these probable clones were screened & confirmed by restriction digestion. Briefly the Clone DNA (4 µg of plasmid DNA is required to discharge at least 1 µg of insert) was taken and digested with *Hind*III/*Bam*HI.

Component	Amount
DNA	4 µg
HindIII	36 units
BamHI	36 units
10X Assay buffer	40 µl
Water :	X µl
Total reaction volume	400 µl

The released insert was gel eluted and ligated to pBIN19 vector at *Hind*IIII/BamHI sites. • 10 µl of ligation reaction mix was transformed into 100 µl of DH5α competent cells. Incubated at 4ºC (ice) for 30 min and heat shock given at 42ºC for 45 sec. Quick chilled on ice for 2 min and volume made up to 1ml with LB**.** Incubated at 37ºC for 1 hr in a shaking incubator. The cells were then pelleted at 3000rpm for 3min; Supernatant discarded and cells plated on Kanamycin plate. Plates incubated overnight at 37ºC.

Screening for clones:

- Probable clones were screened by restriction digestion and checked for release of the gene.
- The SmHQT gene was finally sequence confirmed and used further for all agroinfiltration experiments.

2. Development of a p19 construct for using in co-infiltration experiments:

Overlapping PCR-based technique was used for development of p19 construct. The gene was synthetically synthesized following recursive PCR based protocol described elsewhere [37].

The optimized genes were then cloned in cloning vector (pEASY-blunt vector) and amplified; sequence confirmed. The same was sequence-confirmed. The sequence-confirmed gene was sub-cloned in pBIN19 vector and confirmed by sequencing (Figure 2).

Cloning & Sequencing:

The optimized gene was then cloned in cloning vector (pUC57 vector; Addgene) at HindIII/BamHI sites. Probable clones were screened by restriction digestion and further confirmed by sequencing.

Digestion of PCR product & Vector:

Component	Amount
DNA	4 µg
HindIII	36 units
BamHI	36 units
10X Assay buffer	40 µl

Water :	X µl
Total reaction volume	400 µl

Reaction was incubated at 37ºC for 2 hrs.

Ligation with vector:

Component	Amount
Vector	100 ng
Insert	65 ng – 70 ng
Chromous Quick Ligase	1 µl
2X Chromous Quick Ligase assay buffer	10 µl
Water	X µl
Total reaction volume	20 l

Reaction was incubated at room temperature for 30 min.

Transformation in competent cells:

- 10 µl of ligation reaction mix was transformed into 100 µl of DH5α competent cells.
- Incubated at 4ºC (ice) for 30 min and heat shock given at 42ºCfor 45 sec.
- Quick chilled on ice for 2 min and volume made-up to 1ml with LB broth.
- Incubated at 37 ºC for 1 hr in shaking incubator for recovery.
- The cells were then pelleted at 3000rpm for 3min; Supernatant discarded and cells plated on AXI plate.
- Plates incubated overnight at 37ºC.

AXI Plate: To 100ml of LB add 100 µl ampicillin (Initial concentration: 100mg/ml), 120 µl X-gal (Initial concentration: 100mg/ml), 24 µl IPTG (Initial concentration: 1M).

Screening for clones:

- Probable clones were screened by restriction digestion & checked for release of the gene.
- Positive clones were sequence confirmed and processed further for Sub-Cloning in expression vector (pBIN19).

Sequence Data: Gene – p19:

Highlights:

- Yellow Highlight – Restriction enzyme sites
- Blue Highlight – p19 gene sequences
- Black Highlight – Vector Backbone

TCACGACGTTGTAAACGACGGCCAGTGAATTGACGCGTATTGG
GATGGAACAAGCTTAAATTCTCCAGGATTTCTCGACCTAGTTC
GTTTATCTGGTGACTTGCGCTACCGTTGCTTTGCGTAGAGAATT
TCTCTCCATAATTATTATCTTTAGTTGTGGGGTTTGAAGGTTGG
GTCTACCTTTCGGGGGGATAAATTGTAACTTCCAACAAACAAG
CGACATGGAACGAGCTATACAAGGAAACGACGCTAGGGAACA
AGCTAACAGTGAACGTTGGGATGGAGGATCAGGAGGTACCAC
TTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAG
TGGCGGCTACATAACGATGAGACGAATTCGAATCAAGATAAT
CCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGGGAAAGTTGTAT

TTAAGAGATATCTCAGATACGACAGGACGGAAGCTAGCCTGC
ACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTAACTATGC
AGCATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTAT
AGTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGT
CGCGAACTCTTCAGCATCTCTGTGAGATGGCAATTCGGTCTAA
GCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGGAAAGTAA
TGTATCAAGAGGATGCCCTGAAGGTACTGAGACCTTCGAAAA
AGAAAGCGAGTAAGGATCCGTTCCATCCCAATGGCGCGCCGA
GCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT
TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAA
AGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATT
AATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTG
TCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGA
GGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGA
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CAGA

