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Development and use of introgression populations for the detection of QTLs related to important agronomic traits in eggplant

PhD dissertation by

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ABSTRACT

Eggplant (*Solanum melongena* L.) is one of the most important commercial solanaceous vegetable crops grown widely in Asia and Mediterranean region. Despite its economic importance, the availability of experimental populations and genomic tools for breeding is still very limited compared to other major crops. Due to the progressive alteration of global ecosystem by climate change, plants are constantly exposed to stressful environmental conditions that impact negatively on their productivity. The genetic bottleneck occurred during eggplant domestication, which limits the availability of genetic resources for its genetic improvement, makes this crop extremely vulnerable to climate change, and, therefore, new strategies are needed for reducing its genetic erosion. In this context, crop wild relatives (CWRs) have demonstrated to be a valid genetic resources for plant breeding, as their use allows to broaden the genetic diversity of the crop and, in parallel, develop improved varieties adapted to climate change. To achieve this objective, in this doctoral thesis we reported on the development and evaluation of eggplant advanced materials obtained by using crop wild relatives.

In the first chapter, we have conducted a phenotypic evaluation in two environments of a set of 16 eggplant ILs with introgression from *S. incanum*, a close wild relative. Seventeen agronomic traits were scored to test the performance of ILs compared to the recurrent parent and identify QTLs for the investigated traits. We found significant morphological differences between parents, and the hybrid was heterotic for vigour related traits. Although significant genotype x environment interaction (G x E) was detected for most traits, the ILs generally exhibited few phenotypic differences with recipient parent, even in the presence of large introgression fragments from the wild parent. Low to moderate heritability values were found for the agronomic traits. In total, we detected ten stable QTLs, two of which were for plant-related traits and four for both flower- and fruit-related traits. In general, *S. incanum* introgressions improved the performance of most plant- and flower-related traits and decreased that of fruit-related traits. For three QTLs related to fruit pedicel length and fruit weight, we found evidence of synteny to other QTLs previously reported in eggplant populations. Seven QTLs were new, of which four related to plant height, flower calyx prickles, and fruit pedicel length, did not colocalized with any previous identified QTLs in eggplant populations, and three related to stem diameter, peduncle length, and stigma length, were the first reported in eggplant for these traits.

In the second chapter, the set of eggplant ILs with introgression from *S. incanum* was evaluated for fruit shape in two environments. Specifically, we performed a detailed phenotyping of the fruits of the parents, hybrid, and ILs using 32 morphological descriptors of the phenomics tool Tomato Analyzer. Large

differences in fruit morphology were found between ILs parents, and the hybrid exhibited negative values of heterosis for many fruit shape traits, being phenotypically closer to *S. incanum* parent. For most fruit shape descriptors, we observed significant differences between ILs and recipient parent, even in the presence of small wild donor fragments. Although the contribution of the environment and G × E interaction were significant for almost all descriptors, we found that their effects on fruit shape were relatively low, and the observed variations in fruit shape was mainly genetically regulated. Hierarchical clustering revealed nine clusters of highly correlated traits and six ILs groups. A total of 41 QTLs were mapped. Of these, sixteen associated to Basic Measurement and Fruit Shape Index descriptors were syntenic to other previously reported in several intraspecific and interspecific eggplant populations, while twenty-five QTLs related to Blockiness, Homogeneity, Asymmetry and Internal Eccentricity descriptors were new. In addition, in the genomic regions where stable QTLs were identified, we reported the presence of deleterious mutations in genes of *SUN*, *OVATE* and *FAS* families described in tomato, proposing eleven potential candidate genes controlling fruit shape variation in eggplant.

Finally, in the third chapter, we described the development of the first eggplant MAGIC population, obtained by intercrossing seven *S. melongena* accessions from different origins and displaying different morphological characteristics and one of the close wild relative *S. incanum*. A "funnel" approach, in which four F1 hybrids were obtained, were used to obtain two double hybrids generations. One of the double hybrids generation had the cytoplasm of *S. melongena*, while the other had the cytoplasm of *S. incanum*. These segregating double hybrids (149 individuals per double hybrid) were intercrossed using a chain-pollination scheme to develop the quadruple hybrid generation (S0), which was formed by 209 progenies. Two plants per S0 progeny were selfed to obtain the S1 progeny (n=402) and a single seed descend approach was used for two more generations to obtain 391 S2 and 305 S3 progenies. Two individuals per S3 progeny were grown. For those S3 progenies that were phenotypically uniform one plant was sampled for DNA extraction, while for those displaying some segregation two plants were sampled, making a total of 420 S3 individuals (348 with *S. melongena* cytoplasm and 72 with *S. incanum* cytoplasm) being sampled. The biased distribution probably reflected negative effects on fertility resulting from the interaction of the *S. incanum* cytoplasm with a mostly *S. melongena* nuclear genome. The S3 individuals were genotyped with the eggplant 5k probes Single Primer Enrichment Technology (SPET) platform, which yielded 7,724 SNPs. The genotyping results revealed a low heterozygosity (6.9%) and a lack of genetic structure, with no genetic differentiation between the two cytoplasm groups. Inference of haplotype blocks of the nuclear genome revealed an unbalanced representation of the founder genomes, suggesting a cryptic selection in favour or against specific parental genomes.

In summary, in the present doctoral thesis we have developed and evaluated advanced materials of great interest for eggplant breeding. We consider that the information, knowledges, and tools presented by this work provide the bases for future studies and will contribute to the development of a new generation of eggplant varieties adapted to climate change.

RESUMEN

La berenjena (*Solanum melongena* L.) es uno de los cultivos comerciales de hortalizas solanáceas más importantes que se cultiva ampliamente en Asia y la región del Mediterráneo. A pesar de su importancia económica, la disponibilidad de poblaciones experimentales y herramientas genómicas para el mejoramiento es aún muy limitada en comparación con otros cultivos importantes. Debido a la alteración progresiva del ecosistema global por el cambio climático, las plantas están constantemente expuestas a condiciones ambientales estresantes que impactan negativamente en su productividad. El cuello de botella genético ocurrido durante la domesticación de la berenjena, que limita la disponibilidad de recursos genéticos para su mejoramiento genético, hace que este cultivo sea extremadamente vulnerable al cambio climático, por lo que se requieren nuevas estrategias para reducir su erosión genética. En este contexto, los parientes silvestres de los cultivos (CWRs) han demostrado ser un recurso genético válido para la mejora vegetal, ya que su uso permite ampliar la diversidad genética de los cultivos y, en paralelo, desarrollar variedades mejoradas adaptadas al cambio climático. Para lograr este objetivo, en esta tesis doctoral informamos sobre el desarrollo y la evaluación de materiales avanzados de berenjena obtenidos mediante el uso de parientes silvestres.

En el primer capítulo, realizamos una evaluación fenotípica en dos ambientes de un conjunto de 16 ILs de berenjena con introgresión de *S. incanum*, un pariente silvestre. Se evaluaron diecisiete caracteres agronómicos para comparar el rendimiento de las ILs con el parental recurrente e identificar QTLs para los caracteres investigados. Encontramos diferencias morfológicas significativas entre los parentales, y el híbrido resultó heterótico para los caracteres de vigor. A pesar de que la interacción entre genotipo y ambiente (G x E) resultó significativa para la mayoría de los caracteres, en general las ILs mostraron pocas diferencias fenotípicas con el progenitor receptor, incluso en presencia de grandes fragmentos de introgresión del progenitor silvestre. Se encontraron valores de heredabilidad bajos a moderados para los caracteres agronómicos. En total, detectamos diez QTLs estables, dos de los cuales estaban relacionados con caracteres de planta y cuatro para caracteres de flor y fruto. En general, las introgresiones de *S. incanum* mejoraron los valores medios de la mayoría de los caracteres de planta y flor, y disminuyeron el de los caracteres de fruto. Para tres QTLs relacionados con la longitud del pedicelo del fruto y con el peso del fruto, encontramos evidencia de sintenia con otros QTLs identificados previamente en poblaciones de berenjena. Siete QTLs eran nuevos, de los cuales cuatro relacionados con la altura de la planta, con la espinosidad del cáliz de la flor y con la longitud del pedicelo del fruto no colocalizaron con ningún QTL previamente identificado en las poblaciones de berenjena, y tres relacionados con el diámetro del tallo, con la longitud del

pedúnculo y del estigma, fueron los primeros identificados en berenjena para estos caracteres.

En el segundo capítulo, el conjunto de IL de berenjena con introgresiones de *S. incanum* se evaluó para la forma del fruto en dos ambientes. Específicamente, realizamos un fenotipado detallado de los frutos de los parentales, del híbrido y de las ILs utilizando 32 descriptores morfológicos de la herramienta fenómica Tomato Analyzer. Se encontraron grandes diferencias morfológicas en los frutos de los parentales, y el híbrido presentó valores negativos de heterosis para muchos de los caracteres de forma del fruto, siendo fenotípicamente más cercano al parental *S. incanum*. Para la mayoría de los descriptores de forma del fruto observamos diferencias significativas entre las ILs y el parental receptor, incluso en presencia de pequeños fragmentos de introgresión del parental silvestre. A pesar de que la contribución del ambiente y la interacción G × E fueron significativas para casi todos los descriptores, encontramos que sus efectos sobre la forma del fruto fueron relativamente bajos y que las variaciones observadas estaban reguladas sobre todo genéticamente. El agrupamiento jerárquico reveló nueve grupos de caracteres altamente correlacionados y seis grupos de ILs. Se mapearon un total de 41 QTLs. De estos, dieciséis asociados a los descriptores de Basic Measurements y Fruit Shape Index resultaron sinténicos a otros identificados previamente en varias poblaciones intraespecíficas e interespecíficas de berenjena, mientras que veinticinco QTLs relacionados con los descriptores de Blockiness, Homogeneity, Asimmetry y Internal Eccentricity, fueron nuevos. Además, en las regiones genómicas donde se identificaron QTL estables, detectamos la presencia de mutaciones deletéreas en genes de las familias *SUN*, *OVATE* y *FAS* descritos en tomate, proponiendo once potenciales genes candidatos implicados en el control de la variación de forma del fruto en berenjena.

Finalmente, en el tercer capítulo, describimos el desarrollo de la primera población MAGIC de berenjena, obtenida cruzando siete accesiones de *S. melongena* de diferentes orígenes que presentaban diferentes características morfológicas, y una del pariente silvestre *S. incanum*. Se utilizó un esquema de "embudo", en el que se obtuvieron cuatro híbridos F1, para partir de las cuales se obtuvieron dos generaciones de híbridos dobles. Una generación de híbridos dobles presentaba el citoplasma de *S. melongena*, mientras que la otra presentaba el citoplasma de *S. incanum*. Estos híbridos dobles segregantes (149 individuos por híbrido doble) se cruzaron utilizando un esquema de polinización en cadena para desarrollar la generación híbrida cuádruple (S0), que se formó con 209 progenies. Para cada progenie S0 se autofecundaron dos plantas para obtener la progenie S1 (n=402) y durante dos generaciones más se utilizó un enfoque de descendencia de semilla única para obtener 391 progenies S2 y 305 progenies S3. Por cada progenie S3 se cultivaron dos individuos. Para aquellas progenies S3 que eran fenotípicamente uniformes, se tomaron muestras de una planta para la extracción de ADN, mientras que para aquellas que mostraban alguna segregación, se tomaron muestras de dos plantas, llegándose a un total de 420 individuos S3 (348 con citoplasma de *S. melongena* y 72 con citoplasma de *S. incanum*) muestreados. La distribución sesgada probablemente reflejaba los efectos negativos sobre la fertilidad resultantes de la interacción del citoplasma de *S. incanum* con un genoma nuclear mayoritariamente de *S. melongena.* Los individuos S3 se genotiparon con la plataforma SPET (Single Primer Enrichment Technology) de 5k sondas de berenjena, la cual produjo 7.724 SNP. Los resultados del genotipado revelaron una baja heterocigosidad (6,9%) y falta de estructura genética, sin diferenciación genética entre los dos grupos de citoplasma. La inferencia de bloques de haplotipos del genoma nuclear reveló una representación desequilibrada de los genomas de los fundadores, lo que sugiere una selección críptica a favor o en contra de genomas parentales específicos.

En resumen, la presente tesis doctoral demuestra que hemos desarrollado y evaluado materiales avanzados de gran interés para la mejora de la berenjena. Consideramos que la información, los conocimientos y las herramientas que se presentan este trabajo sientan las bases para futuros estudios y contribuirán al desarrollo de una nueva generación de variedades de berenjena adaptadas al cambio climático.

RESUM

L'albergínia (*Solanum melongena* L.) és un dels cultius comercials d'hortalisses solanácees més importants que es cultiva àmpliament a Àsia i la regió del Mediterrani. Malgrat la seua importància econòmica, la disponibilitat de poblacions experimentals i eines genòmiques per al millorament és encara molt limitada en comparació amb altres cultius importants. A causa de l'alteració progressiva de l'ecosistema global pel canvi climàtic, les plantes estan constantment exposades a condicions ambientals estressants que impacten negativament en la seua productivitat. El coll de botella genètic ocorregut durant la domesticació de l'albergínia, que limita la disponibilitat de recursos genètics per al seu millorament genètic, fa que aquest cultiu siga extremadament vulnerable al canvi climàtic, per la qual cosa es requereixen noves estratègies per a reduir la seua erosió genètica. En aquest context, els parents silvestres dels cultius (CWRs) han demostrat ser un recurs genètic vàlid per a la millora vegetal, ja que el seu ús permet ampliar la diversitat genètica dels cultius i, en paral·lel, desenvolupar varietats millorades adaptades al canvi climàtic. Per a aconseguir aquest objectiu, en aquesta tesi doctoral presentem el desenvolupament i l'avaluació de materials avançats d'albergínia obtinguts mitjançant l'ús de parents silvestres.

En el primer capítol, realitzem una avaluació fenotípica en dos ambients d'un conjunt de 16 IL d'albergínia amb introgresions de *S. incanum*, un parent silvestre. Es van puntuar dèsset caràcters agronòmics per a avaluar el rendiment de les ILs en comparació amb el parental recurrent i identificar els QTL per als caràcters investigats. Trobarem diferències morfològiques significatives entre els parentals, i l'híbrid va resultar heteròtic per als caràcters de vigor. A pesar que la interacció entre genotip i ambient (G x E) va resultar significativa per a la majoria dels caràcters, en general les ILs van mostrar poques diferències fenotípiques amb el progenitor receptor, fins i tot en presència de grans fragments d'introgresió del progenitor silvestre. Es van trobar valors de heredabilitat baixos a moderats per als caràcters agronòmics. En total, detectarem deu QTL estables, dos dels quals estaven relacionats a caràcters de planta i quatre per a caràcters de flor i fruit. En general, les introgresions de *S. incanum* van millorar els valors mitjos de la majoria dels caràcters de planta i flor, i van disminuir el dels caràcters de fruit. Per a tres QTL relacionats amb la longitud del pedicel del fruit i amb el pes del fruit, trobem evidència de sintenia amb altres QTLs identificats prèviament en poblacions d'albergínia. Set QTL eren nous, dels quals quatre estaven relacionats amb l'altura de la planta, amb la espinositat del calze de la flor i amb la llargària del pedicel del fruit no van colocalitzar amb cap QTL prèviament identificat en les poblacions d'albergínia, i tres relacionats amb el diàmetre de la tija, amb la llargària del peduncle i de l'estigma, van ser els primers reportats en albergínia per a aquests caràcters.

En el segon capítol, el conjunt de IL d'albergínia amb introgresions de *S. incanum* es va avaluar per a la forma del fruit en dos ambients. Específicament, realitzarem un fenotipado detallat dels fruits dels parentals, de l'híbrid i de les ILs utilitzant 32 descriptors morfològics de l'eina fenómica Tomato Analyzer. Es van trobar grans diferències morfològiques en els fruits dels parentals, i l'híbrid va presentar valors negatius de heterosis per a molts dels caràcters de forma del fruit, sent fenotípicamente més pròxim al parental *S. incanum*. Per a la majoria dels descriptors de forma del fruit observarem diferències significatives entre les ILs i el parental recipient, fins i tot en presència de xicotets fragments d'introgresió del parental silvestre. A pesar que la contribució de l'ambient i la interacció G × E van ser significatives per a quasi tots els descriptors, trobem que els seus efectes sobre la forma del fruit van ser relativament baixos i que les variacions observades estaven regulades sobretot genèticament. L'agrupament jeràrquic va revelar nou grups de caràcters altament correlacionats i sis grups de ILs. Es mapejaren un total de 41 QTL. D'aquests, setze estaven associats als descriptors de Basic Measurements i Fruit Shape Index van resultar sintènics a altres reportats prèviament en diverses poblacions intraespecífiques i interespecífiques d'albergínia, mentre que vint-i-cinc QTLs relacionats amb els descriptors de Blockiness, Homogeneity, Asimmetry i Internal Eccentricity, van ser nous. A més, a les regions genòmiques on es van identificar QTL estables, detectarem la presència de mutacions deletèries en gens de les famílies *SUN*, *OVATE* i *FAS* descrits en tomaca, proposant onze gens candidats potencialment implicats en el control de la variació de forma del fruit en albergínia.

Finalment, en el tercer capítol, descrivim el desenvolupament de la primera població MAGIC d'albergínia, obtinguda creuant set accessions de *S. melongena* de diferents orígens i que presenten diferents característiques morfològiques, i una del parent silvestre *S. incanum*. Un esquema de "embut", en el qual es van obtindre quatre híbrids F1, es va utilitzar per a obtindre dues generacions d'híbrids dobles. Una generació d'híbrids dobles presentava el citoplasma de *S. melongena*, mentre que l'altra presentava el citoplasma de *S. incanum*. Aquests híbrids dobles segregants (149 individus per híbrid doble) es van creuar utilitzant un esquema de pol·linització en cadena per a desenvolupar la generació híbrida quàdruple (S0), que es va formar amb 209 progènies. Per cada progènie S0 es van autofecundar dues plantes per a obtindre la progènie S1 (n=402) i durant dues generacions més es va utilitzar un enfocament de descendència de llavor única per a obtindre 391 progènies S2 i 305 progènies S3. Per cada progènie S3 es van cultivar dos individus. Per a aquelles progènies S3 que eren fenotípicamente uniformes, es van prendre mostres d'una planta per a l'extracció d'ADN, mentre que per a aquelles que mostraven alguna segregació, es van prendre mostres de les dues plantes, arribant a un total de 420 individus S3 (348 amb citoplasma de *S. melongena* i 72 amb citoplasma de *S. incanum*) mostrejats. La distribució esbiaixada probablement reflectia els efectes negatius sobre la fertilitat resultants de la interacció del citoplasma de *S. incanum* amb un genoma nuclear majoritàriament de *S. melongena*. Els individus S3 es van genotipar amb la plataforma SPET (Single Primer Enrichment Technology) de 5k sondes d'albergínia, que va produir 7.724 SNP. Els resultats del genotipat van revelar una baixa heterocigositat (6,9%) i falta d'estructura genètica, sense diferenciació genètica entre els dos grups de citoplasma. La inferència de blocs de haplotips del genoma nuclear va revelar una representació desequilibrada dels genomes dels fundadors, la qual cosa suggereix una selecció críptica a favor o en contra de genomes parentals específics.

En resum, en la present tesi doctoral hem desenvolupat i evaluat materials avançats de gran interés per a la millora de l'albergínia. Considerem que la informació, els coneixements i les eines que es presenten aquest treball estableixen les bases per a futurs estudis i contribuiran al desenvolupament d'una nova generació de varietats d'albergínia adaptades al canvi climàtic.

GENERAL INTRODUCTION

1. THE EGGPLANT

1.1 *Taxonomy*

Eggplant (*Solanum melongena* L.) is an angiosperm plant native to Southeast Asia. It belongs to the Solanaceae family and is classified in the taxa indicated in Figure 1 (Vorontsova and Knapp, 2016).

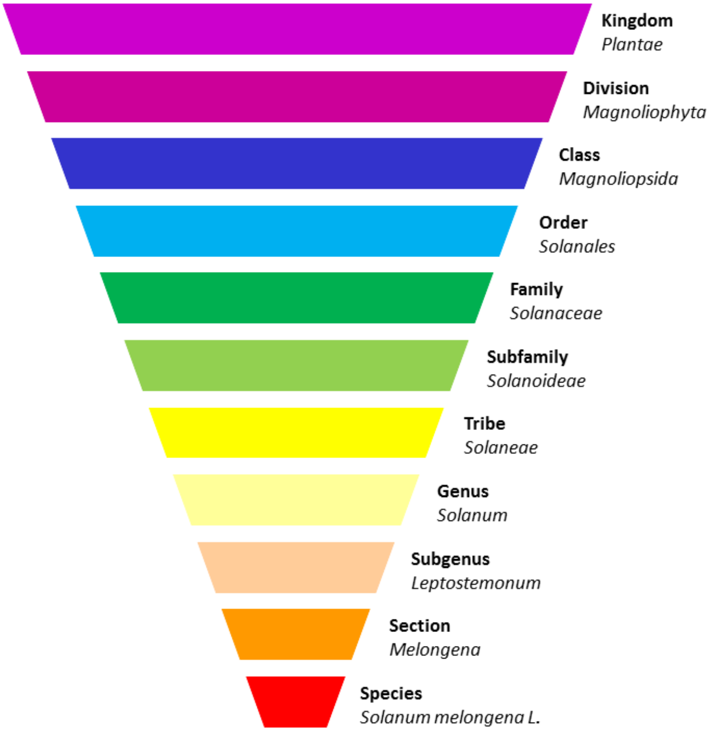

Figure 1. Taxonomic classification of eggplant (Vorontsova and Knapp, 2016).

The Solanaceae family contains 90 genera and an estimated number of 3000 species. The genus *Solanum* is one of the most species-rich in flowering plants (Frodin 2004), and its ca. 1400 species include herbaceous, shrubby, and arboreal plants distributed worldwide (Knapp et al., 2013). In addition to eggplant, the genus contains species of great economic importance for humans, such as tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.). Among the 13 clades of the genus *Solanum*, eggplant is a member of the largest clade *Leptostemonum* (subgenus *Leptosmonum* Bitter), commonly known as the 'spiny solanum', which includes 450 recognized species (Knapp et al., 2013). Eggplant is a diploid species with a basic chromosome number of n = 12 and a genome size of approximately 1.21 Gb (Barchi et al., 2019).

1.2 *Botany*

Eggplant is a perennial that often is cultivated as an annual (Daunay and Janick, 2007). The plant grows 40 to 150 cm tall and branched, with a tough herbaceous, erect, and spiny stem. Its green, simple, ovate, flat, and slightly lobed leaves are arranged alternately on the branches and can measure 10 to 20 cm long and 5 to 10 cm broad. Anthocyanins, prickles, and hairiness on vegetative parts vary quantitatively. Leaf prickles and hairiness are more pronounced in wild types (Naujeer, 2009).

The flowers measure 3-5 cm in diameter and are white to purple with a spiny calyx and yellow stamens (Naeem and Ugur, 2019). Corolla generally has five lobes, but six, seven and eight lobed corolla is commonly found in globose and round fruited types (Figure 2). Inflorescences generally display solitary hermaphrodite flowers, but some cultivars present one to five andromonoecious cymes. Flowers normally are self-fertile, although crosspollination by insects does occur (Daunay et al., 2004).

The fruit is a berry of variable shape (spherical, long, oblong, oval) and size (from 0.5 g to 1500 g), with a glossy smooth skin. Absence or presence as well as distribution pattern of anthocyanins and chlorophylls control fruit color, which can be white, green, violet, dark purple and black, or combinations of these colours in stripes (Daunay et al., 2004) (Figure 3). At physiological ripe stage, fruit color varies from yellow to brown as a consequence of pigments degradation. The flesh surrounding the seeds is yellow, green, or white in color, and turns brown after cutting due to the oxidation of phenolic compounds. The seeds are small, numerous and deformed, and contain nicotinoid alkaloids which are responsible for the slightly bitter taste of the fruit.