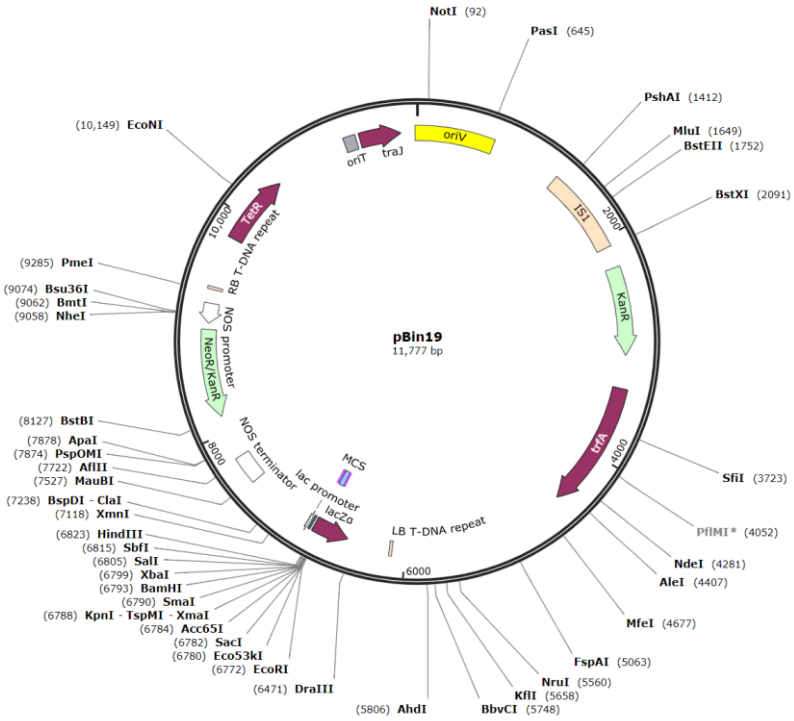

Figure 2 : Vector map pBIN19

Sub-Cloning of Sequence Confirmed p19 in pBIN19:

- The pUC57+p19 clone was restriction digested (HindIII/BamHI); released gene was then cloned into pBIN19 at (HindIII/BamHI).
- Probable clones were screened & confirmed by restriction digestion.

Release of insert from sequence confirmed pBS clone and sub cloning in pBIN19:

- Clone DNA (4 µg of plasmid DNA is required to release at least 1 µg of insert) was taken and digested with HindIII/BamHI

Component	**Amount**
DNA	4 µg
HindIII	36 units
BamHI	36 units
10X Assay buffer	40 µl
Water :	X µl
Total reaction volume	400 µl

- The released insert was gel eluted and ligated to pBIN19 vector at HindIIII/BamHI sites.
- 10 µl of ligation reaction mix was transformed into 100 µl of DH5α competent cells. • Incubated at 4ºC (ice) for 30 min and heat shock given at 42º for 45 sec.
- Quick chilled on ice for 2 min and volume made up to 1ml with LB**.**
- Incubated at 37 ºC for 1 hr in a shaking incubator.
- The cells were then pelleted at 3000rpm for 3min; Supernatant discarded and cells plated on Kanamycin plate.
- Plates incubated overnight at 37 ºC.

Screening for clones: • Probable clones were screened by restriction digestion & checked for release of the gene.

- The p19 gene was finally sequence confirmed and used further for all Agroinfiltration experiments.

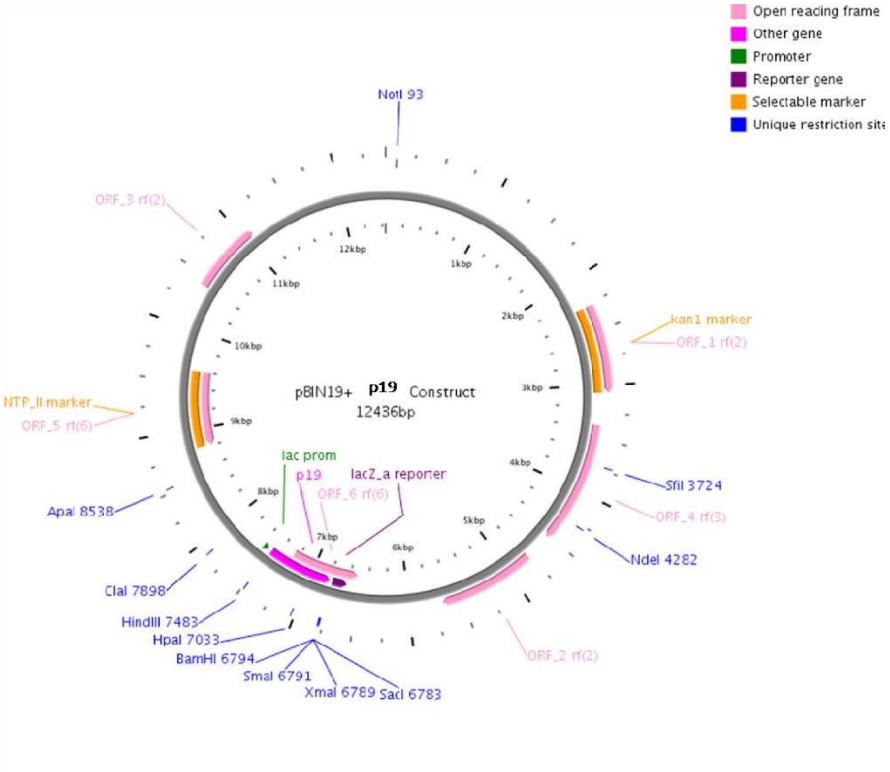

Figure 3: Representation of vector pBIN19 with p19 (protein of tomato bushy stunt virus).

3. Use of a control construct (GUS bearing) & Agroinfiltration in Eggplant fruit

Preparation of Electro-Competent Cells & Transformation:

Agrobacterium tumefaciens GV3101 was used for all further studies. The strain is cultured on LB broth/LB agar in presence of Rifampicin (20 mg/ml stock) and Gentamycin (50 mg/ml stock), at 28ºC for 24-48Hrs. Single colony was picked from an LB-agar plate and 5ml starter culture is prepared by growing in at 28ºC for 24-48 h, in a shaker incubator. 100ml LB was inoculated with 1% inoculums of overnight grown agrobacterium culture (in presence of antibiotics) and grown to an O.D of 0.7. The culture was spun down at 4ºC for 10 minutes at 5000 rpm. The pellet was re-suspended in 100ml ice-cold sterile distilled water and spun at 4ºC for 10 minutes at 5000 rpm. The pellet was re-suspended in 50ml ice-cold sterile distilled water and spun at 4ºC for 10 minutes at 5000 rpm. The pellet was re-suspended in 10ml ice-cold sterile distilled water and spun at 4ºC for 10 minutes at 5000 rpm. The pellet was re-suspended in 1ml ice cold sterile glycerol and 50 µl aliquots were made. To the 50µl cells, 1µg of purified plasmid DNA was added on ice and the mixture was transferred to a pre-chilled electroporation cuvette.

Electroporation was carried out using a Bio-Rad electroporator, with the conditions of capacitance:25µF, voltage: 2.4kV, resistance:200 Ohm and pulse length of 5 msec. Immediately after electroporation,1ml sterile LB was added and the culture was incubated for 4 hours at 28ºC with gentle agitation. The cells were pelleted at 3000 rpm for 5 mins and plated on selective LB-agar plates. The LB agar plates were made with Rifampicin; Gentamycin and Kanamycin. The plates were incubated at 28ºC for 3-4days.

Observation:

- The plates showed mixed colonies indicating contamination and hence competent cells were prepared further using the freeze-thaw protocol.

Preparation of Freeze/Thaw Competent Cells & Transformation:

Agrobacterium tumefaciens GV3101 was streaked on a LB agar plate in presence of Rifampicin (20mg/ml stock) and Gentamycin (50mg/ml stock), and incubated at 28ºC for 24-48Hrs. Thereafter, a single colony was picked from an LB-agar plate and 5ml starter culture is prepared by growing in at 28ºC for 24-48Hrs, in a shaker incubator. The 100ml of LB was inoculated with 1% of inoculums of overnight grown agrobacterium culture (in presence of antibiotics) and grown to an O.D of 0.7. Further, the culture was spun down with 4ºC for 10 minutes at 5000 rpm. The pellet was re-suspended in 10ml of ice-cold sterile 20mM $CaCl_2$ and spun at 4ºC for 10 minutes at 5000 rpm. The cell pellet was re-suspended in 1ml of ice-cold sterile 20mM $CaCl_2$ and 100µl aliquots were made. Around 1µg plasmid DNA was added to the cells and the cells/DNA mix was frozen on dry ice for 5 minutes. The frozen cells/DNA mix was thawed to room temperature for 5-10 minutes and the mix was transferred to a tube containing 2ml LB medium and incubated with shaking at 28ºC for 2-4 hours. The cells were pelleted at 3000rpm for 5 mins and plated on selective LB-agar plates. The LB agar plates were made with Rifampicin; Gentamycin and Kanamycin. The plates were incubated at 28ºC for 3-4 days.

Observation:

- The plates showed individual colonies and the same were replica plated for future use.
- The colonies were screened by PCR for confirmation and once confirmed for presence of plasmid DNA, were further sub-cultured for use in agroinfiltration experiments.

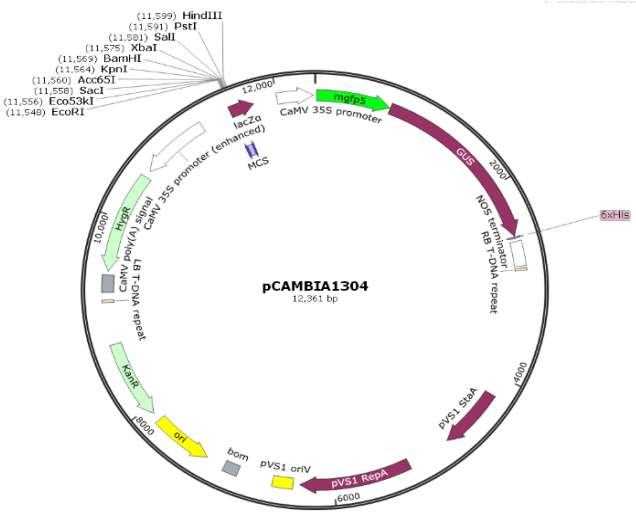

Figure 4: Vector details of pCAMBIA 1304 used for the standardization of agroinfiltration protocols.