Eggplant is a warm-weather plant with a growing season of at least 120 days. For an optimal growth, it requires sandy loam or silt loam soil with a pH between 5.5 and 6.8, and an average temperature of 22-30 °C. Being heliophyte, eggplant needs to be positioned in an area with very intensive insolation. Although the frequency and amount of irrigation should be managed to avoid root rot caused by waterlogging, regular and abundand watering is required, especially during fruit development (Chen et al., 2002).

Figure 2. Diversity in shape, color and number of lobes of the eggplant flower.

Figure 3. Diversity in shape, size, and color of the eggplant fruit.

1.3 *Eggplant wild relatives*

Crop wild relatives are wild plant species closely related to cultivated plants and represent a rich source of novel genetic diversity for crop breeding (Daunay and Hazra, 2012). Wild relatives grow in a multitude of environments and often harbor important genes for resistance or tolerance to biotic and abiotic stresses (Dempewolf et al., 2017). Cultivated plants derive from the domestication of wild relatives, a long process through which humans have gradually changed the characteristics of wild relatives by selecting for certain desirable traits (Smýkal et al., 2018; Kersey et al., 2020). The wild species from which a cultivated plant was derived, in some cases may be well known and easily identified in current wild plant populations, while others have arisen via hybridization and polyploidization (Kantar et al., 2017; Smýkal et al., 2018).

Eggplant wild relatives have been found in a wide range of environmental conditions, including desertic areas and other highly stressful environments (Vorontsova and Knapp, 2016; Plazas et al., 2020). Some of them are of great interest for breeding, in particular for traits related to adaptation to climate change but also pest and disease resistance (Tani et al., 2018; Ghani et al., 2020; Rakha et al., 2020; Kouassi et al., 2021; Plazas et al., 2019, 2022). Although several efforts have been made to transfer these traits to cultivated eggplant, the use of wild relatives in eggplant breeding is still limited, and no commercial cultivar with traits introgressed from wild relatives have been released until now (Rotino et al., 2014; Syfert et al., 2016; Taher et al., 2017; Boyaci, 2020).

Depending on phylogenetic relationships and crossability with crops, wild relatives can be classified into primary, secondary and tertiary genepools (Harlan and de Wet, 1971). Species in the primary genepool (GP1) typically have no or only limited crossing barriers with the crop and the offspring is fertile. Secondary genepool (GP2) is composed of species that are crossable with GP1, although the success of the crosses and the viability or fertility of the hybrids may be reduced. Finally, tertiary genepool (GP3) consist of more distantly related species which have strong reproductive barriers with the crop. The resulting hybrids are abnormal, lethal, or sterile, and crossing may require specific breeding techniques to succeed. In the case of eggplant, the primary genepool includes all cultivated species and its wild ancestor *S. insanum* (Ranil et al., 2017). The secondary genepool of eggplant comprises more than 40 species, including *S. anguivi, S. campylacanthum*, *S. dasyphyllum*, *S. incanum*, *S. lichtenstenii*, *S. linnaeanum*, *S. pyracanthum*, *S. tomentosum* and *S. violaceum* (Syfert et al., 2016). Among the species of great interest in the tertiary genepool of eggplant there are *S. elaeagnifolium*, *S. sisymbriifolium* and *S. torvum* (Daunay and Hazra, 2012; Rotino et al., 2014; Plazas et al., 2016; Toppino et al., 2021). 2019).

The work of this doctoral thesis is mainly focused on the use of the wild species *S. incanum*, which belongs to the secondary genepool of eggplant. *S. incanum* is naturally distributed in desert and dryland areas from northern Africa to Pakistan (Vorontsova and Knaap, 2016; Gramazio et al., 2017). The interest in this species resides in its drought tolerance (Lester and Hasan, 1991; Daunay, 2008; Knaap et al., 2013), which is an important breeding objective in eggplant. In addition, *S. incanum* is considered a powerful source of phenolic compounds (Stommel and Whitaker, 2003; Ma et al., 2011; Prohens et al., 2013; Meyer et al., 2015), and presents resistance to some fungal and bacterial disease (Yamakawa and Mochizuki, 1979; Collonnier, 2001; Mishra et al., 2021). Due to these favorable features, *S. incanum* has been used for several genetic studies (Gramazio et al., 2014, 2016, 2017), and, recently, its entire genome has been sequenced (Gramazio et al., 2019).

1.4 Origin and domestication

The identity of the wild progenitors of cultivated eggplant (*S. melongena*) has been subjected to considerable taxonomic confusion which gave rise to a number of similar but varied theories on the origin and domestication of this crop. Although most of the wild relatives of eggplant have been found between Africa and the Middle East, the wild ancestor of the cultivated eggplant is *S. insanum*, a wild species distributed in Southeast Asia and Madagascar (Ranil et al., 2017). Due to its morphological similarity to and close affinity with cultivated eggplant and other quite similar wild relatives, *S. insanum* has been treated in different ways at taxonomic level (Knapp et al., 2013). The early phylogenetic analysis, which considered *S. insanum* conspecific to *S. melongena* or recognized it at species level under a variety of different names, supported that cultivated eggplant had been domesticated from *S. incanum* (Lester and Hasan, 1991; Weese and Bohs, 2010; Meyer et al., 2012). Human migrations would have brought *S. incanum* from the Middle East to the Indo-Burman region, where under natural and artificial selection it probably evolved, giving rise to modern cultivars and spiny forms. Further revisions of the taxonomy provided a detailed description of the taxa and resolved the problem related to nomenclature and species boundaries of eggplant relatives (Vorontsova and Knapp, 2012, 2016; Vorontsova et al., 2013; Knapp et al., 2013). Recent phylogenetic studies including more species from Africa and Asia confirmed the hypothesis that *S. insanum* is the wild progenitor of the cultivate eggplant, and defined the group of African species (including *S. incanum*) as sister to the group formed by cultivated eggplant and its wild progenitor (Aubriot et al., 2016; Aubriot et al., 2018; Page et al., 2019).

Although extensive information exists on eggplant domestication, some questions about its process are still unclear. Vavilov (1935) indicated the Indo-Burman region as the center of domestication of eggplant. Evidence based on ancient Chinese literature revision and molecular markers analysis suggested that eggplant had two centers of domestication in India and China, and an additional independent center of domestication in Philippines (Wang et al., 2008; Weese and Bohs, 2010; Meyer et al., 2012; Kumar et al., 2020; Rakha et al., 2021). Although diversity in agronomic traits and microsatellite markers detected comparing accessions of China, India, Indonesia, Indo-China and Sri Lanka reinforced the hypothesis of multi-local domestication (Hurtado et al., 2012; Cericola et al., 2013), in a recent study based on genotyping-by-sequencing (GBS) and RNAseq data of a broad sampling of wild, weedy and cultivated eggplant, strong evidence of a single origin in South-East Asia have been found (Page et al., 2019). From the Indo-Chinese center of domestication, eggplant was then introduced into the Mediterranean region by Arabs, and its cultivation gradually spread into Western Asia, Africa, Europe and, finally, in America (Prohens et al., 2005; Taher et al., 2017). et al., 2019).

1.5 *Economic importance*

Eggplant is a horticultural crop of great economic importance. It ranks sixth among all vegetables in global production and represents the third most cultivated vegetable within the Solanaceae family after tomato and potato (FAOSTAT, 2019). According to the latest FAOSTAT data, world eggplant production increased by 27% over ten years, going from 43.5 million tons in 2009 to 55.2 million tons in 2019 (Figure 4). About 94.2% of world eggplant production takes place in Asia, with only 1.66% generated in Europe. China is once again confirmed as world leader (35.5 million tons), and together with India (12.7 million tons) account for 87.4% of the world eggplant production (FAOSTAT, 2019).

In UE, the total harvested area for eggplant was estimated in 27,716 hectares (ha), with a total production of 915,951 tons (FAOSTAT, 2019). Italy and Spain are the main European producers with 300,620 and 245,150 tons, respectively. Romania ranks third (79,660 tons), followed by Ukraine (66,420 tons) and Netherlands (64,000 tons) (FAOSTAT, 2019) (Figure 5) In Spain, eggplant is the eleventh most cultivated vegetable, before garlic, cauliflower, and pumpkin (MAPA, 2020). In the last year, cultivated area of eggplant as well as its total production increased by 6.6% and 15.1%, respectively (Table 1 and Table 2) (MAPA, 2020). Andalusia represents the first national producer with a total of 245,760 tons and 2,871 ha of cultivated area, followed by the Valencian Community with 12,702 tons and 251 ha (Table 1 and Table 2) (MAPA, 2020).

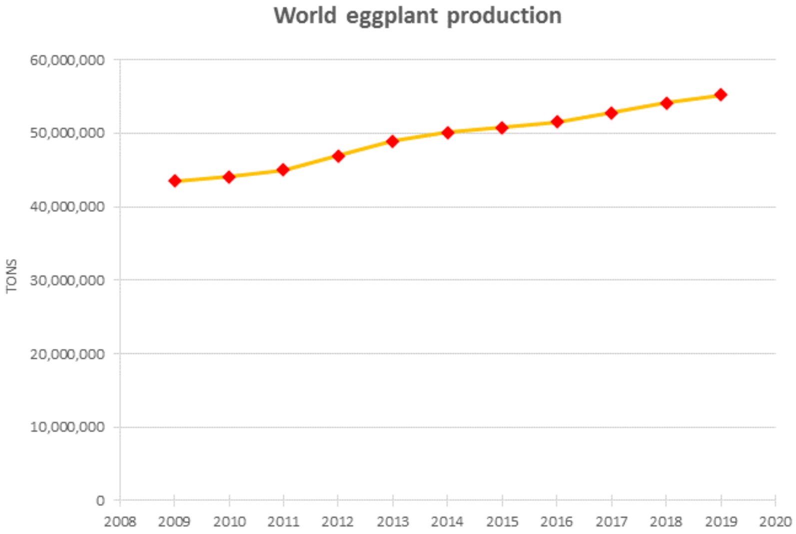

Figure 4. World eggplant production (Tons) between 2009 and 2019 (FAOSTAT, 2019).

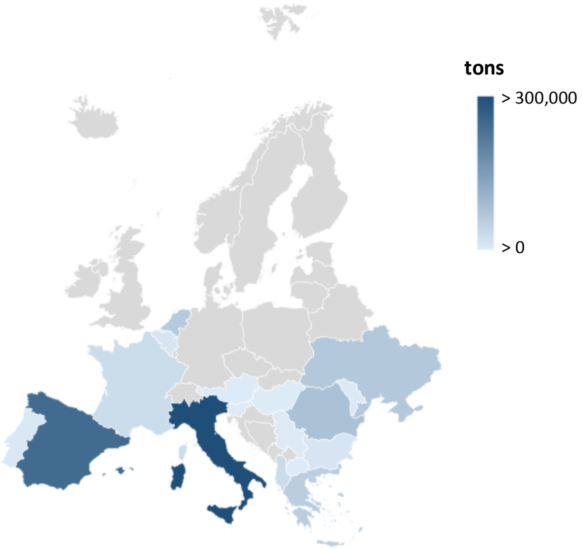

Figure 5. Heatmap of eggplant production in EU countries (Tons) (FAOSTAT, 2019).

Table 1. Total eggplant production in Spain by region (Tons) (MAPA, 2020).

Region	**2020**	**2019**	**Difference**	**Dif. (%)**	**Position**
Andalucia	245,760	207,832	37,928	18.2	1 (-)
Valencian Community	12,702	12,475	227	1.8	2 (-)
Extremadura	4,817	5,256	–439	-8.4	3 (+1)
Canary Islands	4,429	3,676	753	20.5	4 (+1)
Navarre	4,202	5,869	–1,667	-28.4	5 (-2)
Catalonia	3,275	3,580	–305	-8.5	6 (-)
Murcia	1,923	1,979	–56	-2.8	7 (-)
Castile La Mancha	1,728	1,269	459	36.2	8 (+1)
Balearic Islands	1,356	1,442	–86	-6.0	9 (-1)
Aragon	964	918	46	5.0	10 (-)
La Rioja	846	602	244	40.5	11 (-)
Madrid	61	61	0	0.0	12 (+2)
Galicia	60	60	0	0.0	13 (-1)
Castile and León	34	82	–48	-58.5	14 (-1)
Basque Country	23	25	–2	-8.0	15 (-)
Asturias	20	20	0	0.0	16 (-)
Cantabria	-	-	-	-	17 (-)
Spain	**282,200**	**245,146**	**37,054**	**15.1**	-

Table 2. Total cultivated area of eggplant in Spain by region (Hectares) (MAPA, 2020).

Region	2020	2019	Difference	Dif. (%)	Position
Andalucia	2,871	2,637	234	8.9	1 (-)
Valencian Community	251	250	1	0.4	2 (-)
Catalonia	126	135	−9	-6.7	3 (-)
Canary Islands	90	79	11	13.9	4 (+1)
Navarre	83	102	−19	-18.6	5 (-1)
Extremadura	67	73	−6	-8.2	6 (-)
Balearic Islands	58	59	−1	-1.7	7 (-)
Castile La Mancha	57	42	15	35.7	8 (+1)
Murcia	43	45	−2	-4.4	9 (-1)
Aragon	24	23	1	4.3	10 (-)
La Rioja	19	14	5	35.7	11 (-)
Basque Country	5	5	0	0	12 (-)
Asturias	2	2	0	0	13 (+1)
Castile and León	2	4	−2	-50.0	13 (-)
Madrid	2	2	0	0	13 (+1)
Galicia	1	1	0	0	14 (-)
Cantabria	-	-	-	-	15 (-)
Spain	**3,701**	**3,473**	**228**	**6.6**	

2. CROP IMPROVEMENT UNDER CLIMATE CHANGE SCENARIOS

2.1 *Climate change impact on crop production*

Climate change represents a major challenge of the twenty-first century for world community. Industrial revolution is unequivocally one of the main causes of the climate change (Raza et al., 2019; Raven and Wagner, 2021; Naz et al., 2022). In the last 200 years, the excessive utilization of fossil fuels and accumulation of dangerous greenhouse gases (especially CO_2) in the Earth's atmosphere have warmed the planet, resulting in an increase in global average temperature of 1.41 °C (IPCC, 2019). In addition, massive deforestation and daily human activities maximize greenhouse effect, and earth's temperature rises more and more (Anderson, 2020). The last two centuries are considered as the warmest period of civilization, and the Earth's average temperature is expected to rise by 2 °C by 2100 (Malhi et al., 2021).

Climate change effects are being manifested through frequent extreme weather events, such as spells of drought and flood, cold or heat waves, and fluctuations in sea levels, which alter the global ecosystem (Vaughan et al., 2018; Arunanondchai et al., 2018). The constant exposure of plants to such stressful environmental conditions has a large impact on their morpho-physiological and biochemical functioning (Gunderson et al., 2010; Liancourt et al., 2015; Barlow et al., 2015; Salehi-Lisar et al., 2016; Raza et al., 2019). Plant growth and metabolisms are very susceptible to excess increase or decrease of atmospheric CO_2 levels, fluctuations in temperature and precipitation (Hasanuzzaman et al. 2013; AbdElgawad et al., 2016). For several crop species, high levels of CO_2 promote increased yields by acting positively on photosynthesis (Jabolonski et al., 2002; Ainsworth and Long, 2005; Zhang et al., 2020) and enhancing water use efficiency due to need of fewer open stomata for gas exchange (Jin et al., 2018; Anderson et al., 2020). Despite this, the negative effects of CO_2 accumulation prevail on the positive ones, severely influencing agriculture and food production (Figure 6). Stress induced by high temperature affects crop reproductive development, and the consequent decrease in yield is mainly associated with pollen infertility (Gupta et al., 2015; Sita et al., 2017; Fahad et al., 2018; Kumar et al., 2019). Fluctuations in temperature and changes in precipitation patterns generate suitable climatic conditions that favor the emergence of novel pest and plant disease and the growth of newer invasive weeds (Juroszek et al., 2020; Hunjan and Lore, 2020). Moreover, rising sea levels as a consequence of melting glaciers will lead to the loss of arable lands and salinization of the aquifers (Kirwan and Gedan, 2019; Fagherazzi et al., 2019; Okur and Örçen, 2020).

Overall, climate change is already having a significant impact on agricultural production worldwide, putting global food security at serious risk for future generations especially in view of the speedy increase in the world's population.

Estimates suggest the world population will rise to 9.7 billion people by 2050 (United Nations, 2019), and global crop production will have to increase substantially to keep up with this demand while simultaneously reducing its environmental impact (Hunter et al., 2017).

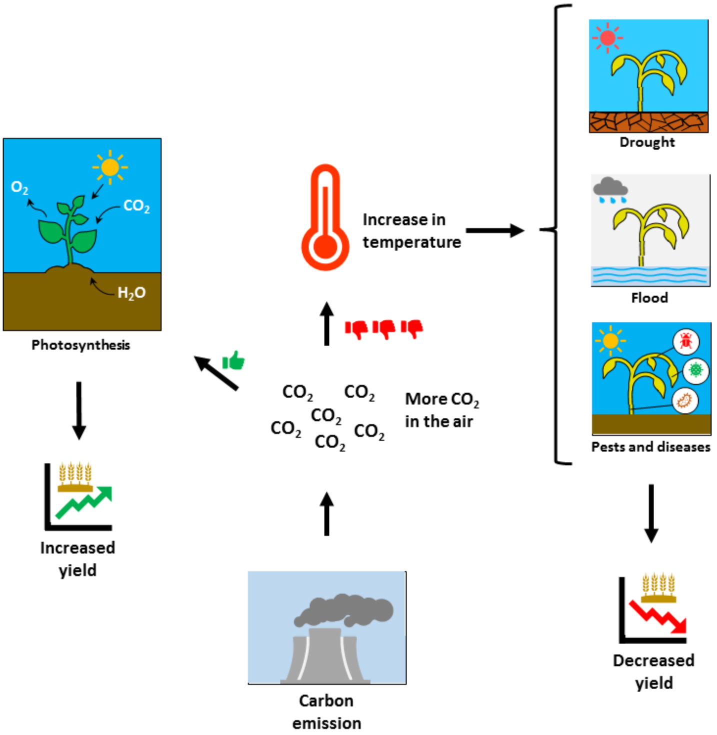

Figure 6. Positive and negative effects of CO_2 accumulation on crop production.

In this context, developing new plants that resist to emerging biotic and abiotic stresses without altering their productivity represent an important goal for scientists and breeders (Degani et al., 2019; Fahad et al., 2021; Rivero et al., 2022). Understanding the genetic basis of simple and complex traits underlying stress-resistance processes and efficiently introducing them into new elite cultivars are strategies to achieve this objective (Scossa et al., 2021). Modern breeding technologies and methodologies emerged in the last twenty years had a significant impact on plant genetics research and breeding programs. In particular, 'Omics' approach remains a key strategy for crop upgrading and development. The combination of omics technologies, encompassing fields such as genomic, transcriptomic, metabolomic and phenomic, have allowed significant steps forward in the elucidation of a multitude of traits, signalling pathways, gene expression and metabolite composition that are crucial in biotic and abiotic stress responses in crops (Dutta et al., 2020). The recent genome editing technique based on the CRISPR-Cas9 system facilitates crop improvement through the rapid, precise, and targeted modifications of genomes (Massel et al., 2021). Although this technique outperforms traditional breeding in some aspects, the legislation applicable to gene editing is under discussion in many countries, and there is also a high degree of skepticism among consumers about its safety (Scheben and Edwards, 2019). Other strategies consisting of interventions in cultural practices are very beneficial for crop adaptability. The adoption by farmers of useful approaches such as alterations in timing and methods for sowing, a collection of crops with short life cycles, crop rotation, optimum irrigation management, and variation in cropping schemes can considerably decrease the adverse effects of extreme environmental conditions (Duku et al., 2018; Teixeira et al., 2018; Marcinkowski and Piniewski, 2018; Deligios et al., 2019). In addition, there are alternative approaches that can contribute to the development of a new generation of crops adapted to climate change. These approaches are described in the next sections.

2.2 *Introgressiomics: a breeding strategy for adapting crops to climate change*

Crop domestication can be described as a complex evolutionary process in which natural and artificial selection has led to the development of new plants that meet human needs (Smykal et al., 2018). During this process, genetic bottlenecks of varying degrees, which occur due to the use of limited numbers of individuals of progenitor species or events of reproductive isolation and strong selection pressures, have resulted in a reduction in genetic diversity in modern crops, limiting their potential for developing novel varieties with improved traits (Zhang et al., 2017) (Figure 7). Luckily, such lost diversity can be recovered from their wild ancestors and relatives, which are a reservoir of favorable traits for adaptability of modern cultivars (Hajjar and Hodgkin, 2007; Warschefsky et al., 2014; Fonseka et al., 2020).

Climate changes are enhancing the genetic erosion of both modern crops and wild species. Environmental variations in agroecosystems caused by climate change are happening so rapidly that makes cultured varieties unable to adapt to these new conditions, threatening to disappear (Bellon and van Etten, 2014). Destruction or reduction of habitats caused by human activity may lead to extinction of genetically differentiated populations or even of entire species of wild relatives (Ford-Lloyd, 2011). To prevent this, numerous projects and initiatives carried out for decades by different organizations in collaboration with universities and other national and international research centers allowed the collection, classification, and conservation in germplasm banks of more than 7 million cultivated and wild related species (FAO, 2010). In parallel, broadening the genetic base of crops by exploiting this preserved diversity may be a useful strategy for improving crops in a changing climate.

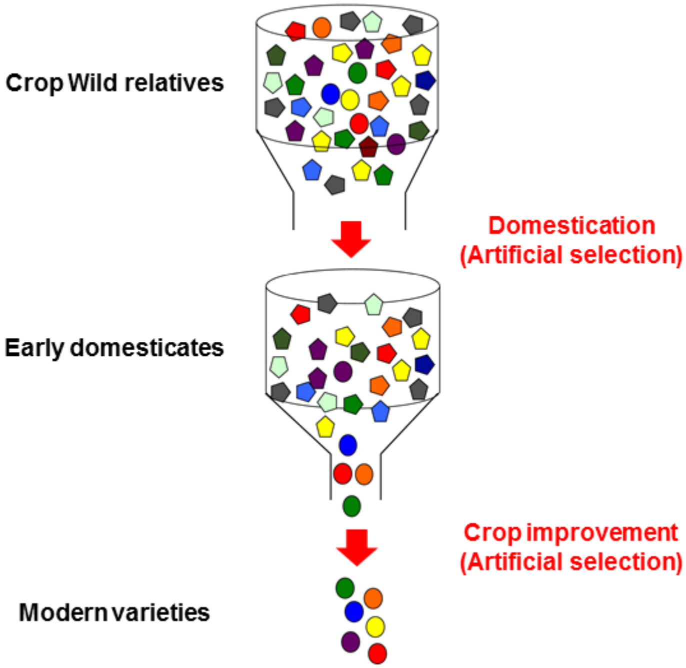

Figure 7. Reduction in genetic diversity during crop domestication process due to bottleneck events (based on Zhang et al., 2017).

Prohens et al., (2017) have suggested a novel approach, 'Introgressiomics', consisting in a mass scale development of plant materials and populations with introgression from crop relatives into the genetic background of crops. Introgressiomics can be considered as a form of advanced preemptive breeding as it aims to (i) generate introgression materials encompassing the maximum genetic diversity possible and (ii) provide breeders with such genetic diversity in a form more readily incorporable in breeding pipelines.

Introgressiomics follows a multi-step process. The first step consists in the identification of the CWRs set to be utilized, which depends on the strategy to be adopted in the program: focused or unfocused (Prohens et al., 2017). For the focused strategy, wild materials which harbor alleles that may contribute to improving a certain trait of interest are selected on the basis of genotypic, phenotypic, climatic, and eco-geographical data (Hijmans et al., 2005; Street et al., 2016), and the offspring are evaluated for that trait. For the unfocused strategy, a set of wild material with a broader representation of diversity is chosen for crossing, and the resulting progeny are then screened for traits of interest. An important aspect to consider in CWRs set creation are the genepool to which they belong, as it may affect the success of the crosses and hybridization.

In the following step, crop and selected CWRs are hybridized for creating introgression materials. Compared to the hybridization between crops and CWRs from the primary genepool, those between crops and CWRs from secondary and tertiary genepools present more difficulties due to pre-zygotic (pollen germination, development of pollen tube or fertilization) and post-zygotic (development of embryo) reproductive barriers (Khush and Brar, 1992; Prohens et al., 2017). Several techniques allow to overcome these barriers, such as stigma treatment and pollen mixture with compatible pollen for pre-zygotic, and embryo rescue and in vitro culture for post-zygotic (Prohens et al., 2017). When pre-zygotic and post-zygotic barriers cannot be overcome, the use of bridge cross method have demonstrated to be very effective in obtaining hybrid plants (Shivanna and Bahadur, 2015).

Once the interspecific hybrids have been obtained, a certain number of backcrosses are needed for developing introgression materials (Dempewolf et al., 2017). However, obtaining the interspecific hybrids does not guarantee to proceed with the development of backcross populations since they may partially or totally sterile due to genetic incompatibilities (nuclear and cytoplasmatic) and irregular chromosome pairing (Maheshwari and Barbash, 2011; De Storme and Mason, 2014; Chen et al., 2018). In the case of hybrids with partial sterility, their use as female parent can increase the success rate of fertilization, allowing to obtain viable progeny (Prohens et al., 2017). Another strategy is the duplication of the hybrid genome, which sometimes leads to the restoration of pollen fertility (Shivanna and Bahadur, 2015). This approach involves some additional steps, since the resulting hybrid is a tetraploid and, therefore, its ploidy may not coincide with that of the recurrent parent (the cultivated species). Thus, it is necessary to obtain the tetraploid version of the recurrent parent and, after one or two generations of backcrosses, restore the diploid endowment to the population by culturing anthers or microspores (Prohens et al., 2017). The low fertility of the interspecific hybrids can be gradually restored in backcross generations, with increasing levels of fertility as the genome of the recurrent cultivated parent is being recovered (Liedl and Anderson, 1993).

Finally, introgression populations, such as Chromosome Substitution Lines (CSLs), introgression lines (ILs) and multi-parent advanced generation inter-cross (MAGIC) (Cavanagh et al., 2008), are obtained through the use of marker-assisted selection. These reservoirs of wild alleles may allow to quickly obtain new generations of cultivars adapted to future challenges, including climate change.

2.3 *Speed breeding: a method to accelerate crop research and breeding*

Although CWRs are an excellent source of diversity, their use in breeding has several drawbacks. In addition to reproductive barriers that complicate CWR introduction into crops, the development of introgression populations is a time-consuming process. To accelerate applied and basic research on plants, activities such as crossing, development of experimental populations and phenotyping can be performed according to speed breeding method (Hickey et al., 2019). The term 'speed breeding' refers to the techniques that accelerate crop production by shortening generation times (Watson et al., 2018).

Temperature, photoperiod, and light are important external signals that control plant growth and development (Chen et al., 2004; Penfield, 2008). Speed breeding techniques involve the use of growth chambers and controlled environment greenhouses with optimized light quality and intensity for regulating these signals. Vegetative development occurs at a higher optimal temperature than reproductive development. Thus, higher temperatures are used to accelerate vegetative growth and lower temperature are maintained during reproductive growth (Hickey et al., 2019). Adjustment in photoperiod and light wavelength promotes flowering, triggering a transition to reproductive development (Song et al., 2015; Jähne et al., 2020). As observed during our research activity, even a limitation of plant growth space to a 1,5 L pot in association with a reduction in water supply anticipates flowering and fruit set, allowing a rapid generation cycling. Dormancy of fresh seeds can be broken through the application of germination protocols which provide seed soaking, cold stratification, and use of germination-promoting hormones (Penfield, 2017). Furthermore, integrating speed breeding and single-seed descent (SSD) method can effectively accelerate the generation of homozygous lines with fixed trait (Watson et al., 2018; Ghosh et al., 2019; Ahmar et al., 2020).

3. USE OF INTROGRESSION POPULATIONS FOR QTL MAPPING

3.1 *QTL mapping*

Most traits of agricultural relevance (productivity and quality, tolerance to environmental stress, and some forms of disease resistance) are quantitative in nature and are controlled by collective effect of numerous genes located on the same/different chromosome. The chromosomal regions having gene(s) which govern(s) the expression of the quantitative traits are known as quantitative trait loci (QTLs). Since the advent of molecular markers, breeders have aimed to identify QTLs with genetically linked DNA-markers for incorporating genes into improved cultivars via marked-assisted selection (MAS), and for better understanding the genetics of complex traits (Collard et al., 2005). The process of constructing mapping populations and conducting QTL analysis to detect an association between phenotype and the genotype of the markers is known as QTL mapping (Mc Couch, 1995; Paterson, 1996). Linkage analysis and association mapping are the two most commonly used methods for QTL mapping. In QTL mapping, markers are used to split the mapping population into different genotypic group based on the presence or absence of a particular marker locus, and correlation statistics are applied to determine whether significant differences between groups exist for the trait being measured (Young, 1996). The precision of QTL mapping largely depends on the genetic variation covered by a mapping population, the size of a mapping population, and number of marker loci used (Sehgal et al., 2016; Kulwal, 2018).

Mapping population consists of individual progenies that are originated from two or more parents of one species or related species (Boopathi, 2020). In general, parents used for developing mapping populations are highly homozygous and differ for a number of economic and agronomically important traits. The selection of a mapping population is critical to successful mapping study and depends on many factors such as plant species and its reproductive mode, traits to be mapped, and type of marker system used. In a given population do not exist an ideal number of individuals that are required to perform an accurate mapping analysis. Generally, a population of 50 – 250 individuals is used in preliminary mapping studies, while a larger population size (approximately >500) is needed for high-resolution and fine mapping (Nadeem et al., 2018). Although a large population size often results in a better resolution of QTL mapping (Broman et al., 2019), its characterization and maintenance also imply high efforts. Therefore, in establishing a population will be necessary to find the right balance between population size and efforts required for its development.

3.2 *Linkage mapping and biparental populations*

Linkage mapping analysis in plants are commonly performed using biparental populations, and generally consist of several steps: (i) developing an appropriate mapping population from two parental strains with high genetic variability and contrasting phenotype for the target trait(s), (ii) phenotyping the mapping population for trait(s) of interest under different environmental conditions (greenhouse, screen-house, and/or field conditions), (iii) selecting an adequate marker system that allows the genotyping of the population, and (iv) using an appropriate statistical method to detect and locate QTLs linked to the trait(s) of interest (Collard et al., 2005). Different biparental populations developed from the F1 hybrid can be used (Figure 8):

- F2 population: derived from the self-fertilization of F1 individuals.

- Double haploid lines (DHs): lines produced by regenerating plants through the induction of chromosome doubling from pollen grains (haploids). As only one allele is available for all the genes, DHs are completely homozygous.

- Recombinant inbred lines (RILs): inbred generation derived by selfing individual F2 plants following a single seed descent method. In each generation, recombination events reduce heterozygosity until no further segregation can occur. The result is a set of completely homozygous RILs with fragments of either parental genome. After eight or nine rounds of selfing, a level of more than 98% homozygosity is generally obtained.

- Backcross (BC) populations: obtained by F1 backcrossing to one of the parents. With each round of backcrossing, the proportion of the donor genome is reduced by 50%. BC populations can be screened directly or selfed for testing even the expression of recessive traits

- Introgression lines (ILs): their development involves several generations of backcrossing followed by two or more round of self-fertilization. The result is a series of lines that represents the complete genome of a wild or exotic donor parent in the background of a cultivated parent, where each single line solely contains a single segment of the donor.

Each of the mentioned population have its own advantages and disadvantages. Although F2 and BC populations are easy to construct and require only a short time to produce, they are considered to be temporary population and due to their high heterozygosity cannot be propagated indefinitely through seeds. On the other hand, since they are highly homozygous, DHs, RILs and ILs represent permanent populations that can be multiplied and reproduced without genetic changing occurring. This allows to conduct replicated trials across different locations and years and exchange their seeds between different laboratories, ensuring to researchers the use of the exact same material. The major disadvantage of RILs and ILs is the length of time needed for their development. Although the development of DHs takes less time than RILs and ILs, their production is only practicable in species that are susceptible to tissue culture. Furthermore, the use of wild species in the development of IL populations can lead to the introgression of undesirable traits caused by linkage drag which can be eliminated only after several round of crossing.

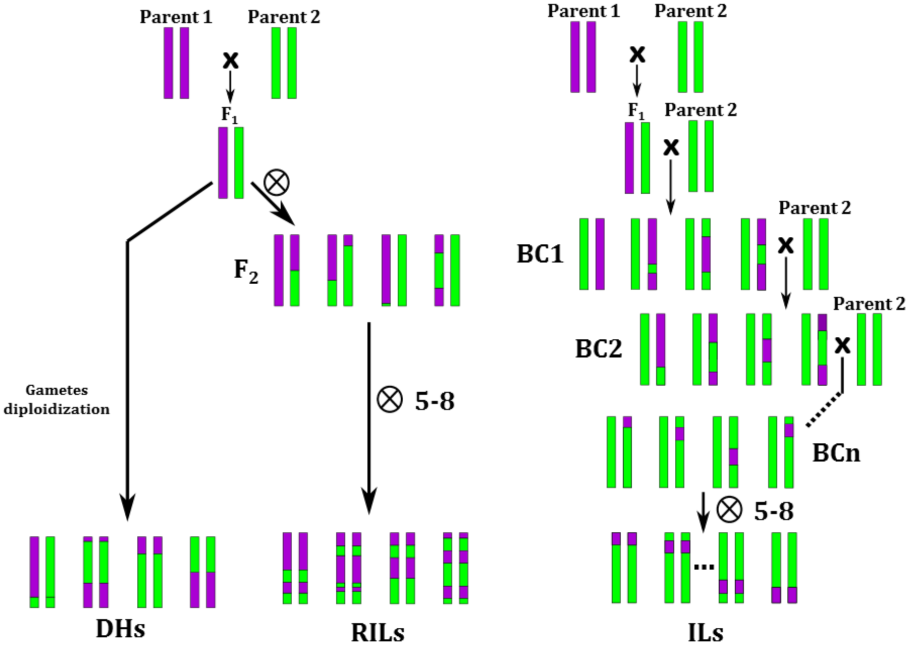

Figure 8. Representation of the breeding schemes for biparental populations development. Double haploids (DHs), F2, and recombinant inbred lines (RILs) are developed by successive generations of selfing (left side). Backcross populations (BC) are obtained by F1 backcrossing to one of the parents, while introgression lines (ILs) are developed by subjecting advanced BC population (BCn) to several rounds of self-fertilization (right side).

Despite the common use in mapping studies of all mentioned biparental populations, ILs have demonstrated a greater efficiency in QTL estimation compared to the others (Boopathi, 2020). The main reasons are:

- Depending on the average introgression segment size of the ILs (i.e., the resolution of the ILs population), QTL can be mapped into a smaller interval compared to classical QTL mapping.

- Due to the small portion of the wild genome present in each line, epistatic and linkage drag effect are reduced, and thus, phenotypic variations between ILs can be attributed with high accuracy to a specific introduced segment.

- QTL may be divided into separate monogenic components, making the measurement of phenotypic traits more reliable.

- ILs can be crossed to different tester lines allowing to explore the effects of heterozygosity on the phenotype.

3.3 *Association mapping and multiparental populations*

Although linkage mapping has proved and remains a powerful approach for dissecting genes underlying complex traits, it suffers from some limitations. First of all, allelic variation is restricted to genetic base of the two parents used for creating the mapping population. Furthermore, QTL resolution is limited to 10-30 cM in F2, BC1, DH and RILs populations, as the analysis mainly depend on the small number of recombination events resulting from fewer generations to establish this populations (Hall et al., 2010; Pascual et al., 2015; Cockram and Mackay, 2018; Han et al., 2020). In addition, in some species the creation of mapping populations is not possible or takes longer than expected, retarding the dissection of complex traits (Neale and Savolainen, 2004).

The limitations of linkage mapping can be overcome through association mapping, a population-based method aimed to identify trait–marker relationships based on linkage disequilibrium (LD). Association mapping takes advantages of high genetic diversity of large and more representative natural populations including collections of selected individuals with unknow kinship (i.e., panels), which have undergone many generations of recombination (Korte and Farlow, 2013; Varshney et al., 2018; Ibrahim et al., 2020; Kaur et al., 2021). Therefore, QTLs are identified based on historical recombination events between SNPs and QTL at the population level, assuming that only markers that are in LD with the trait of interest will remain associated with the trait over multiple generations of recombination (Nordborg and Tavaré, 2002). As a result, mapping resolution increases, and causal polymorphism within a gene that is responsible for the difference in two alternative phenotypes can be identified (Sehgal et al., 2016). However, in association mapping it is important to account for relatedness and population structure, which can increase the rate of false positives results (spurious associations) (Platt et al., 2010; Vilhjalmsson and Nordborg, 2013; Korte and Farlow, 2013).

Important steps in association mapping include: (i) generation of a mapping population consisting of individuals from a germplasm collection covering a broad genetic diversity, (ii) phenotypic characterization of the individuals, preferably in different environments and multiple replications, (iii) genotyping the mapping population with molecular markers, (iv) measurement of LD extent and estimation of the population structure and kinship, and (v) application of an appropriate statistical method based on LD quantification, population structure and correlation between genotypic and phenotypic data to identify ‘marker tags’ located within closed proximity of targeted trait.

With the rapid development and decreasing cost of advanced genomic and bioinformatics techniques, genome-wide association studies (GWAS) have become a powerful tool for unraveling the molecular genetic basis underlying the natural phenotypic variation (Alqudah et al., 2020). The application of GWAS for a more precise mapping of genes related to complex agronomic traits have been carried out in many crops (Tian et al., 2019; Saini et al., 2021). However, its efficiency is limited when pedigrees and parental information are unknown or not available (Dell'Acqua et al., 2015). Furthermore, GWAS have low power for rare alleles conferring interesting phenotypes, since markers used are often excluded based on the minor allele frequency (usually a MAF of < 5%) (Gupta et al., 2019). In order to increase the power of fine mapping, the use of large sample size or the construction of multiparent populations may be helpful (Huang and Han, 2014).

Multiparent populations represent a bridge between linkage mapping and association mapping approaches (Dell’Acqua et al., 2015; Arrones et al., 2020). They are produced by crossing more than two inbred founder lines through different cross designs, resulting in greater genetic diversity, smaller haplotype blocks and higher mapping power than biparental populations (Valdar et al., 2006; Klasen et al., 2012). Various kind of multiparent populations can be used in GWAS, but in the framework of this doctoral thesis I focused on multiparent advanced generation inter-cross (MAGIC) populations.

3.4 *MAGIC populations*

In MAGIC populations, multiple inbred founders (typically from 4 to 16) are intermated for several generation prior to creating inbred lines (panels of RILs) whose genomes are fine-scale mosaics of contributions from all founders. In this way, MAGIC populations incorporate higher allelic diversity than biparental population and the power of QTL detection increases due to greater opportunities for recombination given by multiple intercross cycles between parents. Therefore, MAGIC populations embody a kind of intermediate status between the extreme simplicity of diallelic system of RILs and the much greater complexity of the natural accessions (Han et al., 2020).

An important decision to take before initiating MAGIC population development is the selection of the founder lines, which should cover a wide phenotypic, genetic, and geographic diversity in order to achieve a highly representative panel and exploit the potential of the population (Huang et al., 2015; Cockram and Mackay, 2018; Diouf and Pascual, 2021). The use of founders which are previously re-sequenced and characterized at molecular level allows to better design strategies for identifying causal polymorphisms (Pascual et al., 2015; Arrones et al., 2020). In addition to commercial cultivars or elite breeding lines with desirable traits, wild relatives represent promising materials to be used as founders in MAGIC populations. On the other hand, their use as founders to broaden the genetic diversity can generate genetic incompatibility, which may lead to large reduction in the number of progenies especially in early generations (Kover et al., 2009; Huang et al., 2015; Arrones et al., 2020). Therefore, the choice of founders must ensure not only a high genotypic and phenotypic diversity but also the production of a resource that is practical to manage.

Once selected, the founders are intercrossed following different mating designs to create a MAGIC population. Generally, two main breeding schemes are used (Arrones et al., 2020) (Figure 9):

- funnel scheme: a number of (n) parental lines are inter-crossed for several generation to obtain n/2 F1 hybrids, which are subsequently intercrossed in a set mating design to combine the genomes of all founders in the progeny lines.

- diallel scheme: multiple parents are crossed to produce all possible bi-parental F1s, which are subsequently inter-crossed until all founders are represented in a single generation.

Regardless of the breeding scheme used, the final aim is the achievement of lines with a high degree of homozygosis, whose genomes theoretically comprise contributions from each of the founders in equal proportions (Diouf and Pascual, 2021). In this way, the mixed lines from different funnels are randomly and sequentially intercrossed to increase the number of recombinations. Finally, the individuals resulting from the inter-crossing stage are progressed to create highly homozygous individuals via SSD or DH production (Figure 9). While the production of DH is often the faster way to create homozygous populations, multiple rounds of selfing can introduce additional recombination, although less than during the initial and advanced stages of inter-crossing (Huang et al., 2015). When the highly homozygous individuals are obtained by self-fertilization after hybridization, more than five generations of SSD are required, resulting in RILs with an expected level of genome heterozygosity less than 3% (Huang et al., 2015).

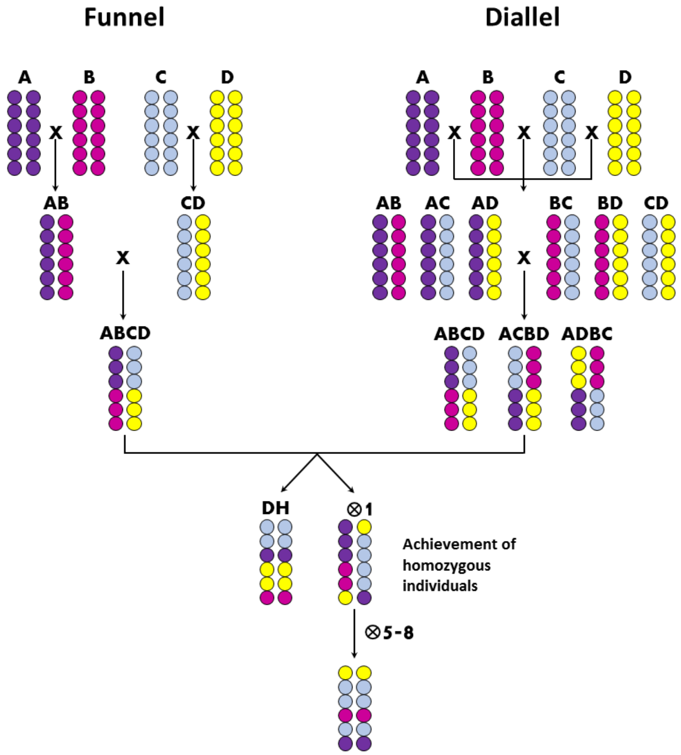

Figure 9. Creation of a 4-way MAGIC population using "funnel" or "diallel" scheme and achieving homozygous individuals by doubled haploids (DH) production, or by several rounds of selfing following the single-seed descent (SSD) method (based on Arrones et al., 2020).

Magic populations have multiple advantages compared to bi-parental and natural populations. They represent immortal resources that offer a more high-resolution mapping of genes and QTLs. In fact, the high number of recombination events occurring during the multiple rounds of inter-crossing and selfing reduces the LD and allows the identification of strong gene-trait associations with greater resolution (Cavanagh et al., 2008; Sallam and Martsch, 2015; Scott et al., 2020). Furthermore, the use of multiple founders confers a high degree of genotypic and phenotypic diversity to a single population and promotes new combinations of alleles (Chen et al., 2013; Huang et al., 2015). Magic populations are also convenient system in which to study interactions (epistasis and G x E) and correlations between traits, and the extent to which their genetic basis is shared (Scott et al., 2020; Diouf and Pascual, 2021). In addition, they provide a promising and useful germplasm to be included in breeding pipeline as pre-breeding materials or directly released as new cultivars (Chen et al., 2013; Ongom and Ejeta, 2017; Scott et al., 2020).

Nevertheless, MAGIC populations have some disadvantages linked to their development. Their great limitation are the higher cost and the longer time required for their development due to the large number of founder lines and longer generations to obtain a homozygous lines (Mackay et al., 2014). The utilization of multiple founders in population development makes the process logistically challenging and requires a high number of molecular markers to analyze the complex genetic structure of the population. To ensure high QTL mapping power, MAGIC populations require larger population size compared to bi-parental populations, which not only hinders the population development but also limits the phenotype to be evaluated (Samantara et al., 2020). Genetic incompatibility of the founders could result in a drastic decrease in the number of progenies, which reduces genetic diversity and give rise to population structure (Koover et al., 2009). Another negative factor is the complexity of the breeding crossing scheme, which may make the management of the crosses difficult due to the numbers of individuals needed in each generation (Templeton, 2006). This problem may increase the chance of mating individual genotypes with similar genotypes, thus resulting in assortative mating which induces the creation of genetic subgrouping and LD distortion (Garnier-Géré et al., 2013). Therefore, given the complexity of the design, the time investment required to development and a number of factors that eventually influence the diversity, power, and resolution of a MAGIC population, is better to clearly define the objectives for the population before undertaking its development (Huang et al., 2015; Boopathi, 2020; Diouf and Pascual, 2021).

3.5 *An overview of the main IL populations developed and evaluated*

In the last decades, numerous IL populations, in some cases mentioned as near isogenic lines (NILs), chromosome segment substitution lines (CSSLs), recombinant chromosome substitution lines (RCSLs) or single segment substitution lines (SSSLs), have been developed in major crops (Table 3). Among the IL populations developed for rice, five (Tian et al., 2006; Cheema et al., 2008; Furuta et al., 2014; Arbelaez et al., 2015; Qin et al., 2019) and two (Arbelaez et al., 2015; He et al., 2017) of them, for which *Oryza rufipongon* and *O. meridionalis*, respectively, were used as the donor parent, allowed the evaluation and the detection of many QTLs related to yield, pericarp color, and agronomic traits. Several QTLs for yield-related traits have been identified in rice also using IL populations obtained through hybridization with *O. glumaepatula* (Rangel et al., 2008), *O. nivara* (Ma et al., 2016), *O. longistaminata* (Ramos et al., 2016), and *O. Barthii* (Bessho-Uehara et al., 2017). An IL population with introgression from *O. minuta* have been developed and tested to look for genomic region associated with resistance to blast blight, bacterial blight, and brown and whitebacked planthopper (Guo et al., 2013). QTLs for plant height and purple apiculus have been mapped in a ILs population of ZhangPu wild rice (Yang et al 2016).

ILs population were developed exploiting synthetic hexaploidy wheat (tetraploid wheat x *Aegilops tauschii*; Pestsova et al., 2006; Liu et al., 2006; Huang et al., 2003, 2004; Ibrahim et al., 2012) or synthetic octaploid wheat (hexaploid wheat x *A. tauschii*; Zhang et al., 2018) as a 'bridge' for transferring some superior genes of *A. tauschii* into common wheat. Using these populations, more than 250 QTLs related to agronomic traits and drought tolerance were identified and located.

A maize IL population was developed to investigate the effects of the introgression of genetic material of the wild species *Zea mays mexicana* on ear traits, protein contents and endosperm amino acid composition (Wang et al., 2008). QTLs involved in flooding tolerance have been identified by analyses of ILs derived from crosses between maize inbred line Mi29 and *Z. nicaraguensis* (Mano and Omori, 2013). Liu et al., (2016) used a population of a teosinte NILs derived from ten *Z. parviglumis* accessions in the B73 background to examine the genetic architecture of kernel traits.