Agroinfiltration in Eggplant fruits:

Loop full culture of *Agrobacterium* clone harbouring the recombinant plasmid was inoculated in 5ml LB broth containing the respective antibiotics and grown at 28ºC for overnight. The overnight grown culture was sub-culture in a fresh 5ml LB broth and allowed to grow to an O.D of 1.6 at 600nm. The cells were then pelleted at 6000rpm for 5 mins at room temperature. The cell pellet was re-suspended in infiltration medium (sterile water) to retain the O.D at 1.6. The final *Agrobacterium* suspension was used for agroinfiltration of Eggplant fruits. A 2ml syringe with a needle was used to inject the Eggplant fruits at 10-15 spots and allowed to grow for 3 to 10days after infiltration (DAI). The fruit samples were harvested from 3 days onwards and screened for positive expression of the GUS gene by X-Gluc staining. 1mM X-Gluc was prepared according to standard protocol and the fruit sections were stained for 30mins at 37ºC and visualized under a light microscope (LYZER LT-1610X). The fruits 3 DAI showed best X-Gluc staining, as compared with the 7 DAI and 10 DAI fruits. Also, post 5 DAI the fruits started to show yellowing in the fruit colour. And hence the 3 DAI was finalized for use on the SmHQT gene studies.

3. Agroinfiltration of SmHQT+p19 in Eggplant fruits:

For the agroinfiltration experiment of SmHQT, the pBIN19 clones harbouring the SmHQT gene and p19 gene, respectively were transformed in *Agrobacterium* using the above mentioned freeze/thaw protocol. The A*grobacterium* harbouring the pBIN19+SmHQT and pBIN19+p19 clones, respectively, were screened by colony PCR using vector specific primers. The PCR positive colonies were replica plated and sub-cultured

for further use in agroinfiltration experiments. The agroinfiltration was performed as per the standardized protocol mentioned above and the fruits were harvested at 3 DAI. 3 DAI fruits were harvested and stored in -80ºC for further studies. Total RNA was extracted from 3 DAI agroinfiltrated fruit (SmHQT + p19) and untreated control fruit. The extracted total RNA was used for transcriptome studies.

NOTE:

- The *Agrobacterium* cultures harbouring only the pBIN19+SmHQT and pBIN19+p19 were used at a ratio of 1:1 for all agroinfiltration experiments.

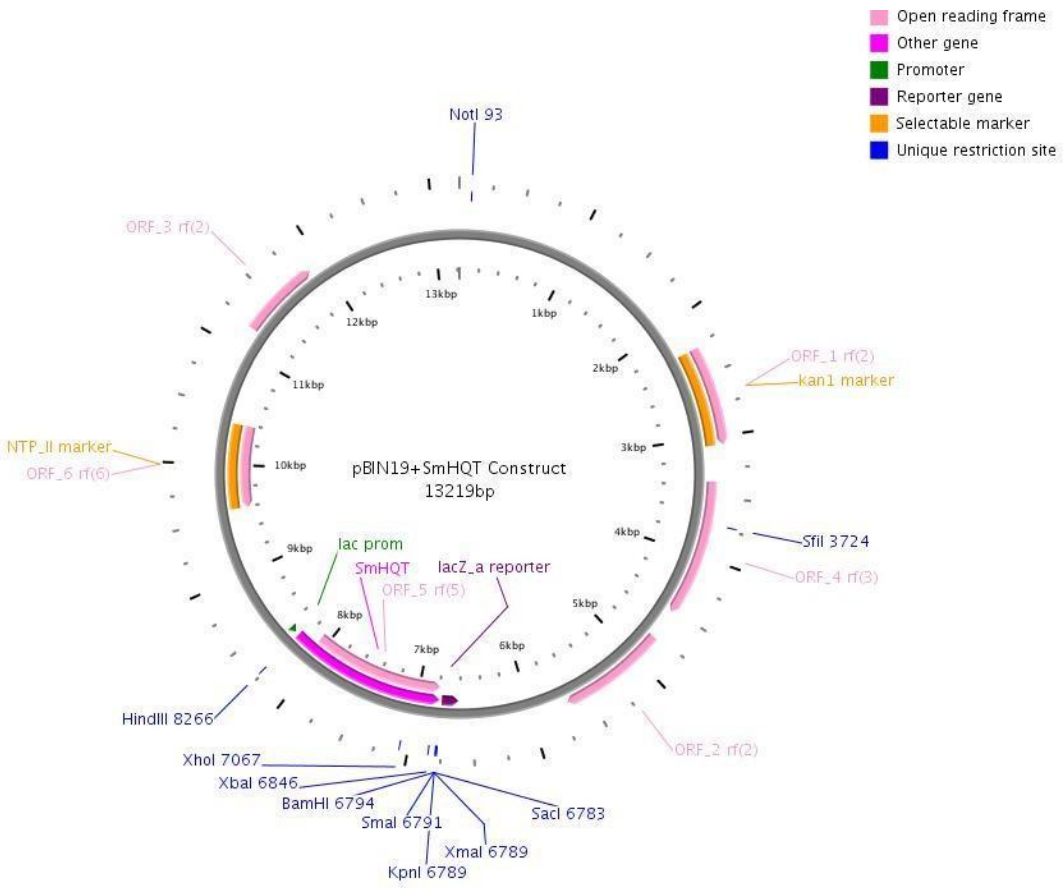

Figure 5: Representation of vector pBIN19+SmHQT Construct.