In barley, Matus et al., (2003) developed a set of 140 RCSLs using an accession *of Hordeum vulgare* ssp*. spontaneum* (Cesarea 26-24) as donor parent and *H. vulgare* ssp. *vulgare* Harrington as recurrent parent with the aim to study the effect of the wild introgressions on traits related to yield, malting quality and domestication. QTLs for agronomic traits were detected by Hori et al., (2005) in a set of RCSLs generated from the initial cross between *H. vulgare* ssp*. spontaneum* strain H602 and the Japanese malting barley Haruna Nijo. von Korff et al. (2004) developed two sets of candidate introgression lines (pre-ILs) by introgressing exotic segments from the exotic barley accession ISR42-8 into two spring barley genetic backgrounds (cultivars Thuringia 42 and Scarlett 42). The set of Scarlett 42 pre-IL was later evaluated with regard to disease resistance, agronomic performance, and malting quality (von Korff et al., 2005, 2006, 2008). Schmalenbach et al., (2008) developed the first ILs population in barley by submitting the above-mentioned Scarlett pre-ILs to additional rounds of backcrossing and selfing, and in parallel, marker-assisted selection. The final population, which consisted of 59 ILs, were used for verification of QTLs for disease resistance, agronomic traits, and malting quality (Schmalenbach et al., 2008, 2009a, 2009b).

A great number of IL populations have been developed in tomato. The first tomato IL population has been developed for *S. pennellii* (Eshed and Zamir, 1994). Over time, this population has been extensively exploited for mapping studies, allowing the identification of over 3000 QTL (Sol Genomics Network; http://solgenomics.net). IL populations have been developed for other tomato wild species, including *S. habrochaites* (Monforte and Tanksley, 2000a; Finkers et al., 2007) and *S. lycopersicoides* (Canady et al., 2005). These populations have been extremely useful for identifying QTLs for a wide variety of traits (Gorguet et al., 2008; Li, 2010, 2011; Santos, 2015; Tripodi et al., 2020), studying QTL x genetic background and QTL x QTL interactions (Monforte et al., 2001), and QTL fine mapping (Monforte and Tanksley, 2000b). In addition, three IL populations have been obtained by introgression of chromosomal segments of *S. pimpinellifolium*, contributing to characterize and map QTLs for fruit quality traits (Barrantes et al., 2014, 2016; Celik et al., 2017; Di Giacomo et al., 2020). Recently, Chetelat et al. (2019) developed two IL populations for transferring *S. sitiens* alleles into tomato and evaluating the effect of the wild introgression on major morphological traits.

In pepper, lines containing introgression from wild species were generated and used for genetic studies, although the coverage of the donor genome was not complete and/or the lines contained multiple exotic introgressions simultaneously (Chaim et al., 2003a, 2003b; Rao et al., 2003; Zygier et al., 2005). Only recently, a set of NILs derived from *Capsicum baccatum* ssp. *pendulum* with *C. annuum* parents (Eggink et al., 2014) and an IL population with introgression from wild-type bell pepper CA157 into genetic background of *C. annuum* CA52 (Ji et al., 2020) have been developed and used for the detection and validation of loci controlling, respectively, biochemical, sensory and physical traits, and response to low temperature.

Table 3. Current status of the main IL populations developed and used for QTLs detection in crops.

Crop	**Parents**		**Final population**	**Reference**	**Target traits**
	Recurrent	**Donor**			
Rice					
	O. sativa ssp. *indica* 'Guichao 2'	*O. rufipogon* 'DXCWR'	159 CSSLs	Tian et al., 2006	yield
	O. sativa ssp. *indica* 'IR64'	*O. rufipogon* 'IRGC 105491'	105 CSSLs	Cheema et al., 2008	yield
	O. sativa ssp. *japonica* 'Koshihikari'	*O. rufipogon* 'W0106'	33 CCSLs	Furuta et al., 2014	agronomic traits
	O. sativa ssp. *japonica* 'Curinga'	*O. rufipogon* 'IRGC 105491'	48 ILs	Arbelaez et al., 2015	agronomic traits, pericarp color
	O. sativa ssp. *indica* '9311'	*O. rufipogon* 'LSWR'	77 ILs	Qin et al., 2019	agronomic traits
	O. sativa ssp. *japonica* 'Curinga'	*O. meridionalis* 'Ng.-W2112'	32 ILs	Arbelaez et al., 2015	agronomic traits, pericarp color
	O. sativa ssp. *indica* 'Huajingxian 74'	*O. meridionalis* ('IRGC104093', 'IRGC105286', 'IRGC105293' and 'IRGC105291')	99 SSSLs	He et al., 2017	agronomic traits
	O. sativa ssp. *indica* 'BG90-2'	*O. glumaepatula* 'RS-16'	35 ILs	Rangel et al., 2008	yield
	O. sativa ssp. *indica* '93-11'	*O. nivara* 'W2014'	131 ILs	Ma et al., 2016	yield
	O. sativa ssp. *japonica* ''Taichung 65'	*O. longistaminata* 'IRGC 110404'	40 CSSLs	Ramos et al., 2016	yield
	O. sativa ssp. *japonica* 'Koshihikari'	*O. barthii* 'W0009'	38 CSSLs	Bessho-Uehara et al., 2017	yield
	O. sativa ssp. *indica* 'IR24'	*O. minuta* '101133'	131 ILs	Guo et al., 2013	resistance to disease (bacterial blight, brown planthopper and whitebacked planthopper)
	O. sativa ssp. *indica* 'Dongnanihui 810'	ZhangPu' wild rice	146 CSSLs	Yang et al., 2016	plant height and purple apiculus
Wheat					
	T. aestivum 'Chinese Spring'	'Synthetic 6x'	84 ILs	Pestsova et al., 2006	agronomic traits
	T. aestivum 'Laizhou953'	'Am3'	94 CSSLs	Liu et al., 2006	agronomic traits
	T. aestivum 'Prinz'	'W-7984'	72 lines	Huang et al., 2003	agronomic traits
	T. aestivum 'Flair'	'XX86'	111 lines	Huang et al., 2004	agronomic traits
	T. aestivum 'Triso'	'Syn084'	223 lines	Ibrahim et al., 2012	drought tolerance
	T. aestivum 'Zhoumai 18'	'T015'	379 ILs	Zhang et al., 2018	agronomic traits
Maize					
	Z. mays ssp. mays 'Ye515'	*Z. mays* ssp. *mexicana*	52 ILs	Wang et al., 2008	ear traits, protein contents, endosperm amino acid composition
	Z. mays ssp. *mays* 'Mi29'	*Z. nicaraguensis* 'CIMMYT 13451'	45CSSLs	Mano and Omori, 2013	flooding tolerance
	Z. mays ssp. *mays* 'B73'	10 accession of *Zea mays* ssp. *parviglumis*	928 NILs	Liu et al., 2016	kernel traits
Barley					

	H. vulgare ssp. *vulgare* 'Harrington'	*H. vulgare* ssp. *spontaneum* 'Cesarea 26-24'	140 RCSLs	Matus et al., 2003	yield, domestication, malting quality
	H. vulgare ssp. *vulgare* 'Haruna Nijo'	*H. vulgare* ssp. *spontaneum* 'H602'	134 RCSLs	Hori et al., 2005	agronomic traits
	H. vulgare ssp. *vulgare* 'Thuringia 42'	*H. vulgare* ssp. *spontaneum* 'ISR42-8'	84 pre-ILs	von Korff et al., 2004	-
	H. vulgare ssp. *vulgare* 'Scarlett 42'	*H. vulgare* ssp. *spontaneum* 'ISR42-8'	301 pre-ILs	von Korff et al., 2004	resistance to powdery mildew (von Korff et al., 2005), agronomic traits (von Korff et al., 2006), malting quality (von Korff et al., 2008)
	H. vulgare ssp. *vulgare* 'Scarlett 42'	*H. vulgare* ssp. *spontaneum* 'ISR42-8'	59 ILs	Schmalenbach et al., 2008	resistance to powdery mildew (Schmalenbach et al., 2008), agronomic traits (Schmalenbach et al., 2009a), malting quality (Schmalenbach et al., 2009b)
Tomato					
	S. Lycopersicum 'M82'	*S. pennellii* 'LA 716'	50 ILs	Eshed and Zamir, 1994	various traits (Sol Genomics Network. http://solgenomics.net)
	S. Lycopersicum 'E6203'	*S. habrochaites* 'LA1777'	99 NILs	Monforte and Tanksley, 2000a	various traits (Monforte and Tanksley 2000b; Monforte et al., 2001; Li, 2010; Tripodi et al 2020)
	S. Lycopersicum 'Moneymaker'	*S. habrochaites* 'LYC4'	30 ILs	Finkers et al., 2007	resistance to *Botrytis cinerea* (Finkers et al., 2007), parthenocarpy (Gorguet et al., 2008), salinity tolerance (Santos, 2015)
	S. Lycopersicum 'VF36'	*S. lycopersicoides* 'LA2951'	90 ILs	Canady et al., 2005	biotic and abiotic stress (Li et al., 2010, 2011)
	S. Lycopersicum 'Moneymaker'	*S. pimpinellifolium* 'TO-937'	54 ILs	Barrantes et al., 2014	Fruit quality (Barrantes et al., 2016)
	S. Lycopersicum 'Tueza'	*S. pimpinellifolium* 'LA1589'	93 NILs	Celik et al., 2017	Fruit quality
	S. Lycopersicum 'Caimanta'	*S. pimpinellifolium* 'LA0722'	22 NILs	Di Giacomo et al., 2020	Fruit quality
	S. Lycopersicum 'NC 84173'	*S. sitiens* 'LA4331' and 'LA1974'	56 ILs	Chetelat et al., 2019	morphological traits
Pepper					
	3 varieties of *C. annuum* ('MT', 'SM' and 'GNM')	*C. baccatum* ssp. *pendulum* 'PEN45'	20 NILs	Eggink et al., 2014	flavor, terpenoid content, brix level
	C. annuum 'CA52'	wild-type bell pepper 'CA157'	22 ILs	Ji et al., 2020	cold resistance

3.6 *An overview of the main MAGIC populations developed and evaluated*

Despite the considerable resource required for their development, numerous MAGIC populations have been developed in economically important crops, demonstrating their great potential in QTL mapping (Table 4). Bandillo et al., (2013) developed three 8-ways MAGIC populations in rice: japonica MAGIC (8 japonica parents); indica MAGIC (8 indica parents); and MAGIC plus (8 indica parents with two additional rounds of 8-way F1 inter-crossing). Furthermore, an additional MAGIC population (16-way Global MAGIC) is being developed by inter-crossing the indica and japonica base populations to increase the overall diversity. With the development of these MAGIC populations, their purpose was to fine map QTLs related to traits for biotic and abiotic stress tolerance, and grain quality. Thereafter, novel 12-way, 8-way, and 4-way MAGIC populations have been developed with the aim to find QTLs related to plant height, yield, grain shape and heading date (Li et al., 2014; Meng et al., 2016; Ogawa et al., 2018; Han et al., 2020).

To date, there are nine MAGIC populations available in wheat. The first two developed populations (one 4-way MAGIC and another one 8-way MAGIC) have been used to map QTLs for plant height and hectolitre weight (Huang et al., 2012). With a similar objective, two more recent MAGIC populations with 338-2125 final RILs have been developed (Milner et al., 2015; Sannemann et al., 2018). Other different MAGIC populations were used to assess QTLs for shoot length and coleoptile characteristics (Rebetzke et al., 2013), and to find candidate QTLs/genes controlling awning and flowering time (Mackay et al., 2014; Thépot et al., 2014). More recently, Shah et al., (2019) constructed an 8-way MAGIC population of more than 3000 RILs aimed to study the complex genetic architecture of recombination and structural variation in wheat.

Three MAGIC populations have been obtained in maize, of which two are 8-way with, respectively, 1636 and 700 F6 RILs (Dall'Acqua et al., 2015; Butrón et al., 2019), and one is 4-way with a final population of 1291 F4:5 RILs (Anderson et al., 2018). These populations have been useful for the identification of QTLs related to pollen shed, grain yield, ear height, flowering time, and resistance to *Fusarium* ear rot. wheat.

Three MAGIC population have been developed in barley (Sannemann et al., 2015; Bülow et al., 2019; Puglisi et al., 2021). While the first one was developed with flowering time traits as a target, the second MAGIC population focused on the study of the adaptation to climate and site-related agronomic changes, and the third aimed at examining the ability of genomic prediction models to predict grain yield.

In the case of vegetables, two MAGIC populations are already available in tomato. The first one, was developed using four accessions of cultivated tomato (*Solanum lycopersicum*) and four of weedy tomato (*S. lycopersicum* var. *cerasiforme*) as founders, with the final objective to study fruit weight (Pascual et al., 2015). The second tomato MAGIC population was developed by Campanelli et al., (2019) through inter-cross of seven *S. lycopersicum* accessions and one wild accession of *S. cheesmaniae*. In this way, they developed the first reported interspecific MAGIC population, with the main goal of exploiting the variability generated for selecting tomato genotypes adapted to different environments and agricultural practices, and ready to be released as new varieties. Furthermore, to our knowledge, the generation of a new MAGIC population in tomato, as well as of the first pepper MAGIC population, are in the final step of development at Universitat Politècnica de València (Spain). These novel population aim to dissect QTLs related to disease resistance, and agronomic and fruit quality traits.

Despite its economic and social importance, eggplant has lagged behind in the development of experimental populations. This led to a delay in the understanding of the genetics and genomics of relevant agronomic traits, which so far has been limited to genome-wide association study (GWAS) and mapping in biparental or RIL populations (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014, 2015; Wei et al. 2020; Toppino et al., 2020). Only recently, the first introgression lines population, using *S. incanum* as donor parent, have been developed (Gramazio et al., 2017). In addition, the creation of three new IL populations with introgression from *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium* are underway (García-Fortea et al., 2019, 2021; Villanueva et al., 2021). These novel resources, together with the development of more materials that broaden the genetic background of eggplant may provide new tools to support researchers in dissection of the genetic base of complex traits in this crop. Therefore, in the framework of this doctoral thesis, the first eggplant study on phenotyping and QTL analysis using an ILs set with introgression from *S. incanum* is presented. Moreover, we reported on the development of the first eggplant MAGIC population derived from inter-crosses of seven cultivated eggplant (*S. melongena*) and one wild relative (*S. incanum*) parents.

Table 4. Current status of the main MAGIC populations developed and used for QTLs detection in crops.

Crop	Design	Founders	Final RIL population	Target traits	Reference
Rice					
	8-way *japonica*, diallel	Elite and modern cultivars	500 S5	biotic/abiotic stress, grain quality	Bandillo et al. 2013
	8-way *indica,* diallel	Elite and modern cultivars	1328 S7	biotic/abiotic stress, grain quality	Bandillo et al. 2013
	8-way MAGIC plus, diallel	Elite and modern cultivars	S4 (in progress)	biotic/abiotic stress, grain quality	Bandillo et al. 2013
	16-way MAGIC global, diallel	Elite and modern cultivars	(in progress)	biotic/abiotic stress, grain quality	Bandillo et al. 2013
	12-way, funnel	Cultivars	1600 S9	plant height, heading date	Li et al. 2014
	8-way, diallel	Breeding lines	1688 S5	yield, plant height, heading date	Meng et al. 2016
	8-way, diallel	Cultivars	981 F6	grain shape	Ogawa et al. 2018
	4-way, diallel	Inbred lines	247 F7	heading date	Han et al. 2020
Wheat					
	4-way, diallel	Cultivars	1579 F6	plant height, hectolitre weight	Huang et al., 2012
	8-way, diallel	Cultivars	-	plant height, hectolitre weight	Huang et al., 2012
	4-way, diallel	Commercial cultivars	1458 F6:7	coleoptile length and thickness, shoot length	Rebetzke et al. 2013
	8-way, diallel	Cultivars	1091 F7	awning	Mackay et al. 2014
	60-way, NAM-like	Breeding lines	1000 S4	flowering time	Thépot et al. 2014
	4-way, diallel	Cultivars	338 F8	plant height, grain yield	Milner et al. 2015
	8-way, funnel	Breeding lines	516 F6:8	powdery mildew resistance	Stadlmeier et al. 2017
	8-way, diallel	Cultivars	2125 F4	plant height	Sannemann et al. 2018
	8-way, diallel	Elite lines and cultivars	>3000 S2:5	number of recombination events	Shah et al. 2019
Maize					
	8-way, diallel	Inbred lines	1636 F6	pollen shed, grain yield, plant and ear height	Dell'Acqua et al. 2015
	4-way, funnel	Inbred lines	1291 F4:5	plant height, ear height, flowering time	Anderson et al. 2018
	8-way, funnel	Inbred lines	700 F6	resistance to *Fusarium* ear rot	Butrón et al. 2019
Barley					
	8-way, funnel	Old landraces and a model cultivar	5000 DH	flowering time	Sannemann et al. 2015
	32-way, funnel	Cultivars	324 F6	climate and site-related agronomic adaptation	Bülow et al. 2019
	8-way, funnel	Old and elite varieties	352 DH	grain yield	Puglisi et al. 2021
Tomato					

	8-way, funnel	Cultivars and wild accessions	397 S3	fruit weight	Pascual et al. 2015
	8-way, funnel	Cultivars and wild accessions	400 F10	resistance genes and fruit shape	Campanelli et al. 2019
	8-way, funnel	Cultivars and wild accessions	in progress	morphoagronomic traits, resistance genes	Universitat Politècnica de València (unpublished)
Pepper					
	8-way, funnel	Landraces	in progress	fruit traits	Universitat Politècnica de València (unpublished)

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OBJECTIVES

The present doctoral thesis has two main objectives:

1. Characterization of a set of eggplant introgression lines (IL) carrying genome fragments of its wild relative *S. incanum*.

    1.1. Evaluation of morpho-agronomic traits of the set of ILs, the recipient and donor parents and the F1 hybrid, grown under two environments, and identification of stable QTLs for the traits evaluated.

    1.2. Phenomic analysis of fruit shape of the IL set, the recipient and donor parents and the F1 hybrid in two environments, and detection of QTLs and candidate genes controlling the traits evaluated.

2. Development of the first eggplant MAGIC population by intercrossing seven cultivated eggplant (*S. melongena*) and one wild relative (*S. incanum*) parents.

RESULTS

Chapter 1: Performance of a set of eggplant (*Solanum melongena*) lines with introgressions from its wild relative *S. incanum* under open field and screenhouse conditions and detection of QTLs

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1. Abstract

Introgression lines (ILs) of eggplant (*Solanum melongena*) represent a resource of high value for breeding and the genetic analysis of important traits. We have conducted a phenotypic evaluation in two environments (open field and screenhouse) of 16 ILs from the first set of eggplant ILs developed so far. Each of the ILs carries a single marker-defined chromosomal segment from the wild eggplant relative *S. incanum* (accession MM577) in the genetic background of *S. melongena* (accession AN-S-26). Seventeen agronomic traits were scored to test the performance of ILs compared to the recurrent parent and of identifying QTLs for the investigated traits. Significant morphological differences were found between parents, and the hybrid was heterotic for vigour-related traits. Despite the presence of large introgressed fragments from a wild exotic parent, individual ILs did not display differences with respect to the recipient parent for most traits, although significant genotype × environment interaction (G × E) was detected for most traits. Heritability values for the agronomic traits were generally low to moderate. A total of ten stable QTLs scattered across seven chromosomes was detected. For five QTLs, the *S. incanum* introgression was associated with higher mean values for plant- and flower-related traits, including vigour prickliness and stigma length. For one flower- and four fruit-related-trait QTLs, including flower peduncle and fruit pedicel lengths and fruit weight, the *S. incanum* introgression was associated with lower mean values for fruit-related traits. Evidence of synteny to other previously reported in eggplant populations was found for three of the fruit-related QTLs. The other seven stable QTLs are new, demonstrating that eggplant ILs are of great interest for eggplant breeding under different environments.

Keywords: *Solanum melongena*; *S. incanum*; introgression lines; stable QTL analysis; agronomic traits; G × E interaction; synteny.

2. Introduction

Eggplant (*Solanum melongena* L., Solanaceae; 2n = 2x = 24) ranks fifth among all vegetables and second, after tomato, among Solanaceae vegetables in global production (FAOSTAT, 2019). However, despite its importance, still few genetic and genomic studies have been performed in eggplant (Gebhardt et al., 2016; Gramazio et al., 2019a). In fact, even though the first draft of the genome was released in 2014, indicating a genome size of 1.13 Gb (Hirakawa et al., 2014), just last year the first high-quality chromosome-anchored reference genome with an estimated genome size of 1.21 Gb (Barchi et al., 2019a) and the first resequencing study (Gramazio et al., 2019b) were made available for the eggplant-breeding community. As well, compared to other important close crops, like tomato, potato, or pepper, eggplant has lagged behind in the development of experimental populations (Gramazio et al., 2018). This led to a delay in the understanding of the genetics and genomics of relevant agronomic traits, which so far has been limited to genome-wide association study (GWAS) and mapping in biparental populations (Frary et al., 2014; Portis et al., 2015; Barchi et al., 2019b). However, in the last years, significant efforts have been done to develop new materials that will help in dissecting complex and quantitative traits, many of them using eggplant wild relatives as a source of variation (Toppino et al., 2008; Liu et al., 2015; Kouassi et al., 2016; Plazas et al., 2016; García-Fortea et al., 2019). Only recently, the first introgression line (IL) population, using *S. incanum* L. as a donor parent, has been developed in eggplant (Gramazio et al., 2017a). *Solanum incanum* is a wild species naturally distributed in desertic and dryland areas from northern Africa to Pakistan that belongs to the secondary genepool of common eggplant (Syfert et al., 2016; Vorontsova and Knapp, 2016). The interest in this species lies in its drought tolerance (Daunay, 2008; Knapp et al., 2013), high content of bioactive phenolic compounds (Stommel and Whitaker, 2003; Ma et al., 2011; Prohens et al., 2013; Meyer et al., 2015), and resistance to some diseases (Rotino et al., 2014; Taher et al., 2017). As a result, *S. incanum* has been used for a plethora of breeding and genetic studies (Gisbert et al., 2011; Salas et al., 2011; Meyer et al., 2015; Gramazio et al., 2014, 2016, 2017b, 2019b).

In the last decade, due to the challenges of adapting agriculture to climate change, several initiatives, like "The Crop Wild Relatives (CWR) Project" (https://www.cwrdiversity.org/project/) (Dempewolf et al., 2014), were launched to unlock the potential of the unexploited eggplant crop wild relatives (CWRs). In this respect, conventional breeding and new approaches, like "introgressiomics" (Prohens et al., 2017), encourage the development of plant materials and populations with introgressions from CWRs into the genetic background of crops to foster genetic studies and to develop resilient varieties.

In this context, IL populations, which consist of a set of fixed and immortal lines that cover the totality or part of a donor parent genome carrying one or a few introgressed fragments into the genetic background of a recipient parent (Eshed and Zamir, 1994; Zamir, 2001; Eduardo et al., 2005), provide a promising opportunity to efficiently incorporate exotic natural variation in the modern breeding programs (Prohens et al., 2017; Zamir, 2001). Due to their high homozygosity, ILs are a stable resource that can be used for a multitude of genetic studies (Zamir, 2001). Indeed, in addition to the introduction of variability in the crops, IL populations have demonstrated greater efficiency in QTL estimation compared to other segregating populations such as F2, double haploid lines, and recombinant inbred lines (RILs) (Eshed and Zamir, 1995; Alonso-Blanco et al., 2006; Gur and Zamir., 2015; Yin et al., 2015). As reported by Zamir (2001), ILs allow simultaneously to carry out QTLs detection and development of improved varieties. Furthermore, the great advantage of IL sets is based on the small portion of the exotic genome present in each line, for which epistatic and linkage drag effects are reduced, and the phenotypic variation between the ILs can be attributed with high accuracy to a specific introduced segment (Zamir, 2001; Tanksley and Nelson, 1996; Ashikari and Matsuoka, 2006; Calafiore et al., 2019). Once QTLs associated with a trait of interest are localized, this information can be utilized for examining gene by gene and gene by environment (G × E) interaction, pleiotropic effects, and mapping strong QTL effects (Gur and Zamir., 2015; Eshed and Zamir, 1996). However, up to now, IL populations have been developed mainly in major cultivated crops (Jena et al., 1992; Pestsova et al., 2004; Szalma et al., 2007) and regarding Solanaceae almost exclusively in tomato (Eshed et al., 1992; Monforte et al., 2000; Chetelat et al., 2019).

The advantage of using IL populations for QTL identification has been demonstrated in many studies. For example, the *S. pennellii* IL population (Eshed and Zamir, 1994) allowed so far, the identification of almost 3000 putative QTLs for different traits, like morphology, stress tolerance, yield, fruit colour, and bioactive compounds (Eshed and Zamir, 1995; Schauer et al., 2006; Rigano et al., 2014; Alseekh et al., 2015; Krause et al., 2018). The screening of ILs led to a plethora of QTL identification studies in many important crops, like rice, maize, and barley, among others, and for a wide variety of different traits (Salvi et al., 2011; Ma et al., 2016; Qiu et al., 2017; De Leon et al., 2017; Honsdorf et al., 2017; Qin et al., 2019). Detection of stable QTLs, i.e., those that are detected in different environments, is particularly relevant for breeders, as its introgression allows genetic advances irrespective of the existence of G × E interaction (Zhao et al., 2016).

In the present paper, we describe the first eggplant study on phenotyping and QTL analysis using an IL population with introgressions from an eggplant wild relative (Gramazio et al., 2017a). The results will provide relevant information on the phenotypic characteristics of the ILs with introgressions from a wild relative and may allow identifying stable QTLs for important traits.

3. Materials and methods

Plant Material

From the IL population of *Solanum incanum* (MM577) developed in the *S. melongena* (AN-S-26) background (Gramazio et al., 2017a), a set of 16 ILs were selected based on a maximization of representation of the genome of *S. incanum* and on seed availability (Figure 1). The recipient parent, AN-S-26, is a non-prickly Spanish local variety of eggplant from the region of Andalusia that has anthocyanin pigmentation in several vegetative parts of the plant, such as stems and leaf veins, and has large obovoid purple fruits. The donor parent, MM577, collected in a desertic region in Israel is prickly, particularly in the calyx, and produces small green rounded fruits. Both accessions and the IL population are maintained at the Universitat Politècnica de València eggplant pre-breeding collection.

Excluding chromosomes 6 and 11, for which no ILs were available, each chromosome was represented by at least one IL, being two for chromosomes 1 and 4 and three for chromosomes 3 and 7 (Table 1 and Figure 1). Individual ILs carried from 0.1% to 10.9% of the donor parent genome, with an average of 4.83%, and covered altogether 58.57% of the *S. incanum* genome. The average size of the introgressions in the chromosomes was 55.94 Mb, with a range of 2 to 125 Mb, being chromosome 10 the less covered (1.8%) and chromosome 8 the most covered (97.2%) (Table 1).

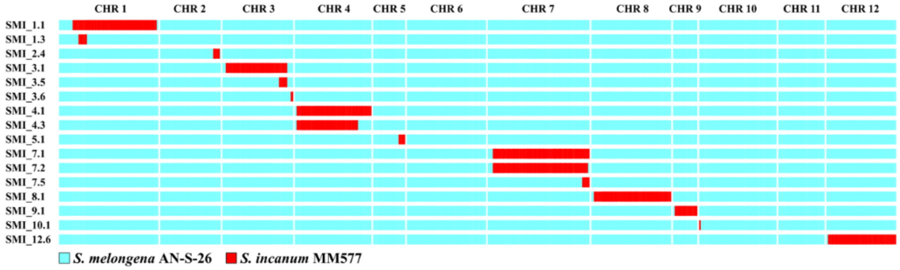

Figure 1. Graphical genotypes of the selected ILs for QTLs identification: The rows indicate IL codes, and the columns indicate the chromosomes. Homozygous introgressions of *S. incanum* MM577 are depicted in red, while the genetic background of the recipient parent (*S. melongena* AN-S-26) is depicted in blue.

Table 1. Statistics of the 16 selected *S. incanum* (MM577) ILs in the genetic background of *S. melongena* (AN-S-26) calculated using the physical distance of the chromosome-anchored reference genome (Barchi et al., 2019a): For further details about the marker-assisted selection of the introgressions, refer to Gramazio et al. (2017a).

ILs	Chr.	Donor parent (%)	IL size (Mb)	IL position (Mb)	Chr. IL size (%)	Total chr. ILs size (Mb)	Total chr. ILs size (%)
SMI_1.1	1	9.9	114	19–133	83.8	114	83.8
SMI_1.3	1	0.7	9	27–36	6.5		
SMI_2.4	2	0.5	6	75–81	7.2	6	7.2
SMI_3.1	3	6.9	79	7–86	81.4	82	84.5
SMI_3.5	3	0.6	8	78–86	8.3		
SMI_3.6	3	0.2	3	93–96	3.1		
SMI_4.1	4	7.0	81	4–105	96.1	101	96.1
SMI_4.3	4	8.8	101	4–85	75.2		
SMI_5.1	5	0.6	8	35–43	18.6	8	18.6
SMI_7.1	7	10.5	121	14–139	88.0	125	88.0
SMI_7.2	7	10.9	125	14–135	85.2		
SMI_7.5	7	0.8	10	129–139	7.0		
SMI_8.1	8	9.2	106	3–109	97.2	106	97.2
SMI_9.1	9	2.5	29	5–34	64.4	29	64.4
SMI_10.1	10	0.1	2	0–2	1.8	2	1.8
SMI_12.6	12	8.1	93	3–96	93.0	93	93.0
Mean		4.8	55.9		51.1	66.6	63.5
Total						666.0	

Field Cultivation and Phenotypic Evaluation of The Traits

Seeds were germinated in Petri dishes, following the protocol developed by Ranil et al. (2015), and subsequently transferred to seedling trays in a climatic chamber under photoperiod and temperature conditions of 16 h light (25 °C) and 8 h dark (18 °C). Field tests were conducted in the campus of the Universitat Politècnica de València, Spain (GPS coordinates: latitude, 39° 28’ 55” N; longitude, 0° 20’ 11” W; 7 m above sea level). Five plants of each of the two parents, the F1 hybrid, and that of the 16 ILs were grown under two different conditions (open field and screenhouse) using a randomized complete block design with five blocks per condition. Plants were spaced 1.5 m between rows and 1.2 m within rows. Water and nutrients were provided through the irrigation system, and pruning was done manually to regulate vegetative growth and flowering. Phytosanitary treatments against spider mites and whiteflies were performed when necessary. During the study period, the average temperature varied between 17.0 °C and 37.9 °C in the open field and between 18.0 °C and 40.0 °C in screenhouse. The levels of relative humidity ranged from 24.1% to 87.1% (open field) and from 26.9% to 94.7%

(screenhouse). The plants were phenotyped using 17 conventional morphological descriptors related to plant, leaves, flower, and fruit using Eggplant Genetic Resources Network (EGGNET) descriptors (Van Der Weerden and Barendse, 2007) (Table 2).

Table 2. List of traits used for ILs characterization with their abbreviations and units.

Descriptor code	Trait	Descriptor scale/unit
Plant descriptors		
PH	Plant height 1 month after transplanting	cm
SD1	Stem diameter 1 month after transplanting	cm
SD5	Stem diameter 5 months after transplanting	cm
SP	Stem prickles	0–9[a]
Leaf descriptors		
LCC	Leaf chlorophyll concentration	SPAD unit
LBL	Leaf blade Lobing	0–9[b]
LSS	Leaf surface shape	1–9[c]
LPU	Leaf prickles on the upper surface	0–9[a]
LPL	Leaf prickles on the lower surface	0–9[a]
Flower descriptors		
CD	Corolla diameter	cm
PL	Peduncle length	cm
SL	Stigma length	cm
FLCP	Flower calyx prickles	0–9[a]
Fruit descriptors		
FCL	Fruit calyx length	cm
FPL	Fruit pedicel length	cm
TY	Total yield	g
FW	Fruit weight	g

[a]Measured according to the following scale: 0 = none; 1 = very few; 3 = few; 5 = intermediate; 7 = many; 9 = very many. [b]Measured according to the following scale: 1 = very weak; 3 = weak; 5 = intermediate; 7 = strong; 9 = very strong. [c]Measured according to the following scale: 1 = very flat; 3 = flat; 5 = intermediate; 7 = bullate; 9 = very bullate.

Data analysis

For each trait measured, mean values of the parents (MP) were calculated for both environments. Statistical differences between parents were tested with ANOVA. Mid-parent heterosis values (H_{MP}) were estimated using the equation:

$$(1)\quad H_{MP}\ (\%) = ((F1 - MP)/MP) * 100$$

where F1 is the performance of the F1 hybrid. To determine the significance of heterosis, Student's t-test was carried out at the significance level of $p < 0.05$.

In order to evaluate differences among ILs and the cultivated parent AN-S-26, one-way analyses of variance (ANOVA) were used to evaluate differences among genotypes (G) in either open field or screenhouse environments, while two-way ANOVA was used to analyze IL data to test the differences between genotypes (G), environments (E), and genotype × environment interaction (G × E). Estimation of broad-sense heritability (H^2) was performed for each trait and environment by calculating the variance components from the mean squares (MS) within and between the ILs with a hierarchical ANOVA using the following formulas (Wricke and Weber, 1986):

For one environment (open field or screenhouse):

$$(2)\quad H^2 = V_G/(V_G + V_E)$$

For both environments (open field and screenhouse):

$$(3)\quad H^2 = V_G/(V_G + V_E + V_{G\times E})$$

All the statistical analyses were performed using the Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA).

QTL detection

For QTL detection, the mean of the replicates for each trait, IL, and environment was compared with the recipient parent AN-S-26 (SM) using a Dunnett's test at $p < 0.05$. QTL detection was assumed when the means of the ILs varied significantly from the recipient parent in both environments. The relative increase over the recipient parent and allelic effects were calculated as follows:

$$(4)\ \text{Increase over SM (\%)} = (\text{mean (IL)} - \text{mean (SM)}/\text{mean (SM)}) \times 100$$

$$(5)\ \text{Allelic effect} = (\text{mean (IL)}/\text{mean (SM)})/2$$

4. Results

Parents Phenotype and Heterosis

Significant differences ($p < 0.05$) between the recipient parent (*S. melongena* AN-S-26) and the donor parent (*S. incanum* MM577) of the IL population were found for all the traits in both environments, except for leaf blade lobing (LBL), in which no differences were detected for any of the environments, and for plant height (PH) and leaf chlorophyll concentration (LCC), for which significant differences were observed only in the open field (Table 3 and Supplementary data S1).

Stem diameter values after 1 month (SD1) and 5 months (SD5) since transplanting were, respectively, lower and higher in MM577 than in AN-S-26 (Table 3). In both environments, MM577 showed higher levels of prickliness (SP, LPU, LPL, and FLCP), smaller corolla diameter (CD), shorter peduncle (PL), more bullate leaves (LSS), and longer stigma (SL) than AN-S-26. In the open field, MM577 exhibited a much lower yield (TY, 28.40 g versus 1919.40 g of AN-S-26) with smaller fruits (FW, 3.73 g versus 73.10 g), shorter pedicel (FPL, 1.92 cm versus 7.66 cm), and calyx (FCL, 1.22 cm versus 6.33 cm). MM577 did not set fruit in the screenhouse, and therefore, the comparison of both parents under screenhouse was not possible (Table 3).

The hybrid displayed significant positive values for heterosis over the mid-parent for vigour traits (PH, SD1, and SD5); prickliness (SP, LPU, LPD, and FLCP); and LCC, LBL, CD, and SL in both environments (Table 3). Significant positive values of heterosis ranged from 22.8% (CD) to 900.0% (LPU and LPD) in open field and from 12.3% (LCC) to 900.0% (LPU and LPD) in the screenhouse. LSS displayed significant negative values for heterosis under both environments (−66.6% in both environments). For fruit traits evaluated only in the open field, due to the lack of fruit set in screenhouse, significant negative heterosis was observed for FPL and FW (−43.4% and −55.4%, respectively) and no significant values were observed for differences in FCL and TY.

Table 3. Comparison of the mean values of the recipient parent *S. melongena* AN-S-26 (SM) and the donor parent *S. incanum* MM577 (SI) and hybrid mid-parent heterosis (H_{MP}) in the open field and screenhouse conditions.

Trait	Open field			Screenhouse		
	SM	**SI**[a]	**H_{MP} (%)**[a]	**SM**	**SI**[a]	**H_{MP} (%)**[a]
Plant						
PH	45.78	35.24*	28.8*	48.04	47.34^{ns}	50.1***
SD1	1.28	0.87*	38.0*	1.08	0.88*	38.5**
SD5	1.60	2.24***	100.6***	1.37	1.82**	107.3***
SP	0.00	9.00***	100.0***	0.00	9.00***	100.0***
Leaf						
LCC	48.44	55.49*	35.1*	47.35	48.75^{ns}	12.3**
LBL	5.00	5.00^{ns}	40.0***	5.00	5.00^{ns}	40.0***
LSS	1.00	5.00***	−66.6***	1.00	5.00***	-66.6***
LPU	0.00	1.00***	900.0***	0.00	1.00***	900.0***
LPL	0.00	1.00***	900.0***	0.00	1.00***	900.0***
Flower						
CD	4.71	3.58*	22.8**	4.95	3.84***	21.8**
PL	2.85	1.76*	15.5^{ns}	2.68	1.87**	11.4^{ns}
SL	0.20	0.60*	80.4*	0.22	0.69**	67.8***
FLCP	0.00	5.00***	260.0***	0.00	5.00***	260.0***
Fruit						
FCL	6.33	1.22***	$−10.1^{ns}$	5.39	na	na
FPL	7.66	1.92***	−43.4***	7.27	na	na
TY	1,919.40	28.40**	$−3.5^{ns}$	1,429.20	na	na
FW	73.10	3.73***	−55.4***	64.58	na	na

[a] ***, **, *, ^{ns} indicate respectively, significantly difference from SM at *p* values <0.001, <0.01, and <0.05 or not significative (*p* ≥ 0.05); na = data not available.

Analysis of Variance and Heritability

Five qualitative traits, one related to plant (SP) and four related to leaves characteristics (LBL, LSS, LPU, and LPL) displayed variation neither within or among ILs and the *S. melongena* parent nor between environments. Therefore, they were not subjected to ANOVA. Significant ($p < 0.05$) F-values (ranging from 2.12 to 11.86) were observed in the open field between genotypes for all the 12 remaining traits (PH, SD1, SD5, LCC, CD, PL, SL, FLCP, FCL, FPL, TY, and FW) (Table 4). Similar results, with significant F-values ranging from 2.45 to 18.82 were detected in screenhouse for 11 traits (PH, SD1, SD5, LCC, PL, SL, FLCP, FCL, FPL, TY, and FW), except for CD which had a nonsignificant F-value. A wide range of heritability (H^2) values was obtained in each of the environments, ranging from 0.18 (TY) to 0.69 (FPL) and from 0.09 (CD) to 0.78 (SL), respectively, in the open field and screenhouse (Table 4).

The two-way ANOVA revealed significant differences between genotypes for all traits. Differences between environments were statistically significant for all traits, except for SD1, PL, and FCL. The G × E interaction was also significant for all traits except for LCC, FCL, and FW, although significant F-values displayed a narrower range (from 1.74 to 2.95) than the genotypic or environmental effects. Genotypic and environmental differences were more significant than G × E interaction for LCC, FLCP, TY, and FW and showed similar statistical significance for PH ($p < 0.001$). The statistical significance of genotype differences exceeded that of the environmental differences for SD1, SD5, LCC, PL, SL, FLCP, FCL, and FPL and was relatively lower for CD and TY.

Plant traits (PH, SD1, and SD5) displayed highly significant genotypic differences ($p < 0.001$), variable levels of significant G × E interaction, and low to moderate heritability ($H^2 = 0.22$ to 0.38). The environmental differences were nonsignificant for SD1 and highly significant for PH (Table 4). Among leaves traits, LCC exhibited highly significant and significant values, respectively, for genotypic and environmental differences, with nonsignificant G × E interaction and low heritability ($H^2 = 0.2$).

All evaluated traits related to flower morphology displayed significant genotype differences, with low (CD) or high (PL, SL, and FLCP) values. The significance level of G × E interaction ranged from low (CD and FLCP) to moderate (PL), while a high level was detected for SL ($p < 0.001$). The environmental differences were significant for SL, FLCP, and CD but nonsignificant in the case of PL. Heritability was between 0.31 and 0.50 except for CD, which exhibited a low heritability value of 0.03.

Considerable variation for H^2 values was found for fruit traits. These traits displayed a low (TY) or moderate (FCL, FPL, and FW) heritability with highly significant genotypic differences. FCL and FW had a nonsignificant G × E interaction which for TY and FPL were respectively low and moderate. Results showed relevant statistical significance ($p < 0.001$ and $p < 0.01$) of the environmental differences except for FCL, which was the only trait exhibiting nonsignificant differences.

Table 4. F-Values, their probability, and broad-sense heritability values (H^2) obtained for SM and ILs data from the one-way (genotype) analysis of variance (ANOVA) in the open field or screenhouse and from the two-way (genotype, environment, and their interaction G × E) ANOVA by using open field and screenhouse conditions combined: Five traits (SP, LBL, LSS, LPU, and LPL) were excluded from the analysis as they were monomorphic.

Trait	**Open field**		**Screenhouse**		**Open field + Screenhouse**			
	Genotype[a]	H^2	**Genotype**[a]	H^2	**Genotype**[a]	**Environment**[a]	**G × E**[a]	H^2
Plant								
PH	4.65***	0.43	9.89***	0.64	11.05***	353.63***	2.95***	0.38
SD1	2.34**	0.21	5.33***	0.46	6.06***	0.38[ns]	1.75*	0.27
SD5	4.44***	0.41	2.45**	0.23	5.36***	5.47*	1.96*	0.22
Leaf								
LCC	2.82**	0.27	2.52**	0.23	4.03***	6.23*	1.30[ns]	0.20
Flower								
CD	2.50**	0.23	1.47[ns]	0.09	2.10*	62.12***	1.74*	0.03
PL	3.65***	0.35	9.70***	0.63	9.50***	0.64[ns]	2.19**	0.36
SL	4.42***	0.41	18.82***	0.78	15.60***	6.50*	2.81***	0.50
FLCP	3.20***	0.31	6.46***	0.52	7.92***	8.07**	2.11*	0.31
Fruit								
FCL	6.69***	0.54	2.88**	0.30	6.86***	0.50[ns]	0.96[ns]	0.38
FPL	11.86***	0.69	8.20***	0.62	16.20***	10.16**	2.38**	0.53
TY	2.12*	0.18	3.86***	0.39	2.39**	51.11***	1.76*	0.05
FW	5.44***	0.47	2.96**	0.31	6.35***	38.25***	1.63[ns]	0.30

[a] ***, **, *, [ns] indicate respectively, significantly difference from SM at p values <0.001, <0.01, and <0.05 or not significative ($p \geq 0.05$).

QTL Detection

The comparison of the IL values with the recipient parent using the Dunnett's test allowed the detection of 10 stable QTLs (Table 5). The QTLs were identified in seven ILs bearing introgressed fragments from *S. incanum* in seven chromosomes and corresponded to plant, flower, and fruit traits.

Two QTLs for plant-related traits were located on chromosomes 8 (*ph8*) and 2 (*sd5.2*). The QTL *ph8* was identified in IL SMI_8.1 and exhibited a considerable increase effect on PH (30.9% in the open field (OF) and 34.3% in screenhouse (GH)), with an allelic effect of 7.07–8.23 cm, while the QTL *sd5.2* detected in SMI_2.4 accounted for 40.8% increase in OF and 25.6% in GH in SD5 values, with a positive allelic effect of 0.17–0.33 cm.

For flower-related traits, four QTLs were identified on chromosomes 1, 8, 3, and 5 (*pl1*, *sl8*, *flcp3*, and *flcp5*, respectively). The QTL *pl1* located in SMI_1.3 accounted for a decrease of PL of 35.8% in OF and of 26.8% in GH. On the other hand, a high increase over AN-S-26 was found for a QTL detected in SMI_8.1 (*sl8*) that increased SL by 86.9% in OF and by 196.4% in GH. The QTLs found in ILs SMI_3.1 (*flcp3*) and SMI_5.1 (*flcp5*) showed the strongest effects for FLCP with increases of 240.0% in OF and of 180.0% in GH, and of 300.0% in OF and of 180.0% in GH, respectively.

For fruit-related traits, four putative QTLs (*fpl4*, *fpl8*, *fpl12*, and *fw2*) were detected associated to the *S. incanum* introgressions on chromosomes 4, 8, 12, and 2 (SMI_4.1, SMI_8.1, SMI_12.1, and SMI_2.4, respectively). Three of these QTLs (*fpl4*, *fpl8*, and *fpl12*) induced a moderate decrease in FPL, ranging from 35.9% to 41.3% in OF and from 31.4% to 41.6% in GH. One QTL was involved in FW variation (*fw2*), resulting in a 39.5% in OF and 39.1% in GH reduction of FW with a negative allelic effect between −12.64 g and −14.45 g.

Two of the QTLs detected (*sd5.2* and *fw2*) were present in the IL SMI_2.4, while three others (*ph8*, *sl8*, and *fpl8*) were present in SMI_8.1. Three of these QTLs (*ph8*, *sd5.2*, and *sl8*) have a positive allelic effect on the trait, while *fpl8* and *fw2* have a negative allelic effect.

Table 5. List of putative QTLs detected in the IL population.

Trait	Environment	QTL	Chr.	Position (Mb)	Increase over SM (%)	Allelic effect (units)	IL
Plant							
PH	*Open field*	*ph8*	8	3–109	30.9	7.07 (cm)	SMI_8.1
	Screenhouse	*ph8*	8	3–109	34.3	8.23 (cm)	SMI_8.1
SD5	*Open field*	*sd5.2*	2	75–81	40.8	0.33 (cm)	SMI_2.4
	Screenhouse	*sd5.2*	2	75–81	25.6	0.17 (cm)	SMI_2.4
Flower							
PL	*Open field*	*pl1*	1	27–36	−35.8	−0.51 (cm)	SMI_1.3
	Screenhouse	*pl1*	1	27–36	−26.8	−0.36 (cm)	SMI_1.3
SL	*Open field*	*sl8*	8	3–109	86.9	0.09 (cm)	SMI_8.1
	Screenhouse	*sl8*	8	3–109	196.4	0.22 (cm)	SMI_8.1
FLCP	*Open field*	*flcp3*	3	7–86	240.0	1.2[a]	SMI_3.1
	Screenhouse	*flcp3*	3	7–86	180.0	0.9[a]	SMI_3.1
	Open field	*flcp5*	5	35–43	300.0	1.5[a]	SMI_5.1
	Screenhouse	*flcp5*	5	35–43	180.0	0.9[a]	SMI_5.1
Fruit							
FPL	*Open field*	*fpl4*	4	4–105	−35.9	−1.37 (cm)	SMI_4.1
	Screenhouse	*fpl4*	4	4–105	−34.3	−1.25 (cm)	SMI_4.1
	Open field	*fpl8*	8	3–109	−41.3	−1.58 (cm)	SMI_8.1
	Screenhouse	*fpl8*	8	3–109	−31.4	−1.14 (cm)	SMI_8.1
	Open field	*fpl12*	12	3–96	−38.4	−1.47 (cm)	SMI_12.6
	Screenhouse	*flp12*	12	3–96	−41.6	−1.51 (cm)	SMI_12.6
FW	*Open field*	*fw2*	2	75–81	−39.5	−14.45 (g)	SMI_2.4
	Screenhouse	*fw2*	2	75–81	−39.1	−12.64 (g)	SMI_2.4

[a] Scale units according to the following scoring for calyx prickliness: 0 = none; 1 = very few; 3 = few; 5 = intermediate; 7 = many; 9 = very many.