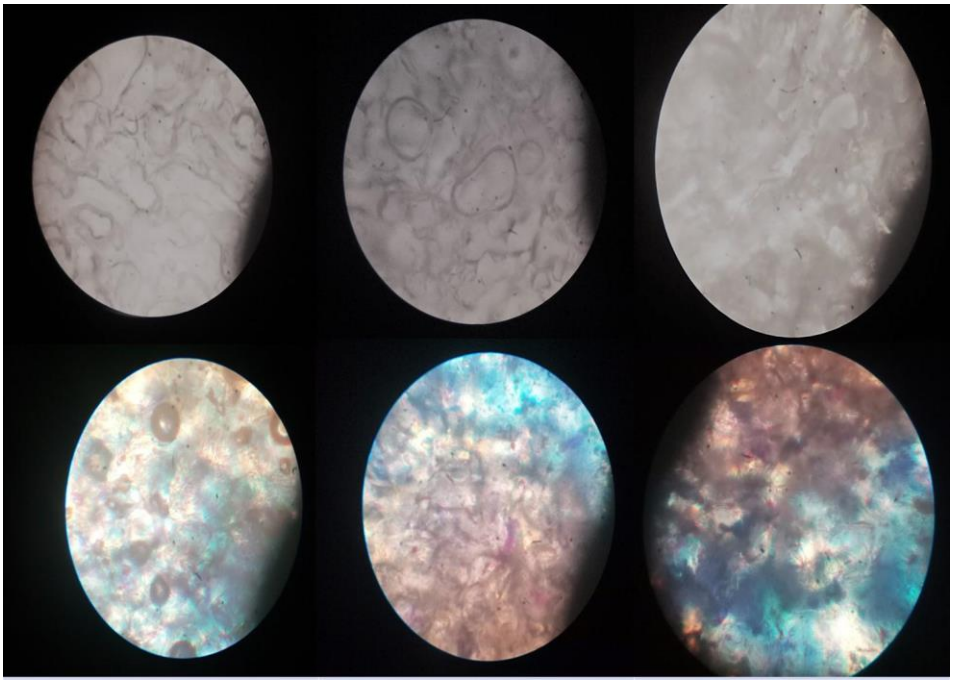

Figure 6: Comparison of control(above) and transgenic (below) fruit slices via X-Gluc staining

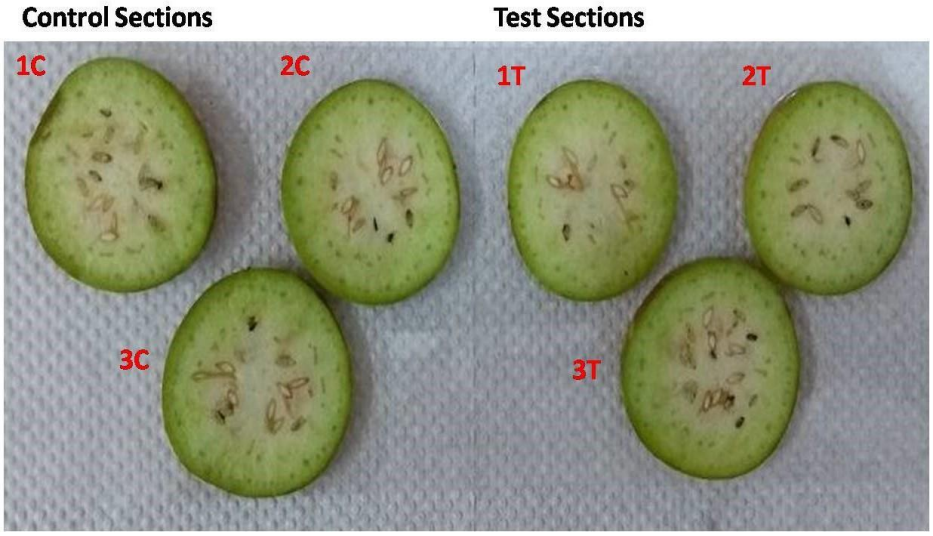

Figure 7: Slices of control(left) and transgenic (right) 3 days after agroinfiltration of fruit.

5. Conclusion

We have shown that eggplant fruits can be successfully agroinfiltrated. Agroinfiltration, method developed here is user friendly and this transient gene expression system developed for the eggplant fruits will be useful in swiftly and precisely identifying the fruit tissue-specific gene functions along with protein production. Further, if this technique is coupled with plant omics tools e.g. with transcriptomics (RNA-seq) it will provide the detailed information of gene activity and with metabolomics it will provide an overall shift in the fruit metabolism because of the insert of a particular gene. Also, this method could apply to other members of family Solanaceae those were never discovered from the genetic transformation viewpoint.

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DISCUSSIONS

Vegetables embrace a number of health benefits for humans because they contain important nutraceutical like phenolic acids[1]. Phenolic acids can be the derivatives of either cinnamic acid and to a lesser extent of benzoic acid [2]. Eggplant is among the top ten vegetables with the high content of these phenolic acids [3]. As the modern consumers are becoming more aware regarding the values of phenolic acid-rich eggplant the need for the development of eggplant varieties with a higher content of plant phenolics has become an important breeding aim for the eggplant breeders [4].

Under-exploited diversity for phenolic acids content has been found among cultivars and wild relatives of eggplant [5]. Conventionally, to improve the phenolic acid content of eggplant a breeding scheme includes the screening of germplasm if available from all possibly genepools, together with morphological characteristics [6]. Thereafter, the crossing of the best genotypes identified because of screening. Whereas, to find out gene action for the traits of interest. Further, modern biotechnological and genomics tools can supplement in the identification of genomic regions and key genes affecting traits [7]. Also, to get rid of the unwanted traits associated with that genomic region or genes of interest [8].

Although eggplant has good intercross ability with its wild relatives. But, not any commercial variety is cultivated with the introgression from the wild relatives [9–11]. Therefore, in our work, we have included the wild relatives of eggplant. Further, the application of

conventional, biotechnological and genomics approaches to eggplant breeding that will facilitate the development of eggplant varieties with enhanced content in phenolic acids.

Morphological screening of eggplant genepools

Use of crop wild relatives in broadening the genetic base of major crops is well known. The wild relatives are the important sources of variation for the traits of interest for the eggplant[10,12]. Unfortunately, eggplant is a member of family Solanaceae there is no commercial variety in our knowledge that has genomic region from wild species of eggplant. One the prime reason could be the insufficient characterisation of wild species for important morphological traits [13]. In our study which is the largest study up to now involving six accessions of cultivated eggplant, 21 accessions of 12 wild species, and 45 interspecific hybrids of cultivated eggplant with seven wild species. In order to morphologically characterize cultivated eggplant, wild relatives and the interspecific hybrids we have used the conventional descriptors, based on EGGNET [14] and IPGRI [15], and Tomato Analyzer traits [16] software program in order to morphometrically test the eggplant fruits[17].

Large differences were found for the traits in the genotypes characterised. The wild species and their interspecific hybrids were highly vigours, with more prickles, also, the number of flowers per inflorescence were much more in wild species and interspecific hybrids. While, for the traits associated with the fruit shape, compared to wild species the cultivated eggplant was more diverse. Further, the interspecific hybrids

were more diverse than the cultivated eggplant for the Tomato Analyzer traits. The interspecific hybrids involving primary genepool species were more close to cultivated eggplant while the interspecific hybrids involving secondary genepool species were like wild species. The wild species, *S. anguivi, S. campylacanthum, S. pyracanthos* and *S. violaceum* could be of interest for improving potency in the cultivated eggplant. In case of fruit weight, the wild species with greater fruit weight identified were *S. insanum*, *S. dasyphyllum* and *S. lichtensteinii*. Also, a rapid recovery of fruit size and weight is possible as early as with the first backcross [18].

In conclusion, the characterization with conventional descriptors and the Tomato Analyzer phenomics tool has allowed a detailed characterisation of eggplant, close wild relatives and their interspecific hybrids. The high variation among wild species allowed identifying sources of variation and most promising species for traits of interest for eggplant breeding. That interspecific hybrids with primary genepool species *S. insanum* are intermediate or close to eggplant for many traits, may facilitate the use of this species in introgression breeding and supports previous evidence that this species is the ancestor of cultivated eggplant. Also, the high vigour of most interspecific hybrids may be directly exploited by using them as rootstocks. The information got here on phenotypic characteristics and heterosis of wild species and interspecific hybrids are of interest for eggplant breeding. Given the adaptation of many wild species to stressful conditions, their utilisation in eggplant breeding

may cause the development of a new generation of cultivars adapted to climate change challenges.

Biochemical characterization

Eggplant is among the top ten vegetables rich in phenolic acids [3] and varieties with enhanced phenolic acid content are desired in eggplant consumers [2,4]. Although, wild relatives have been reported owning several-fold phenolic acids than modern eggplant verities [6[10]]. Therefore, we have used the wild species and their interspecific hybrids to gain in-depth about the composition and fruit flesh colour and browning traits. In eggplant, chlorogenic acid is the predominant phenolic acid in the fruit flesh and the same occurs in the primary genepool species *S. insanum*, which is its wild ancestor [2]. We have measured the chlorogenic acid content in the eggplant using high-performance liquid chromatography (HPLC), as chlorogenic acid is the dominant phenolic acid in the eggplant's fruit flesh. This holds true for the cultivated eggplant and the primary genepools species *S. insanum*. Surprisingly, for the secondary and the tertiary genepool species, the chromatogram peak for the chlorogenic acid was much lower indicating other derivatives of hydrocinnamic acid via different secondary peaks. Therefore, these species can be important donors of the genes for the secondary phenolic acids for the cultivated eggplant. We have observed the higher content of total phenolics in the wild species, further, some wild species have shown several times more content of total fruit phenolics compared to the cultivated genotypes.

We have found that the cultivated eggplant along with the primary genepool species is with white flesh as compared to the secondary and tertiary genepool wild relatives and interspecific hybrids. White flesh verities are desirable for the processing industry. Similarly, the wild relatives of eggplant were with higher PPO activity. The PPO activity might have evolved as a plant defence to herbivores [19]. Similar to the morphological traits the interspecific hybrids involving primary genepool wild relative behaved like cultivated eggplant for the biochemical traits. While the interspecific hybrids with secondary and tertiary genepool species were like the wild species [10]. Amazingly, no significant correlations have been observed between total phenolics content or chlorogenic acid content with any of the fruit flesh colour or browning traits, which suggests that these traits may be independent A white fruit flesh colour is desirable for most eggplant markets [20], and the cultivated eggplant had a much higher luminosity (L*0) and therefore a lower distance to pure white (DW0) than the wild species. Wild species of Solanum crops usually have chlorophylls and carotenoids in the fruit flesh [21], which as with eggplant result in less white flesh. Here, the primary genepool species presented better characteristics, with a fruit flesh colour closer to pure white than those of secondary and tertiary genepool species. Association between target traits is important for breeding [22]. Surprisingly, no significant correlations have been observed between total phenolics content or chlorogenic acid content with any of the fruit flesh colour or browning traits, which suggests that these traits may be

independent. In fact, some interspecific hybrids had a high content in total phenolics and chlorogenic acid content and limited browning.