5. Discussion

IL populations have demonstrated to be a useful and powerful genetic resource for the identification of QTLs in several crops (Balakrishnan et al., 2019). The present work provides a first phenotypic evaluation involving 17 agronomic traits of the first set of eggplant ILs (Gramazio et al., 2017a). This has allowed testing the performance of materials with the same genetic background carrying exotic introgressions in its genome as well as detecting stable QTLs for the investigated traits.

Our results revealed significant differences between the recipient parent (*S. melongena* AN-S-26) and donor parent (*S. incanum* MM557), especially for fruit size and prickles-related traits, demonstrating that profound changes in fruit morphology and prickliness density took place during the domestication (Wang et al., 2008; Page et al., 2019). In agreement with previous works (Prohens et al., 2013; Plazas et al., 2016; Kaushik et al., 2016), we found that the hybrid was in general heterotic for vigour traits, suggesting that this is a common phenomenon in interspecific hybrids between eggplant and its wild relatives. In this regard, hybrids between *S. incanum* and *S. melongena* have been proved as valuable rootstocks for improving eggplant production (Gisbert et al., 2011). However, the hybrid was pricklier than the donor parent, with heterosis values ranging from 100.0% to 900.0%. A similar phenomenon has already been reported in interspecific hybrids of eggplant with *S. macrocarpon*, *S. aethiopicum*, and *S. tomentosum* (Lester, 1986; Prohens et al., 2012; Plazas et al., 2016), where the interspecific hybrids are pricklier than any of the parents. Other studies using segregating populations of *S. linneanum* (Doganlar et al., 2002) and *S. incanum* (Gramazio et al., 2014) suggested that a major QTL located in linkage group 6 accounts for prickliness variability between cultivated eggplant and these two wild relatives and that, consequently, prickliness could be easily selected and removed over backcross generations. Although the hybrid did not set fruits in the screenhouse, in the field, it displayed negative heterosis for the traits related to fruit size, indicating a greater similarity with donor parent. This has also been observed in other interspecific hybrids of (Kaushik et al., 2016) as well as in other related crops such as tomato (Frary et al., 2000; Gur and Zamir., 2015).

In general, the ILs displayed few phenotypic differences with recipient parent, indicating that, even with large introgressed fragments, the effect on the phenotype is minimal for traits of agronomic importance, such as lack of prickles and yield. Similar observations have been made on tomato, where large introgressions have had no effect on most of the relevant morphologic and agronomic traits (Schouten et al., 2019). Broad-sense heritability for PH, PL, SL, FCL, and FPL was moderate, suggesting that, even with a significant G × E effect on these traits, they should respond positively to selection. In this respect, as expected (Kearsey and Farquhar, 1998), stable QTLs were found for traits in which heritability values were high while no or few QTLs were found for traits with low heritability.

In general, excluding PL, the QTLs identified for plant- and flower-related traits were associated with improvement in these traits performance. While Frary et al. (2003) in an F2 between *S. melongena* and *S. linnaeanum* detected a major QTL controlling plant height located on chromosome 5 (*ht5.1*), in the present study, we found a QTL on chromosome 8 (*ph8*) that increased plant height by 30.9%–34.26% with respect to the *S. melongena* parent. Although previous studies described QTLs affecting several plant-related traits in eggplant (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2015), here, we detected the first QTL that influences stem diameter (*sd5.2*), an important trait related to vigour and for grafting (Fassio et al., 2016; Alam et al., 2017). The QTL *pl1* was associated with a decrease in flower peduncle length (PL) and located in the IL SMI_1.3 that overlaps with IL SMI_1.1, for which no effect was detected. A possible explanation is that the larger introgression (SMI_1.1) could include additional QTLs which interact with the QTL present in small introgression (SMI_1.3). This interaction could result in a loss of significant QTL effects (Gur and Zamir, 2015). Stigma length was found to be affected by a locus on chromosome 8 (*sl8*), which increased stigma length between 86.9% and 196.4%. Unlike other traits analyzed in the present work, to our knowledge, PL has not been studied in eggplant or other Solanaceae crops. Therefore, this is the first time that a QTL related to this trait was described. The QTL *sl8* did not colocalize with any previous identified QTLs (Chen and Tanksley, 2004; Xu et al., 2017), suggesting that a new stable locus controlling stigma length was detected.

On the other hand, Doganlar et al. (2002) and Frary et al. (2014) in an F2 between *S. melongena* and *S. linnaeanum* found that a QTL hotspot that mapped chromosome 6 controls the density of prickles in several plant tissues, including flower calyx prickles (FLCP). No ILs with introgressions in chromosome 6 were available to us, although a major QTL related to prickliness has been mapped to chromosome 6 in the BC1 population used to obtain the present IL population (Gramazio et al., 2014). However, our set of ILs allowed detecting two new QTLs for the presence of prickles in the calyx in chromosomes 3 and 5 (*flcp3* and *flcp5*), demonstrating the power of ILs to detect QTLs that may become unnoticed in other types of populations (Eshed and Zamir, 1995; Alonso-Blanco et al., 2006; Gur and Zamir, 2015; Yin et al., 2015). In our IL set, chromosome 3 was represented by three ILs, two of which overlapped (SMI_3.1 and SMI_3.5). The QTL locus *flcp3* was found in IL SMI_3.1, and this result should help to further delimit the genetic region where this QTL is located.

The QTLs detected for fruit-related traits were associated with a decrease in the values of the traits associated with the *S. incanum* alleles. Although the three loci for fruit pedicel length (FPL) were detected on three different chromosomes, the allelic effect was approximately the same for all of them. For two loci mapped on chromosomes 4 and 8 (*fpl4* and *fpl8*), evidence of synteny with loci detected in an intraspecific population of eggplant from the cross 305E40 × 67/3 (Portis et al., 2014) was found. Even in the case of *fpl4*, which maps in one of two overlapping ILs of the set used in this study (SMI_4.1 and SMI_4.3), our study provided useful information for reducing the chromosomal region affecting FPL where this QTL is located.

Fruit weight (FW) is a trait that has been extensively studied in several solanaceous crops. In tomato, although a large number of QTLs have been mapped (Grandillo et al., 1999; Illa-Berenguer et al., 2015; Cambiasso et al., 2019), this trait was found to be controlled primarily by only three loci, which were identified by positional cloning in chromosomes 2, 3, and 11 (Frary et al., 2000; Chakrabarti et al., 2013; Mu et al., 2017). Putative orthologous loci were detected in eggplant using interspecific populations (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014) and GWAS analysis (Portis et al., 2015). In this study, a QTL locus that controlled FW was located on chromosome 2 (*fw2*), evidencing the conservation of these important loci among Solanaceae and suggesting that the phenotype of this trait in eggplant is controlled by a limited number of genes with major effects.

6. Conclusions

The information obtained here on phenotypic characteristics of the *S. melongena* and *S. incanum* parents of the IL set and the heterosis of the interspecific hybrid is of great interest for eggplant breeding. In addition, we observed that, even with the introgression of large fragments from a wild exotic parent, individual eggplant ILs did not present considerable phenotypical variations with respect to recipient parent for most traits, confirming that desirable traits such as lack of prickles and yield did not undergo significant changes in most ILs. Despite significant G × E interaction in most traits, new stable QTLs have been detected and three of them (*fpl4*, *fpl8*, and *fw2*) appeared to be syntenic to other ones previously reported in eggplant populations [8,71,80]. An important next step would be to develop subILs in order to fine map the detected QTLs and tol ultimately identify the gene/s accounting for the QTL effect.

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8. Additional files

Supplementary data S1. Average values of the phenotypic evaluation of the two parents (*S. melongena* AN-S-26 and *S. incanum* MM577), the interspecific hybrid, and the 16 ILs in the open field and screenhouse. https://www.mdpi.com/2073-4395/10/4/467/s1

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Chapter 2: Fruit shape morphometric analysis and QTL detection in a set of eggplant introgression lines

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1. Abstract

Eggplant fruit shape is an important quantitative agronomic trait. The use of introgression lines (ILs) for QTLs identification is a powerful tool for the elucidation of the genetic control of eggplant fruit shape. In the present study, a set of 16 eggplant ILs, each harboring a single marker-defined chromosomal segment from the wild eggplant relative *S. incanum* in the genetic background of *S. melongena*, was evaluated for fruit shape in two environments (open field and screenhouse). A detailed phenotyping of the fruits of the two parents, hybrid and ILs was performed using 32 morphological descriptors of the phenomics tool Tomato Analyzer. Several morphological differences were found between parents, and the hybrid displayed negative heterosis for many fruit shape traits, being more similar to the *S. incanum* parent. Significant differences for most fruit shape descriptors were found between ILs and the recipient parent. For many descriptors, the genotype factor had the highest contribution to the percentage of the sum of squares. Although the contributions of the environment and the G × E interaction were significant for almost all descriptors, their effects on fruit shape were relatively low. Hierarchical clustering revealed nine clusters of highly correlated traits and six ILs groups. A total of 41 stable QTLs spread over ten chromosomes were detected. Of these, sixteen QTLs associated to Basic Measurement and Fruit Shape Index descriptors were syntenic to other previously reported in several intraspecific and interspecific eggplant populations, while twenty-five QTLs, including Blockiness, Homogeneity, Asymmetry and Internal Eccentricity, were new. In addition, mutations associated to genes belonging to *SUN*, *OVATE* and *YABBY* families described in tomato were reported in the QTLs genomic regions identified in eggplant. Eleven *SUN* and *YABBY* genes were proposed as potential candidate controlling fruit shape variations in eggplant. Our results provide novel and highly relevant insights on the genetics of fruit shape in eggplant and have important implications for eggplant breeding.

Keywords: *Solanum melongena*; *S. incanum*; introgression lines; fruit shape, morphometric analysis, Tomato analyzer, QTLs

2. Introduction

Eggplant (*Solanum melongena* L., Solanaceae; 2n = 2x = 24) ranks fifth among all vegetables and second, after tomato. Fruit shape is a trait of great agronomic and commercial relevance in many vegetable crops grown for this organ (Snouffer et al., 2020). Although fruit shape can be affected by the environment, it is largely genetically determined (Wu et al., 2018; Pan et al., 2020). Together with fruit size, fruit shape was among the major traits under selection during the domestication of fruit-bearing crops, resulting in a broad diversity of fruit shapes in most of these cultivated species. In this way, in tomato, starting from small rounded fruits typical of wild relatives, the domestication process led to a gradual selection and accumulation of mutations associated with larger size and diverse shapes, giving rise to a wide variability of combinations of fruit shape and size of present-day cultivars (Tanksley, 2004; Klee and Resende, 2020; Mata-Nicolás et al., 2020).

The accurate study of the fruit shape requires objective and precise phenotypic analysis, requiring a detailed set of morphological descriptors (Brewer et al., 2006). However, fruit shape is frequently evaluated by measuring simple traits, like fruit length and width, and by identifying shape patterns that could be matched with qualitative descriptors (IPGRI, 1996; Scott, 2010; UPOV, 2013). Although these traits provide relevant information and are easily measurable, they do not allow a detailed characterization of the fruit shape (Costa et al., 2011). With the development of modern phenomics tools, many additional fruit shape features, which are often difficult to score by hand, can be accurately measured, providing a more precise and comprehensive characterization of fruit morphology. In this respect, a free software tool, Tomato Analyzer, allows phenomics studies of tomato fruit shape through high-throughput quantitative measurements of many fruit traits from scanned images of fruit sections (Brewer et al. 2007; Gonzalo and van der Knaap 2008; Rodríguez et al. 2010a, 2010b). Although Tomato Analyzer has proven to be very useful for morphological and morphometric characterization of tomato fruit (Rodríguez et al., 2011, 2013; Figas et al., 2015; Nankar et al., 2020), it has also been successfully used for the characterization of other vegetable crops like eggplant (Hurtado et al., 2013), melon (Diaz et al., 2017; Oren et al., 2020), or pepper (Tripodi and Greco, 2018; Pereira-Diaz et al., 2020).

Over the past decades, different segregating populations have been used for dissecting quantitative trait loci (QTL) of physiological and agricultural interest in plants. In particular, F2 populations or recombinant inbred lines (RILs), which are easier to develop, have allowed the detection of numerous QTLs (Nadeem et al., 2018). On the other hand, introgression line (IL) populations, a set of fixed and immortal lines that cover the totality or part of a donor parent genome carrying one or a few introgressed fragments into the genetic background of a recipient parent, allow a more efficient and precise identification of QTLs compared to other segregating populations like F2 and RILs (Yin et al., 2016; Fasahat et al., 2016; Boopathi, 2020). In IL populations, linkage drag is reduced by the small portion of introgressed donor genome of the lines, and, therefore, the phenotypic variation between ILs can be accurately attributed to specific introduced segments (Zamir, 2001). Once a QTL associated with a trait of interest is localized, this information can be used for a better estimation of gene × gene (epistasis) and gene × environment (G x E) interactions, pleiotropic effects, and mapping strong QTL effects (Gur and Zamir, 2015; Balakrishnan et al., 2019). Furthermore, each IL can be used as a starting point for developing lines with smaller introgression (sub-ILs) for increased mapping resolution (Monforte and Tanksley, 2000; Chakrabarti et al., 2013; Sacco et al., 2013) and performing QTL positional cloning (Salvi and

Tuberosa, 2005). However, the major limitation of using IL populations for quantitative studies is the investment in time and resources required to develop them (Yan et al., 2017; Can et al., 2019; Alqudah et al., 2020).

Tomato (*Solanum lycopersicum* L.) is the model system to study fruit shape in Solanaceae, and specifically for fleshy-fruited plant species, with many studies that have allowed identifying the major "domestication" genes controlling the wide phenotypic diversity of the tomato fruit (Kimura and Sinha, 2008; Kim et al., 2017; Anwar et al., 2019). Numerous QTL mapping studies for dissecting the genetic base of fruit shape have been conducted in tomato using inter-specific F2 populations obtained by crossing the cultivated tomato and different small-fruited wild species (Eshed and Zamir, 1995; Bernacchi et al., 1998; Lippman and Tanksley, 2001; van der Knapp and Tanksley, 2003; Frary et al., 2004). Additional mapping experiments have been performed using complete IL libraries (Barrantes et al., 2016; Celik et al., 2017; Di Giacomo et al., 2020) or reduced set of ILs covering specific QTL regions of different chromosomes (Monforte et al., 2001; Yates et al., 2004; Haggard et al., 2013). In this way, numerous QTLs and/or genes involved in the genetic regulation of fruit morphology in tomato have been identified. Among the major genes controlling variation in tomato fruit shape, *SUN* and *OVATE* control fruit elongation, while *FASCIATED* (*FAS*) control locule number (van der Knaap and Ostergaard, 2018). *SUN*, *OVATE* and *FAS* have been identified by positional cloning and encode a member of IQ Domain, Ovate Family Protein (OFP) families, and YABBY family, respectively (Liu et al., 2002; Xiao et al., 2008; Xu et al., 2015).

In eggplant (*Solanum melongena* L.), fruit shape is also a relevant attribute that determines its commercial use and economic value. Eggplant fruits are fleshy berries commercially classified according to their shape. As occurs with tomato (Paran and van der Knaap, 2007; Mata-Nicolás et al., 2020), a wide diversity exists for fruit shape in eggplant (Daunay et al., 2008; Wang et al., 2008; Hurtado et al., 2012), and small differences in fruit shape may be determinant for the success or failure of a commercial cultivar. The knowledge of the genetic base of fruit shape in eggplant is limited to findings obtained from QTL mapping analysis in a few biparental populations (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014; Wei et al., 2020) and GWAS studies (Portis et al., 2015), which identified some major QTLs associated to simple fruit shape traits. Although phenomics studies utilizing Tomato Analyzer have been performed in eggplant using different eggplant germplasm materials (Prohens et al., 2012; Hurtado et al., 2013; Plazas et al., 2014; Kaushik et al., 2016, 2018), their aim was describing the diversity in collections of materials and segregating populations and not associating genomic regions with underlying genes controlling natural variations of fruit shape.

In this work, we analyze the fruit shape in a collection of eggplant ILs with introgressions from a wild species (*S. incanum* L.) in two different environments (open field and screenhouse) and perform a detailed phenotyping using the Tomato Analyzer tool. Stable QTLs and potential candidate genes are identified in the introgressed genomic regions. The obtained results provide novel and highly relevant insights on the genetics of fruit shape in eggplant and represent a step forward in the understanding of this trait of great interest for eggplant breeding.

3. Materials and methods

Plant material and cultivation conditions

From the IL population of *Solanum incanum* (MM577) developed in the *S. melongena* (AN-S-26) background (Gramazio et al., 2017), a set of 16 ILs were selected based on a maximization of representation of the genome of *S. incanum* and on seed availability. Characteristics of the parents and statistics of ILs set, which overall cover 58.6% of the genome of the wild *S. incanum* and for some chromosomes include overlapping ILs, are described in detail in Mangino et al. (2020). Seed germination was performed using the protocol described in Ranil et al. (2015), which is suitable for wild and wild-derived materials, and seedlings were maintained in a climatic chamber with 16 h light (25 °C) / 8 h dark (18 °C) regime. Five replicates, each one consisting of a plant, for each of the two parents, the F1 hybrid, and the 16 ILs were grown under two different conditions (open field and screenhouse) during the spring-summer season of 2017 at the campus of Universitat Politècnica de València (GPS coordinates: latitude, 39° 28’ 55” N; longitude, 0°20’ 11” W; altitude 7 m a.s.l) using the standard horticultural practices. Plants were spaced 1.5 m between the rows and 1.2 m within the rows and distributed according to a completely randomized block-design with five blocks per condition. Irrigation and fertilization were applied with a drip irrigation system. Weeds were removed manually and phytosanitary treatments against spider mites and whiteflies were performed when necessary.

Tomato Analyzer characterization

At the commercially ripe stage, three fruits per replicate were harvested, cut longitudinally and scanned with a Plustek OpticSlim 1180 (Plustek, Taipei, Taiwan) image scanner at a resolution of 300 dpi (Figure 1). Image data were subjected to a morphometric analysis with Tomato Analyzer v 3.0 software (Rodríguez et al., 2010a). A total of 32 morphological descriptors, categorized into basic measurements (7), fruit shape index (3), blockiness (3), homogeneity (3), proximal fruit end shape (3), distal fruit end shape (2), asymmetry (6), and internal eccentricity (5), were automatically recorded. Manual adjustments were done when the software was unable to accurately identify the outline of a trait. A brief description of each trait, their acronyms and evaluation methodology are described in Table 1 and visualized in Figure 2. A more detailed description of each descriptor is available at the Tomato Analyzer software webpage (https://vanderknaaplab.uga.edu/tomato_analyzer.html).

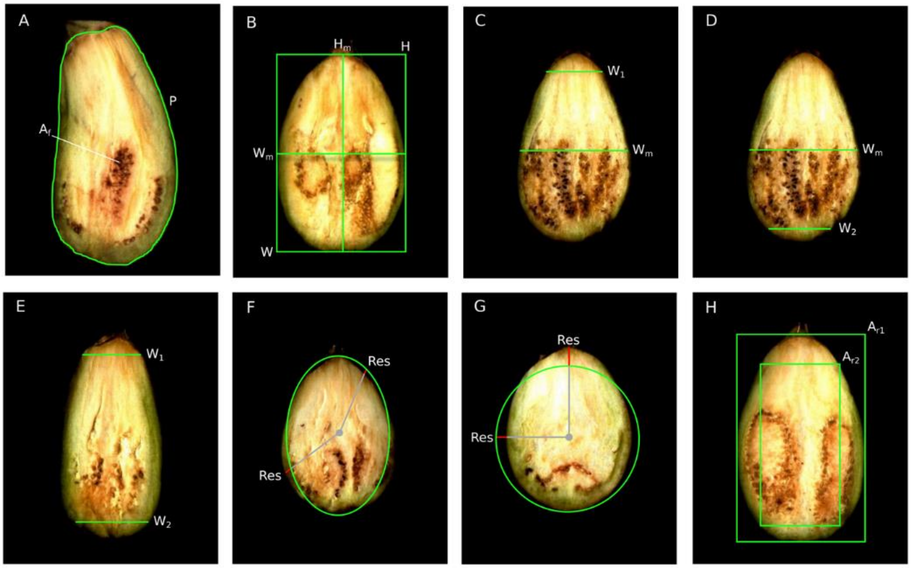

Figure 1. Visual representation of Tomato Analyzer descriptors used in this study and described in Table 1 using eggplant fruit. **A**) Perimeter (P) and fruit area (A_f). **B**) Fruit shape index external I, the ratio (H/W) of the maximum height (H) to maximum width (W), and Fruit shape index external II, the ratio (H_m/W_m) of height mid-width (W_m) to width mid-height (H_m). **C**) Proximal fruit blockiness, the ratio (W_1/W_m) of the width at the upper blockiness position (W_1) to width mid-height (W_m). **D**) Distal fruit blockiness, the ratio (W_2/W_m) of the width at the lower blockiness position (W2) to width mid-height (W_m). **E**) Fruit shape triangle: the ratio (W_1/W_2) of the width at the upper blockiness position (W^1) to the width at the lower blockiness position W_2. **F**) Ellipsoid: the ratio of the error resulting from a best-fit ellipse to the area of the fruit. Error is the average magnitude of residuals (Res) along the fruit's perimeter, divided by the length of the major (longer) axis of the ellipse. **G**) Circular: the ratio of the error resulting from a best-fit circle to the area of the fruit. Error is the average magnitude of residuals along the fruit's perimeter, divided by the radius of the circle. **H**) Rectangular: the ratio of the area (A_{r1}/A_{r2}) of the rectangle bounding the fruit (A_{r1}) to the area of the rectangle bounded by the fruit (A_{r2}). Further details on the descriptors are available at the Tomato Analyzer software webpage (https://vanderknaaplab.uga.edu/tomato_analyzer.html).

Table 1. List of the 32 traits with respective codes and descriptions used for morphometric analysis with Tomato Analyzer v 3.0 software (Rodríguez et al., 2010a) of the 16 ILs, their parents (*S. melongena* AN-S-26 and *S. incanum* MM557) and the interspecific hybrid between them assessed in this study.

Trait (Unit)	Code	Description
Basic Measurements		
Perimeter (cm)	Perimeter	Perimeter length
Area (cm^2)	Area	Fruit area
Width Mid-Height (cm)	Width_MH	The width measured at $^1/_2$ of the fruit's height
Maximum Width (cm)	Max_Width	The maximum orizontal distance of the fruit
Height Mid-Width (cm)	Height_MW	The height measured at $^1/_2$ of the fruit's width
Maximum Height (cm)	Max_Height	The maximum vertical distance of the fruit
Curved Height (cm)	C_Height	The height measured along a curved line through the fruit
Fruit Shape Index		
Fruit Shape Index External I	F_Shape_E_I	The ratio of the maximum height to maximum width
Fruit Shape Index External II	F_Shape_E_II	The ratio of height midwidth to width mid-height
Curved Fruit Shape Index	C_F_Shape	The ratio of curved height to the width of the fruit at mid-curved-height, as measured perpendicular to the curved height line
Blockiness		
Proximal Fruit Blockiness	P_Blockiness	The ratio of the width at the upper blockiness position to width mid-height
Distal Fruit Blockiness	D_Blockiness	The ratio of the width at the lower blockiness position to width mid-height
Fruit Shape Triangle	Triangle	The ratio of the width at the upper blockiness position to the width at the lower blockiness position
Homogeneity		
Ellipsoid	Ellipsoid	The ratio of the error resulting from a best-fit ellipse to the area of the fruit; smaller values indicate that the fruit is more ellipsoid
Circular	Circular	The ratio of the error resulting from a best-fit circle to the area of the fruit; smaller values indicate that the fruit is more circular
Rectangular	Rectangular	The ratio of the area of the rectangle bounding the fruit to the area of the rectangle bounded by the fruit
Proximal Fruit End Shape		
Shoulder Height	Sh_Height	The ratio of the average height of the shoulder points above the proximal end point to maximum height
Proximal Angle Micro (°)	PA_Micro	Proximal fruit end shape angle at position 1% above the tip from the fruit
Proximal Angle Macro (°)	PA_Macro	Proximal fruit end shape angle at position 5% above the tip from the fruit
Distal Fruit End Shape		
Distal Angle Micro (°)	DA_Micro	Distal fruit end shape angle at position 1% above the tip from the fruit
Distal Angle Macro (°)	DA_Macro	Distal fruit end shape angle at position 5% above the tip from the fruit
Asymmetry		

Obovoid	Obovoid	If the area of the fruit is greater below mid-height than above it, a function of width and height
Ovoid	Ovoid	If the area of the fruit is greater above mid-height than below it, a function of width and height
V. Asymmetry	Asv	The average distance between a vertical line through the fruit at mid-width and the midpoint of the fruit’s width at each height
H. Asymmetry. Ob	Asob	If the area of the fruit is greater below mid-height than above it, a function of width and height
H. Asymmetry. Ov	Asov	If the area of the fruit is greater above mid-height than below it, a function of width and height
Width Widest Pos	Width_WP	The ratio of the height at which the maximum width occurs to the maximum height
Internal Eccentricity		
Eccentricity	Eccentricity	The ratio of the height of the internal ellipse to the maximum height
Proximal Eccentricity	P_Eccentricity	The ratio of the height of the internal ellipse to the distance between the bottom of the ellipse and the top of the fruit
Distal Eccentricity	D_Eccentricity	The ratio of the height of the internal ellipse to the distance between the top of the ellipse and the bottom of the fruit
Fruit Shape Index Internal	F_Shape_I	The ratio of the internal ellipse’s height to its width
Eccentricity Area Index	Ec_Area	The ratio of the area of the fruit outside the ellipse to the total area of the fruit

Data analysis

For each trait, means, standard errors and range values were calculated for each parent and F1 hybrid in both environments. Mid-parent heterosis values (H_{MP}) were calculated as:

$$H_{MP} = (F1-MP)/MP$$

where *F1* is the performance of the F1 hybrid and *MP* is the mean value of the parents. Statistical significance of heterosis, as well as statistically significant differences between parents for each environment, were detected using Student's *t*-tests at $p < 0.05$.