Line × Tester genetic study in eggplant

Line × Tester genetic study is an important biometrical strategy to study the inheritance of important quantitative traits [23]. Further, the estimation obtained for general combining ability (GCA), specific combining ability (SCA) and their ratio (GCA/SCA), supports in devising a breeding strategy for future improvement of a crop [24]. In our study, the two lines one with oriental and another with occidental cytoplasm were crossed with four testers representing three wild species namely, *S. insanum*, *S.anguivi*, and *S. lichtensteinii*. The Line × Tester cross produced a total of eight interspecific hybrids. The six parents and eight interspecific hybrids were evaluated for 3 biochemical[5], 12 morphological[14] and 8 tomato analyzer based descriptors[16].

The significant amount of variation was noticed for all the 23 traits studied. The higher values for the SCA component were determined as compared to the GCA component. This further leads to the lower value for the GCA/SCA ratio. Further, *S. anguivi* was most significant for the biochemical traits. Similarly, for most of other traits testers were more significant in values than the cultivated lines although both of the lines were having different cytoplasm. The testers were found to be more significant for most of the traits than the cultivated varieties as wild relatives are known as the reserve house of important genes[25]. The positive and negative SCA and their qualities are likewise essential for a

few characters as some should be more positive than negative. The most reduced variance was seen for the plant stature to the greatest for fruit weight. It was revealed that there was sure heterosis for the 12 characteristics and negative heterosis for the 11 attributes. The positive heterosis was determined for all tomato analyzer based descriptors and negative values for most of the biochemical and morphological descriptors. By and large, in our study, the greater part of the characteristics are appeared to be administered by non-added substance quality activities. Prior investigations declared that both additive and non-additive effects control the biochemical traits in eggplant [26].

Diallel analysis of important morphological and biochemical traits in eggplant

The first generation hybrids are usually heterotic and are identified as better performing under sub-optimal conditions[27,28]. Although, this depends on the careful selecting of the donor parent, that usually starts with the careful testing of the parents based on the genetic variation before crossing. The genetic distances were also estimated in among the parents using molecular markers [29]. But, in eggplant, there is only one study involving genetic distance using AFLP markers and correlating it with yield and fruit weight [30]. Crossing a line into different cross combination gives the information about that line in all its cross combinations. The

cross with its specific value results from the sum of GCA of two lines is used in that cross combination. The SCA estimates are useful for finding the specific cross combinations that in the farm of heterosis for the highest expression of a trait. However, the preferred parents are those in which one parent is with high GCA while the overall cross combination is with a high SCA value [24]. Additive gene action for that traits shows that it is better to use it and perform an efficient selection. This information on the quantitative genetics of eggplant can be used to inference decisions on parental choice for breeding for various morphological traits [30]. Therefore, the present studies were carried out to understand the nature of gene action governing the inheritance of important morphological traits of eggplant as well as to know the combining abilities of parental and their hybrids, respectively, to develop a deep understanding and to correlate this information with their genetic distance obtained by using SNPs. In our work, 9 accessions of cultivated eggplant were used along with the accession of the only primary genepool species *S. incanum*. These parental genotypes were crossed in the half-diallel fashion (i.e. excluding reciprocals). This has resulted in a manageable experimental design and detailed information on the magnitude of general and specific combining abilities and trait heritabilities were obtained. The genetic distances among the parents based were also determined based on the SNPs in order to predict the genetic distance based performance of the eggplant hybrids. The parents and their hybrids were evaluated for morphological and biochemical traits. Also, the morphological traits were based on the morphological descriptors, Tomato Analyzer based fruit descriptors and biochemical traits.

In the case of morphological traits highly significant GCA and SCA, values were recorded for all traits, showing significant additive and non-additive effects controlling traits [31]. Traits with a higher relative proportion of SCA were plant height, stem diameter, number of flowers per inflorescence, prickliness, and yield. For fruit size traits in general values revealed a similar effect of GCA and SCA, showing that, as in other studies with eggplant, both additive and non-additive effects are important. Broad-sense heritability (H^2) values were high, indicating that most of the variation observed is genetically determined and that selection among varieties or hybrids will be efficient [32]. In our study for biochemical traits, we have found that the only wild accessions used i.e. INS2 was having highly significant GCA effects for the traits except for the fruit colour related. INS2 was even positively significant for the flesh browning related traits in which the direction of acceptability and selection was negative. INS2 was determined to be highly significant for the total phenolics and CGA content. *S. insanum* has an immense potential to contribute several favourable genes to modern day eggplant cultivars [33]. The significant SCA effects were scattered among the several cross combinations. For phenolics, the significant SCA effects were recorded in the crosses AN-S-26 × ASI-S-1, and DH 621 × MEL 1. Surprisingly, significant positive SCA effects for CGA were recorded for the different cross combinations H15 × IVIA-371 and IVIA-371 × INS2. This points out the presence of several kinds of phenolic acids in eggplant flesh that might also express more with diverse crosses using wild relatives.