To evaluate the difference among ILs and the cultivated parent AN-S-26, data for all morphological traits were subjected to a two-factorial (genotype and environment) analysis of variance (ANOVA) including the interaction among both main factors. The total sum of squares was partitioned into sums of squares for genotype, environment, genotype × environments (G × E), block and residual effect, and expressed in percentage over total sums of squares. A fixed effects model was used for genotype and environment effect. All statistics were conducted using the Statgraphics Centurion XVII software (Statpoint Technologies, Warrenton, USA).

Pearson linear correlation (*r*) among morphological descriptors were studied. A hierarchical clustering heatmap displaying numeric differences for morphological descriptors across ILs and AN-S-26 parent in the two environments was performed using Clustvis (Metsalu and Vilo, 2015; http://biit.cs.ut.ee/clustvis/) with log-transformed data. Both rows and columns were clustered using correlation distance and average linkage.

QTL detection

For non-overlapping ILs, QTLs detection was performed by carrying out a Dunnett's test to compare the means of each IL, in the open field or screenhouse, with the recipient parent AN-S-26 (SM). For overlapping ILs a system of linear equations was used to assign a mean value to each of the introgressed genomic fragments in which the overlapping lines could be divided and *t* values for each of the introgressed genomic fragments were calculated according to the Dunnett's test procedure. A stable QTL was only reported for the non-overlapping ILs or for the introgressed genomic fragments in the overlapping ILs if the Dunnett's test was significant ($p < 0.05$) and of the same nature (i.e., either positive or negative) in both environments. The relative increase over the recipient parent and allelic effects in each of the environments were estimated as:

$$\text{Increase over recurrent parent (\%)} = (D/SM) \times 100$$

$$\text{Allelic effect} = D/2$$

Where SM is the average value for the recurrent *S. melongena* parent AN-S-26 parent and D is the difference between the mean of the IL and AN-S-26 for non-overlapping lines or the calculated difference over the mean of AN-S-26 caused by the introgressed genomic fragments in the case of overlapping lines.

Analysis of orthologous shape genes located within QTL regions

In order to detect putative eggplant orthologous of tomato genes controlling fruit shape and determine their physical location on QTL region, the cDNA sequences of 74 genes belonging to SUN, OVATE and YABBY gene family described in Huang et al. (2013) were retrieved from the Heinz 1706 tomato reference genome (version SL4.0) in the Sol Genomics Network database (http://www.solgenomics.net). Tomato cDNA sequences were blasted against the 67/3 eggplant reference genome (version V3) database (http://www.eggplantgenome.org), and information regarding orthologous eggplant genes as well as their sequence and physical location on respective chromosomes were obtained. Individual VCF file of parents (Gramazio et al., 2019), including variant effects predicted according to SnpEff software v 4.2 (Cingolani et al. 2012), were filtered out for selecting allelic variants of the identified orthologous genes. Homozygous allelic variants characterized by amino acid substitution or indel between the two parents and classified by high/moderate impact were submitted to SIFT (Sorting Intolerant From Tolerant) (Ng and Henikoff, 2001) and PROVEAN (PROtein Variation Effect Analyzer) (Choi et al., 2012) software in order to predict significant impacts on protein functionality, using a threshold of 0.05 in SIFT and -2.5 in PROVEAN, respectively.

4. Results

Parents fruit characterization and heterosis

For the recipient parent AN-S-26, significant differences ($p < 0.05$) between the two environments were found only for Ellipsoid, Circular and Asv fruit shape descriptors (Table 2). Since MM557 and the hybrid did not set fruit in the screenhouse, the estimation of significant differences between parents as well as mid-parent heterosis under screenhouse conditions was not possible. Eggplant wild relatives and interspecific hybrids often have specific environmental and weather requirements for each plant stage like germination, vegetative development, and fruit set, which are frequently unsynchronized and different to those of the cultivated eggplant. Furthermore, these differences are wider under protected conditions, like the ones under a screenhouse. Significant differences ($p < 0.05$) between the recipient parent AN-S-26 and the donor parent MM557 were found for 24 out of 32 descriptors in the open field. Descriptors for which no significant differences were found were P_Blockiness, D_A_Micro, D_A_Macro, Ovoid, Asov, Eccentricity, P_Eccentricity and D_Eccentricity (Table 2). Compared to AN-S-26, in the open field MM557 exhibited lower values for 21 traits, except for Triangle, PA_Micro and PA_Macro (Table 2).

In the open field, the hybrid displayed significant negative values of heterosis over the mid-parent for three descriptors of Fruit Shape Index (Fruit_Shape_E_I, Fruit_Shape_E_II, C_F_Shape), two of Homogeneity (Ellipsoid, Circular), three of Asymmetry (Obovoid, Asob, Width_WP), and two of Internal Eccentricity (P_Eccentricity and F_Shape_I), ranging from -0.002 (P_Eccentricity) to -0.573 (Asob) (Table 2). Significant positive values of heterosis were detected only for PA_Micro (0.093).

Table 2. Means with standard errors and range values for the recipient parent (*S. melongena* AN-S-26), the donor parent (*S. incanum* MM577) and the interspecific hybrid (F1) of the IL population and hybrid mid-parent heterosis (H_{MP}) in the open field and screenhouse conditions and significance of differences of comparisons of screenhouse vs. open field for AN-S-26, MM577 vs. AN-S-26 for open field, and difference from 0 for H_{MP}.

	AN-S-26				**MM577**		**F1**		
	Open field		**Screenhouse**		**Open field**		**Open field**		
Trait (Unit)	**Mean**	**Range**	**Mean$^{a, b}$**	**Range**	**Mean$^{a, c}$**	**Range**	**Mean**	**Range**	**H_{MP}^{a}**
Basic Measurements									
Perimeter	18.59 ± 1.40	15.36-22.99	21.11 ± 1.01^{ns}	17.25-23.26	6.40 ± 0.35***	5.41-7.29	12.11 ± 0.44	10.85-13.30	-0.020^{ns}
Area	22.48 ± 3.41	14.73-33.70	28.11 ± 2.64^{ns}	18.52-34.45	2.96 ± 0.32***	2.10-3.73	10.24 ± 0.71	8.00-12.02	-0.140^{ns}
Width_MH	4.00 ± 0.34	3.11-5.08	4.20 ± 0.20^{ns}	3.60-4.77	1.84 ± 0.11***	1.53-2.07	3.19 ± 0.10	2.90-3.43	0.108^{ns}
Max_Width	4.10 ± 0.35	3.21-5.25	4.38 ± 0.21^{ns}	3.70-4.96	1.85 ± 0.11***	1.54-2.09	3.21 ± 0.09	2.92-3.45	0.094^{ns}
Height_MW	6.56 ± 0.42	5.69-7.78	7.61 ± 0.36^{ns}	6.27-8.36	2.00 ± 0.09***	1.75-2.26	3.93 ± 0.16	3.44-4.34	-0.072^{ns}
Max_Height	6.65 ± 0.43	5.73-7.90	7.72 ± 0.37^{ns}	6.33-8.41	2.02 ± 0.10***	1.76-2.32	3.96 ± 0.16	3.46-4.37	-0.078^{ns}
C_Height	6.71 ± 0.42	5.8-7.93	7.85 ± 0.34^{ns}	6.53-8.48	2.15 ± 0.09***	1.91-2.37	4.08 ± 0.16	3.62-4.48	-0.071^{ns}
Fruit Shape Index									
F_Shape_E_I	1.64 ± 0.05	1.5-1.79	1.77 ± 0.05^{ns}	1.65-1.89	1.10 ± 0.03***	1.02-1.17	1.23 ± 0.02	1.19-1.28	-0.099*
F_Shape_E_II	1.67 ± 0.06	1.52-1.85	1.82 ± 0.06^{ns}	1.66-1.97	1.10 ± 0.03***	1.02-1.18	1.23 ± 0.02	1.19-1.27	-0.104*
C_F_Shape	1.70 ± 0.06	1.56-1.87	1.87 ± 0.06^{ns}	1.72-2.03	1.18 ± 0.03***	1.10-1.28	1.28 ± 0.02	1.24-1.34	-0.112*
Blockiness									
P_Blockiness	0.61 ± 0.02	0.55-0.66	0.63 ± 0.01^{ns}	0.62-0.65	0.57 ± 0.01^{ns}	0.53-0.61	0.59 ± 0.01	0.55-0.61	0.012^{ns}
D_Blockiness	0.75 ± 0.02	0.70-0.81	0.77 ± 0.02^{ns}	0.71-0.81	0.60 ± 0.01***	0.57-0.63	0.66 ± 0.00	0.65-0.67	-0.029^{ns}
Triangle	0.81 ± 0.02	0.75-0.87	0.83 ± 0.02^{ns}	0.76-0.89	0.95 ± 0.03**	0.87-1.02	0.91 ± 0.02	0.83-0.95	0.033^{ns}
Homogeneity									
Ellipsoid	0.05 ± 0.00	0.04-0.05	0.06 ± 0.00*	0.05-0.06	0.02 ± 0.00***	0.01-0.03	0.02 ± 0.00	0.02-0.02	-0.290***
Circular	0.16 ± 0.01	0.14-0.18	0.19 ± 0.01*	0.16-0.21	0.04 ± 0.01***	0.02-0.06	0.07 ± 0.00	0.06-0.08	-0.292**
Rectangular	0.53 ± 0.01	0.51-0.55	0.52 ± 0.01^{ns}	0.50-0.54	0.48 ± 0.01***	0.47-0.50	0.51 ± 0.00	0.50-0.52	0.008^{ns}
Proximal Fruit End Shape									
Sh_Height	0.04 ± 0.01	0.00-0.06	0.02 ± 0.01^{ns}	0.00-0.04	0.00 ± 0.00*	0.00-0.01	0.01 ± 0.01	0.00-0.04	-0.375^{ns}
PA_Micro	121.7 ± 4.3	110.0-135.8	129. ± 11.8^{ns}	87.0-158.8	156.3 ± 4.9***	146.4-174.6	151.7 ± 2.8	141.3-157.9	0.093*
PA_Macro	111.2 ± 3.9	105.1-125.8	110.7 ± 2.7^{ns}	105.5121.0	135.9 ± 1.8***	130.0-141.1	134.1 ± 2.7	124.6-139.6	0.089^{ns}
Distal Fruit End Shape									
DA_Micro	133.2 ± 19.0	70.3-175.1	130.3 ± 14.3^{ns}	98.8-176.4	151.3 ± 10.2^{ns}	111.8-170.3	135.1 ± 15.2	108.0-179.0	-0.040^{ns}

DA_Macro	135.2 ± 5.4	123.8-154.9	122.9 ± 8.2^{ns}	91.1-136.4	141.9 ± 2.6^{ns}	134.8-148.2	145.5 ± 0.8	143.1-148.0	0.053^{ns}
Asymmetry									
Obovoid	0.22 ± 0.02	0.18-0.27	0.24 ± 0.02^{ns}	0.21-0.29	0.10 ± 0.01***	0.06-0.12	0.11 ± 0.01	0.08-0.14	-0.264**
Ovoid	0.00 ± 0.00	0.00-0.00	0.00 ± 0.00^{ns}	0.00-0.00	0.02 ± 0.02^{ns}	0.00-0.08	0.02 ± 0.02	0.00-0.09	0.489^{ns}
Asv	0.06 ± 0.01	0.04-0.08	0.12 ± 0.02*	0.06-0.17	0.02 ± 0.00**	0.02-0.03	0.03 ± 0.00	0.03-0.04	-0.111^{ns}
Asob	0.24 ± 0.04	0.17-0.38	0.32 ± 0.04^{ns}	0.22-0.42	0.02 ± 0.00***	0.02-0.02	0.06 ± 0.01	0.03-0.07	-0.573**
Asov	0.00 ± 0.00	0.00-0.00	0.00 ± 0.00^{ns}	0.00-0.00	0.01 ± 0.01^{ns}	0.00-0.03	0.00 ± 0.00	0.00-0.02	0.378^{ns}
Width_WP	0.59 ± 0.02	0.54-0.64	0.62 ± 0.02^{ns}	0.58-0.67	0.48 ± 0.01***	0.46-0.50	0.50 ± 0.01	0.49-0.52	-0.063*
Internal Eccentricity									
Eccentricity	0.79 ± 0.00	0.78-0.79	0.78 ± 0.01^{ns}	0.74-0.80	0.79 ± 0.00^{ns}	0.78-0.80	0.79 ± 0.00	0.79-0.79	0.004^{ns}
P_Eccentricity	0.89 ± 0.00	0.88-0.89	0.88 ± 0.01^{ns}	0.85-0.89	0.89 ± 0.00^{ns}	0.89-0.90	0.89 ± 0.00	0.89-0.89	-0.002*
D_Eccentricity	0.89 ± 0.00	0.88-0.89	0.89 ± 0.00^{ns}	0.88-0.89	0.88 ± 0.00^{ns}	0.87-0.89	0.89 ± 0.00	0.88-0.89	0.001^{ns}
F_Shape_I	1.66 ± 0.06	1.51-1.85	1.79 ± 0.05^{ns}	1.64-1.97	1.10 ± 0.03***	1.02-1.18	1.23 ± 0.02	1.18-1.27	-0.106*
Ec_Area	0.40 ± 0.01	0.39-0.41	0.41 ± 0.01^{ns}	0.39-0.44	0.37 ± 0.00**	0.36-0.38	0.38 ± 0.00	0.37-0.39	-0.011^{ns}

[a] ***, **, *, ^{ns} indicate respectively, significant differences at *p* values <0.001, <0.01, and <0.05 or not significative (*p* ≥ 0.05).

[b] Significances correspond to the comparison of screenhouse vs. open field conditions for AN-S-26.

[c] Significances correspond to the comparison of MM577 vs. AN-S-26 for open field conditions.

Analysis of variance

For all the descriptors, the ANOVA revealed that the differences among the ILs and the *S. melongena* parent were statistically significant ($p < 0.05$) for at least one of the factors (genotype, environment and genotype × environment) for all fruit shape descriptors, with the exception of PA_Micro, DA_Macro, Asv, P_Eccentridicty and D_Eccentricity (Table 3 and Figure 2). Important differences between genotypes were found, with significant ($p < 0.05$ or $p < 0.01$) or highly significant ($p < 0.001$) differences for 4 and 23 descriptors, respectively. The contribution of the genotype factor to the total sums of squares ranged from 16.6% (DA_Micro) to 49.08% (F_Shape_E_I). Moreover, the genotype factor was the greatest contributor to the sums of squares for 14 descriptors (>30%), of which three corresponded to descriptors related to Basic Measurements (Height_MW, Max_Height, and C_Height), three to Fruit Shape Index (F_Shape_E_I, F_Shape_E_II and C_F_Shape), three to Blockiness (P_Blockiness, D_Blockiness and Triangle), two to Homogeneity (Ellipsoid and Circular), one to Proximal Fruit End Shape (PA_Macro), and two to Internal Eccentricity (F_Shape_I and Ec_Area) (Table 3).

Significant differences between environments were detected for all the descriptors, except for Sh_Height. With a contribution ranging from 1.75% to 20.9% to the total sums of squares, the environmental factor was not the main contributor to the sums of squares for any of the fruit shape descriptors evaluated (Table 3).

The G × E interaction was statistically significant for all descriptors, except for P_Blockiness, Triangle, Asob and Eccentricity. The G × E contribution to the total sums of squares, which ranged from 10.2% to 24.4% exceed that of the environment contribution for all traits, except for the seven Basic Measurements descriptors (Perimeter, Area, Width_MH, Max_Width, Height_MW, Max_Height and C_Height). Nevertheless, as for the environment, the interaction did not represent the predominant contributor to the total sums of squares for any of the evaluated descriptors (Table 3).

The residual effect had a contribution to the total sums of squares ranging between 28.89% and 61.14% and was the greatest contributor to the total sums of squares for 13 descriptors, of which four corresponded to Basic Measurement descriptors (Perimeter, Area, Width_MH and Max_width), one to Homogeneity (Rectangular), one to Proximal Fruit End Shape (Sh_Height), one to Distal Fruit End Shape (DA_Micro), five to Asymmetry (Obovoid, Ovoid, Asob, Asov and Width_WP), and one to was Internal Eccentricity (Eccentricity) (Table 3).

Table 3. Percentage and statistical significance of the sums of squares over the total for the genotype, environment, genotype × environment (G × E), block and residual effects calculated with a two-way ANOVA to evaluate the differences among ILs and the recurrent parent AN-S-26.

Trait	**Sums of squares**				
	Genotype	**Environment**	**G × E**	**Block**	**Residual**
Basic Measurements					
Perimeter	28.23***	18.75***	18.35***	5.78	28.89
Area	27.63***	18.00***	15.75***	6.48	32.14
Width_MH	33.93***	13.85***	10.90**	7.03	34.29
Max_Width	31.37***	15.75***	11.36**	7.41	34.11
Height_MW	31.52***	20.90***	19.69***	4.73	23.16
Max_Height	30.38***	20.70***	19.58***	5.03	24.31
C_Height	30.63***	20.03***	19.75***	4.89	24.7
Fruit Shape Index					
F_Shape_E_I	49.08***	9.65***	19.11***	1.12	21.04
F_Shape_E_II	47.45***	10.32***	19.79***	0.72	21.72
C_F_Shape	47.85***	8.20***	19.28***	0.6	24.07
Blockiness					
P_Blockiness	44.74***	4.24**	8.43^{ns}	2.21	40.38
D_Blockiness	43.72***	1.75**	17.31***	1.83	35.39
Triangle	44.06***	5.99***	6.23^{ns}	2.44	41.28
Homogeneity					
Ellipsoid	39.64***	2.31***	24.40***	0.88	32.77
Circular	48.94***	9.29***	20.28***	0.78	20.71
Rectangular	40.17***	2.49**	13.04**	2.55	41.75
Proximal Fruit End Shape					
Sh_Height	23.48***	1.25^{ns}	14.32*	3.02	57.93
PA_Micro	7.14^{ns}	0.95^{ns}	6.31^{ns}	4.45	81.15
PA_Macro	43.99***	13.28***	10.2**	1.75	30.78
Distal Fruit End Shape					
DA_Micro	16.64*	2.14*	16.70*	3.38	61.14
DA_Macro	13.48^{ns}	0.13^{ns}	12.02^{ns}	3.57	70.8
Asymmetry					
Obovoid	38.26***	7.30***	11.02*	2.53	40.89
Ovoid	18.82**	4.77***	14.96*	2.57	58.88
Asv	12.29^{ns}	0.04^{ns}	15.92^{ns}	2.25	69.5
Asob	25.29***	11.37***	11.82^{ns}	2.36	49.16
Asov	19.22**	5.97***	14.62*	3.41	56.78
Width_WP	33.22***	5.42***	13.20*	2.1	46.06
Internal Eccentricity					
Eccentricity	17.66**	10.98***	10.72^{ns}	3.73	56.91
P_Eccentricity	15.2^{ns}	0.00^{ns}	8.78^{ns}	6.91	69.11

D_Eccentricity	14.19^{ns}	0.18^{ns}	11.4^{ns}	5.75	68.48
F_Shape_I	46.1***	10.80***	19.86***	1.01	22.23
Ec_Area	39.85***	6.48***	22.98***	2.88	27.81

***, **, *, ^{ns} indicate respectively, significant differences at p values <0.001, <0.01, and <0.05 or not significative ($p \geq 0.05$).

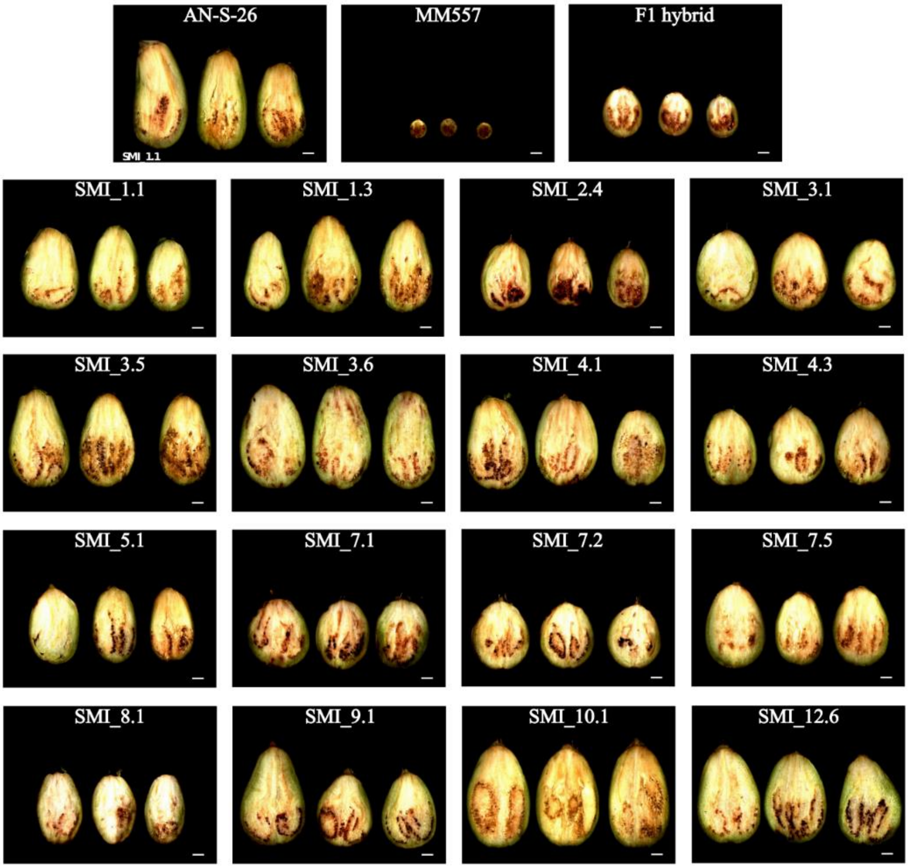

Figure 2. Representation of fruits scanned for the recipient parent (*S. melongena* AN-S-26), donor parent (*S. incanum* MM577), their interspecific hybrid (F1 hybrid) and the 16 ILs used in this study for the phenomic analysis and QTL detection for fruit shape.