Interestingly, phenolics and chlorogenic acid content were not correlated with each other and also were not correlated with any other trait studied i.e., with DW_0, PPO Activity and DB. However, the area percentage of GCA was negatively correlated with all browning and colour related traits (except L^*_0). These results are in agreement with our previous results. Earlier it was also shown that higher phenolics are not associated with the fruit browning [5].

Standardisation of Agroinfiltration procedure for the eggplant

In order to avoid the traditional long breeding cycle and to transfer the gene of interest rapidly without even worrying about the unnecessary genomic region linked to a gene, the genetic engineering methods are of great choice [34]. In planta, if we have to study the gene function of a gene at the specific organ of the plant the agroinfiltration methods are used [35,36]. In eggplant, there is not any standard agroinfiltration protocol to measure the gene expression. Although, eggplant produces a number of fruits that is a system to mass produce a certain protein. The biosynthetic pathway of chlorogenic acid is well known in eggplant [37]. While, the hydroxycinnamoyl CoA-quinate (HQT) is the central enzyme studied to increase the chlorogenic acid content in Solanaceae and in other families. The function of eggplant transferase (SmHQT) enzyme is the esterification of 4-coumaroyl CoA and quinic acid to form CGA, and further to provide the entry molecule of the flavonoid pathway [38]. HQT is well studied in other solanaceous vegetables; with tomato, over-expression of HQT resulted in the overproduction of chlorogenic acid by around a two-fold

[33]. In contrast, the suppression of HQT gene resulted in the reduction of chlorogenic acid content by 90% [2]. Therefore, in our study, we have developed the agroinfiltration method for the eggplant fruit. Further, we have the tested the efficiency of our method with over-expressing the smHQT gene in the eggplant fruit's flesh.

Contribution of this thesis in the improvement of eggplant

The trends of continuous demand of eggplant in the global market will continue in the future. Challenges due abiotic and biotic stress the eggplant breeders have to best use tools for the improvement like, conventional, biotechnological and genomics tools. Further, the information regarding the health related of eggplant and its wild relatives will further make eggplant popular even in the non-traditional production zones. In this direction, the wild relatives can contribute tremendously in the improvement eggplant. This study was the first large-scale approach to characterize the wild relatives from all the three genepools of the eggplant. The wild species and interspecific hybrid were characterized for the number of morphological and biochemical descriptors. This has resulted in the information of potential interest to the plant breeders. The genetics of all important traits in eggplant will help to successfully design a breeding experiment to have an eggplant ideotype with lesser number undesirable traits. Especially the use of primary and secondary genepool

species as testers has shown that the secondary genepool species are better for improving the nutraceutical aspect of eggplant, whereas, the primary genepool species are useful for the morphological traits in the eggplant. The diallel analysis of morphological and biochemical traits that too with using the popularly cultivated parents has helped in the understanding the genetic basis of inheritance of various morphological and biochemical traits in the eggplant. The technique of agroinfiltration developed for the eggplant fruit will be useful in swiftly and precisely identifying the fruit tissue-specific gene functions along with protein production.

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CONCLUSIONS

- Using different approaches (conventional, biotechnological and genomics) and the highly diverse collection of eggplant wild relatives we have shown that there is unexploited diversity in eggplant that could improve the current eggplant ideotype.

- Morphological characterization with conventional descriptors and also using the fruit phenomics tool, i.e. the Tomato Analyzer phenomics tool has allowed a detailed characterization of eggplant, close wild relatives and their interspecific hybrids. The high variation among wild species allowed identifying sources of variation and most promising species for traits of interest for eggplant breeding.

- Also, the high vigour of most interspecific hybrids may be directly exploited by using them as rootstocks. The information obtained here on phenotypic characteristics and heterosis of wild species and interspecific hybrids is of interest for eggplant breeding. Given the adaptation of many wild species to stressful conditions, their utilization in eggplant breeding may cause the development of a new generation of cultivars adapted to climate change challenges.

- Our results reveal that wild relatives of eggplant are highly variable for traits related to phenolics content, and fruit flesh colour and browning and represent a source of variation of interest, in

particular in the case of wild species from the secondary and tertiary genepools, for improving the content in phenolics of cultivated eggplant. However, for the fruit flesh colour and browning traits, the characteristics present in the wild species are detrimental. In addition, the lack of correlation between phenolics content traits on one side and fruit flesh colour and browning on the other suggest that the wild relatives can contribute effectively to the improvement of the bioactive properties of eggplant, while keeping a white fruit colour and low browning.

- Our study provides relevant information for eggplant breeding, in particular for the development of improved F1 hybrids. The highly significant differences observed for GCA and SCA for all traits show that there is a large genetic and gene action diversity in the set of parents and hybrids that can be exploited for breeding.

- With our results, we suggest that hybrids are a fast strategy to develop improved eggplant cultivars. That genetic distances among parents are not good predictors of performing eggplant hybrids shows that many hybrid combinations may have to be tested to identify superior hybrids. The molecular techniques, like the use of markers linked to genes or QTLs of interest, may be a more appropriate strategy for pre-selecting parents in eggplant hybrid breeding programs than the use of genetic distances among parents.

- The effective method developed for the agroinfiltration of eggplant fruit will be useful to acme eggplant fruit as a cost-effective protein production system. The transient protein expression can be measured in the few daytimes, rather, then going for the season-long plant production cycle.

- The present thesis has provided relevant information for improving eggplant, in particular for the development of varities with increased phenolic acids content and opens new research prospectives.