Correlation and hierarchical clustering

To explore the relationships among the fruit shape descriptors, Pearson's correlation coefficients were calculated using data of the ILs and AN-S-26 in both environments (Supplementary data S1) and a hierarchical clustering heatmap analysis (Figure 3) was performed. Both types of analyses provided congruent results, with the hierarchical clustering analysis grouping traits into nine main clusters of correlated traits.

The cluster I comprised all the Basic Measurement descriptors and Eccentricity, among which moderate to strong correlations were found (r = 0.59 to 1.00, p < 0.001) (Supplementary data S1). Two sub-clusters could be distinguished within the cluster II. In the first sub-cluster, all the Fruit shape Index descriptors, F_Shape_I, Circular and Ellipsoid resulted strongly correlated (r = 0.71 to 1.00, p < 0.001) (Supplementary data S1). D_Blockiness, Obovoid, Width_WP and Asob grouped together in the second sub-cluster, displaying moderate to strong correlations (r = 0.59 to 0.95, p < 0.001). The cluster V comprised Triangle, PA_Macro, Ovoid and Asov among which correlations varied from moderate to strong, with r values ranging from 0.37 (p < 0.05) to 0.98 (p < 0.001) (Supplementary data S1). Three descriptors were grouped in each of the clusters VI (P_Blockiness, Rectangular and Ec_Area) and VIII (DA_Micro, P_Eccentrcity and D_Eccentricity). In the cluster VI the correlations ranged from moderate to strong (r = 0.61 to 0.84, p < 0.001) (Table S1). In the cluster VIII, P_Eccentricity exhibited slight or moderate correlation with D_Eccentricity (r = 0.42, p < 0.05) and DA_Micro (r = 0.51, p < 0.01), respectively, while no correlation between D_Eccentricity and DA_Micro was found (Table S1). Asv, PA_Micro, SH_Height and DA_Macro showed no significant correlations with any other descriptors and were placed individually in four separated clusters (III, IV, VII and IX, respectively) (Supplementary data S1).

Hierarchical clustering grouped ILs and parent AN-S-26 in two main branches (Figure 3). The first branch comprised 4 clusters (A, B, C and D). Although clusters A and B consisted mainly of ILs grown in the open field, three of them corresponding to the screenhouse conditions (SMI_3.6_SH, SMI_5.1_SH and SMI_10.1_SH). The values for descriptors of the cluster I were generally high. In addition, cluster A showed high values for descriptors of cluster II. Cluster C grouped the recipient parent from both open field and screenhouse (AN-S-26_OF and AN-S-26_SH), showing high values for descriptors of the clusters II, VI and VII. Cluster D consisted mainly of ILs grown in the screenhouse except for one IL grown in the open field (SMI_8.1_OF), whose corresponding IL in screenhouse (SMI_8.1_SH) was also grouped in the same subcluster. In cluster D, values were especially high for SMI_3.5_SH and SMI_4.3_SH for the descriptors Ovoid and Asov of cluster V, while all the other descriptors were variable within the same cluster. The second branch comprised two clusters (E and F). Clusters E and F consisted only of ILs grown in screenhouse, except for one IL in cluster E (SMI_2.4_OF) that has its correspondent in cluster F. Clusters E and F exhibited low values for descriptors in clusters I and II, especially for the ILs SMI_2.4_SH, SMI_7.5_SH and SMI_12.6_SH. In addition, cluster E exhibited low values even for descriptors in cluster VI, while cluster F showed high values for descriptors of most of the remaining clusters.

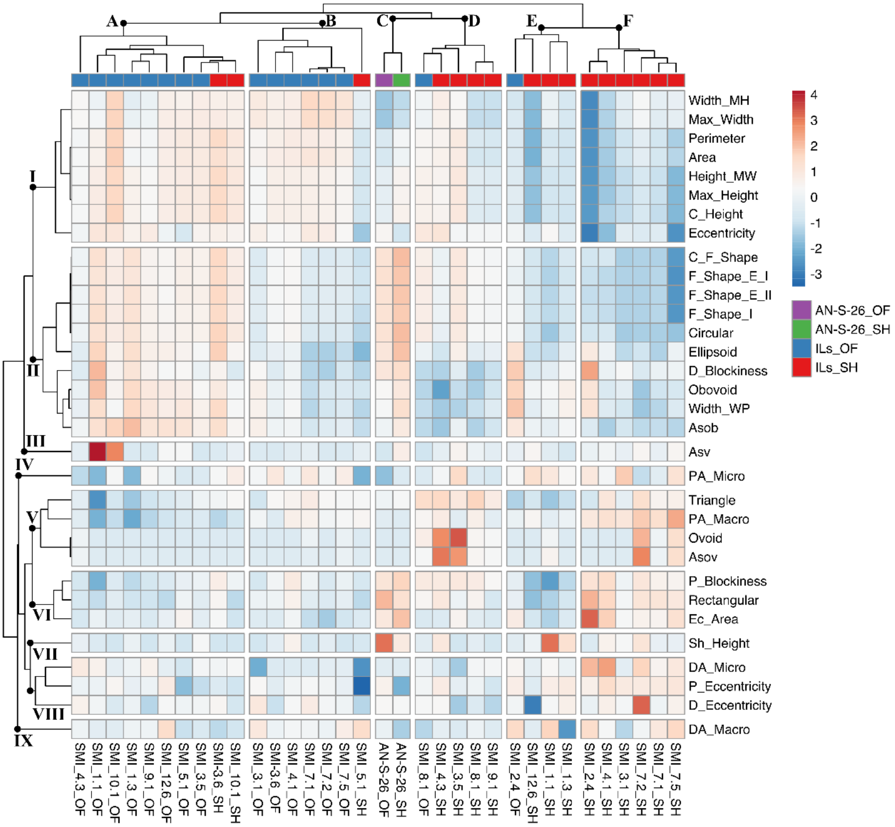

Figure 3. Hierarchical clustering heatmap for the recipient parent (AN-S-26) and the 16 ILs under open field (OF) and screenhouse (SH) conditions for the 32 Tomato Analyzer descriptors assessed in this study.

QTL detection

A total of 41 stable QTLs were found for 13 morphometric traits assessed with Tomato Analyzer in the IL set (Table 4 and Figure 4), with at least one QTL identified for each IL. Four stable QTLs were detected for Basic Measurement descriptors. Two of these QTLs (*wmh3* and *mw3*) were located on chromosome 3 (SMI_3.6), and the two others (*wmh10* and *mw10*) on chromosome 10 (SMI_10.1). The QTLs *wmh3* and *wmh10* accounted for an increase of Width_MH of 32.09% in

OF and of 25.78% in SH, and of 48.96% in OF and of 26.25% in SH, respectively. In the same way, QTLs *mw3* and *mw10* displayed considerable effects for Max_Width, with an increase of 31.64% in OF and 25.52% in SH, and 48.6% in OF and 23.85% in SH, respectively. For Fruit Shape Index descriptors, 12 stable QTLs were detected on chromosomes 2, 3, 4 and 7. For each of the three descriptors (F_Shape_E_I, F_Shape_E_II and C_F_Shape) 4 QTLs spread over eight ILs (SMI_2.4, SMI_3.1, SMI_3.5, SMI_4.1, SMI_4.3, SMI_7.1, SMI_7.2 and SMI_7.5) were identified. These QTLs induced a considerable decrease over the recipient parent, ranging from -6.66% (*fseII3*) to -19.12% (*cfs7*) in OF, and from -10.71% (*fseI3*) to -47.57% (*fseII7*) in SH. For Blockiness descriptors, 4 stable QTLs were found to be distributed on chromosomes 1, 4, 8 and 12. Two QTLs (*pfb1*and *pfb12*) accounted for a decrease of P_Blockiness ranging from -11.45% to -15.45% in OF and from -18.82% to -22.07% in SH. Two QTLs (*dfb4* and *dfb8*) were involved in D_Blockiness variation, resulting in a D_Blockiness reduction of 9.37% in OF and 13.53% in SH, and of 16.31% in OF and 17.13% in SH, respectively. For Homogeneity descriptors, 10 stable QTLs were identified on chromosomes 1, 2, 3, 4, 5, 7, 10 and 12. The QTLs *eli4* and *eli7*, the first located in SMI_4.1 and SMI_4.3 and the latter in SMI_7.1, SMI_7.2 and SMI_7.5, accounted for an Ellipsoid decrease ranging from -22.51% to -33.82% in OF, and from -22.01% to -39.17% in SH. The QTLs *cir2* (SMI_2.4), *cir3* (SMI_3.1 and SMI_3.5), *cir4* (SMI_4.1 and SMI_4.3) and *cir7* (SMI_7.1, SMI_7.2 and SMI_7.5) detected, exhibited large decrease effects on Circular ranging from -14.08% to -41.40% in OF and from -21.66% to -68.53% in SH. A considerable decrease over AN-S-26 parent was found for the QTLs *rec1* (SMI_1.1 and SMI_1.3), *rec5* (SMI_5.1), *rec10* (SMI_10.1) and *rec12* (SMI_12.6) that decreased the Rectangular descriptor values from -6.3% to -10.16% in OF and from -6.55% to -9.41% in SH. For Asymmetry descriptors, one QTL was identified on chromosome 8 (*obv8*). The effect of *obv8* resulted in a change of -35.75% in OF and -44.44% in SH of Obovoid, with a negative allelic effect between -0.04 and -0.05. For Internal Eccentricity descriptors, 10 stable QTLs were found spread in all chromosomes except for chromosomes 5, 6 and 11. The QTLs *fsi2* (SMI_2.4), *fsi3* (SMI_3.1 and SMI_3.5), *sfi4* (SMI_4.1 and SMI_4.3) and *fsi7* (SMI_7.1, SMI_7.2 and SMI_7.5) had a considerable decrease effect on F_Shape_I (from -7.73% to -19.86% in OF and from -10.54% to -46.44% in SH). Similarly, the QTLs *eca1* (SMI_1.1 and SMI_1.3), *eca4* (SMI_4.1 and SMI_4.3), *eca8* (SMI_8.1), *eca9* (SMI_9.1), *eca10* (SMI_10.1) and *eca12* (SMI_12.6) accounted for a decrease of Ec_Area ranging from -4.16% to -5.96% in OF and from -8.11% to -10.08% in SH.

QTLs controlling correlated descriptors co-localized in the same genomic region. In fact, the QTLs detected for Width_Mh and Max_Width co-localized in SMI_3.6 and SMI_10.1. In the same way, all the QTLs detected for F_Shape_E_I, F_Shape_E_II, F_C_Shape, Circular and F_Shape_I co-localized over eight ILs (SMI_2.4, SMI_3.1, SMI_3.5, SMI_4.1, SMI_4.3, SMI_7.1, SMI_7.2 and SMI_7.5).

Table 4. List of putative QTLs for the fruit shape traits analyzed found in the ILs and their physical position into the "67/3" eggplant reference genome, along with the increase over the recipient parent AN-S-26 and their allelic effects in the open field (OF) and screenhouse (SH).

Trait	QTL	Chr.	Physical position (Mb)	Ils carrying the QTL	Increase over AN-S-26		Allelic effect	
					OF	SH	OF	SH
Basic Measurements								
Width_Mh[a]	*wmh3*	3	93 - 96	SMI_3.6	32.09	25.78	0.64	0.54
	wmh10	10	0 -2	SMI_10.1	48.96	26.25	0.98	0.55
Max_Width[a]	*mw3*	3	93 - 96	SMI_3.6	31.64	25.52	0.65	0.56
	mw10	10	0 -2	SMI_10.1	48.6	23.85	1.00	0.52
Fruit Shape Index								
F_Shape_E_I[b]	*fseI2*	2	75 - 81	SMI_2.4	-15.17	-29.99	-0.12	-0.27
	fseI3	3	78 - 86	SMI_3.1, SMI_3.5	-7.79	-10.71	-0.06	-0.09
	fseI4	4	4 - 85	SMI_4.1, SMI_4.3	-13.01	-19.75	-0.11	-0.17
	fseI7	7	129 - 135	SMI_7.1, SMI_7.2, SMI_7.5	-18.44	-44.41	-0.15	-0.39
F_Shape_E_I[b]	*fseII2*	2	75 - 81	SMI_2.4	-13.8	-32.97	-0.11	-0.30
	fseII3	3	78 - 86	SMI_3.1, SMI_3.5	-6.66	-11.25	-0.06	-0.10
	fseII4	4	4 - 85	SMI_4.1, SMI_4.3	-13.63	-21.84	-0.11	-0.20
	fseII7	7	129 - 135	SMI_7.1, SMI_7.2, SMI_7.5	-18.95	-47.57	-0.16	-0.43
C_F_Shape[b]	*cfs2*	2	75 - 81	SMI_2.4	-13.31	-30.44	-0.11	-0.28
	cfs3	3	78 - 86	SMI_3.1, SMI_3.5	-7.80	-11.64	-0.07	-0.11
	cfs4	4	4 - 85	SMI_4.1, SMI_4.3	-13.81	-22.92	-0.12	-0.21
	cfs7	7	129 - 135	SMI_7.1, SMI_7.2, SMI_7.5	-19.12	-45.08	-0.16	-0.42
Blockiness								
P_Blockiness	*pfb1*	1	27 - 36	SMI_1.1, SMI_1.3	-15.45	-18.82	-0.05	-0.06
	pfb12	12	3 - 96	SMI_12.6	-11.45	-22.07	-0.03	-0.07
D_Blockiness	*dfb4*	4	4 - 85	SMI_4.1, SMI_4.3	-9.37	-16.31	-0.04	-0.06
	dfb8	8	3 - 109	SMI_8.1	-13.53	-17.13	-0.05	-0.07
Homogeneity								
Ellipsoid	*eli4*	4	4 - 85	SMI_4.1, SMI_4.3	-22.51	-39.17	-0.01	-0.01
	eli7	7	129 - 135	SMI_7.1, SMI_7.2, SMI_7.5	-33.82	-22.01	-0.01	-0.01
Circular[b]	*cir2*	2	75 - 81	SMI_2.4	-25.05	-54.96	-0.02	-0.05
	cir3	3	78 - 86	SMI_3.1, SMI_3.5	-14.08	-21.66	-0.01	-0.02
	cir4	4	4 - 85	SMI_4.1, SMI_4.3	-27.27	-42.00	-0.02	-0.04
	cir7	7	129 - 135	SMI_7.1, SMI_7.2, SMI_7.5	-41.40	-68.53	-0.03	-0.06
Rectangular	*rec1*	1	27 - 36	SMI_1.1, SMI_1.3	-9.36	-6.55	-0.02	-0.02
	rec5	5	35 - 43	SMI_5.1	-6.30	-7.65	-0.02	-0.02
	rec10	10	0 -2	SMI_10.1	-7.70	-7.79	-0.02	-0.02
	rec12	12	3 - 96	SMI_12.6	-10.16	-9.41	-0.03	-0.02
Asymmetry								

Obovoid	*obv8*	8	3 - 109	SMI_8.1	-35.75	-44.44	-0.04	-0.05
Internal Eccentricity								
F_Shape_I[b]	*fsi2*	2	75 - 81	SMI_2.4	-13.54	-31.78	-0.11	-0.28
	fsi3	3	78 - 86	SMI_3.1, SMI_3.5	-7.73	-10.54	-0.06	-0.09
	fsi4	4	4 - 85	SMI_4.1, SMI_4.3	-13.95	-20.82	-0.12	-0.19
	fsi7	7	129 - 135	SMI_7.1, SMI_7.2, SMI_7.5	-19.86	-46.44	-0.17	-0.42
Ec_Area	*eca1*	1	27 - 36	SMI_1.1, SMI_1.3	-4.40	-8.70	-0.01	-0.02
	eca4	4	4 - 85	SMI_4.1, SMI_4.3	-5.96	-8.58	-0.01	-0.02
	eca8	8	3 - 109	SMI_8.1	-5.67	-8.11	-0.01	-0.02
	eca9	9	5 - 34	SMI_9.1	-5.75	-8.85	-0.01	-0.02
	eca10	10	0 -2	SMI_10.1	-4.94	-10.08	-0.01	-0.02
	eca12	12	3 - 96	SMI_12.6	-4.16	-9.44	-0.01	-0.02

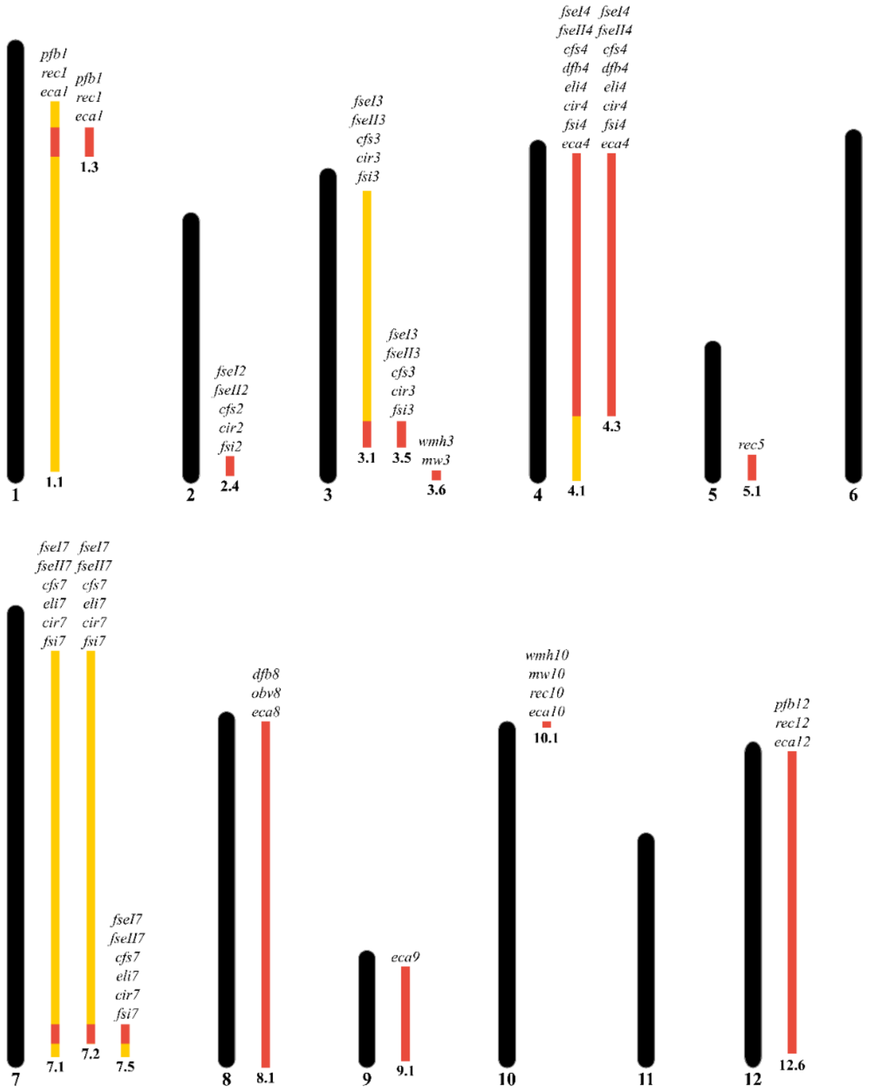

Figure 4. Physical position and size of the ILs (in yellow and/or red), compared to their respective eggplant chromosomes (in black), and genomic regions carrying stable QTLs (in red) identified in each line for the morphometric traits assessed with the phenomics tool Tomato Analyzer. The name of the QTLs carried by each IL is indicated above the corresponding IL, while an abbreviated QTL code (i.e., "1.1" stands for "SMI_1.1") is indicated below.

Analysis of genetic variants in fruit shape related genes

A total of 118 homozygous allelic variants were identified in 43 out of 74 genes belonging to three selected gene families that control fruit shape in tomato (*SUN*, *OVATE* and *YABBY*). To test if a variant has an impact on the biological protein function, the effects of amino acid substitutions and indels were predicted using SIFT and PROVEAN software. A total of 36 variants with predicted high impact effects on protein function were found in 19 eggplant genes, of which 11 of them were located within the introgressed fragments of the ILs (Table 5). SIFT and PROVEAN classified as deleterious, respectively, 27 and 9 variants, with only one variant (L535W) considered deleterious by both software. All the remaining variants were classified as neutral, according to the prediction of both software (Supplementary data S2). SIFT predictions were not available for 4 substitutions (T123_A124insA, del354R, Q119dup and S171del), mainly because the amino acid change involved more than one nucleotide change within a codon.

Table 5. Effect prediction on protein functionality using SIFT (cutoff = 0.05) and PROVEAN (cutoff = -2.5) software for the homozygous variants between the two parents of the IL population in eggplant genes identified as putative orthologous of tomato genes controlling fruit shape belonging to the SUN, OVATE and YABBY gene families. The ILs in which the allelic variant of the *S. incanum* donor parent MM577 is present in the background of the *S. melongena* recurrent parent MM577 are indicated for each gene. If not present in any of the ILs it is indicated by a minus (-) sign.

Gene	Eggplant locus	Variant type	aa Change	Parent alleles	SIFT		PROVEAN		ILs carrying the variant
					Predicted effect	Score	Predicted effect	Score	
SlSUN2	SMEL_001g148580.1	SNP	G317R	MM557	Deleterious	0.00	Neutral	0.618	SMI_1.1
		SNP	D331H	MM557	Deleterious	0.00	Neutral	-0.461	
		del_ins	P346S	MM557	Deleterious	0.00	Neutral	0.524	
SlSUN7	SMEL_002g164780.1	SNP	K26N	MM557	Deleterious	0.00	Neutral	-2.497	SMI_2.4
SlSUN10	SMEL_003g184300.1	SNP	S441N	MM557	Deleterious	0.00	Neutral	-0.545	SMI_3.1, SMI_3.5
SlSUN11	SMEL_003g197880.1	SNP	L285R	MM557	Deleterious	0.00	Neutral	-0.212	SMI_3.6
SlSUN13	SMEL_000g044110.1	SNP	F452S	AN-S-26	Deleterious	0.00	Neutral	0.360	-
SlSUN14	SMEL_004g221920.1	SNP	P140L	MM557	Deleterious	0.00	Neutral	0.203	SMI_4.1
		SNP	P458S	MM557	Deleterious	0.00	Neutral	0.397	
		SNP	K763N	MM557	Deleterious	0.00	Neutral	1.032	
SlSUN15	SMEL_010g349640.1	SNP	D521N	MM557	Neutral	0.07	Deleterious	-3.212	-
SlSUN16	SMEL_006g250960.1	SNP	A93V	MM557	Deleterious	0.00	Neutral	0.749	-
SlSUN19	SMEL_008g297530.1	SNP	S304L	MM557	Deleterious	0.00	Neutral	-1.228	-
		SNP	T352R	MM557	Deleterious	0.00	Neutral	1.839	
		SNP	M355L	MM557	Deleterious	0.00	Neutral	-0.417	
		SNP	S364N	MM557	Deleterious	0.00	Neutral	-0.939	
		SNP	C394G	MM557	Deleterious	0.00	Neutral	1.344	
SlSUN22	SMEL_008g305570.1	SNP	V12I	MM557	Deleterious	0.00	Neutral	0.010	SMI_8.1
		SNP	I297V	MM557	Deleterious	0.00	Neutral	0.353	
SlSUN23	SMEL_008g318520.1	SNP	N60T	MM557	Deleterious	0.00	Neutral	1.190	SMI_8.1
		SNP	R445G	MM557	Deleterious	0.00	Neutral	4.057	
SlSUN25	SMEL_009g322090.1	SNP	E235K	MM557	Neutral	0.86	Deleterious	-3.547	-
		SNP	Y242S	MM557	Neutral	0.71	Deleterious	-4.767	
SlSUN26	SMEL_009g331590.1	SNP	R20K	MM557	Deleterious	0.00	Neutral	0.367	SMI_9.1
		SNP	S77A	MM557	Deleterious	0.00	Neutral	0.411	
		ins	T123_A124insA	MM557	-	-	Neutral	1.722	
		SNP	E454Q	MM557	Deleterious	0.00	Neutral	0.306	
SlSUN31	SMEL_012g395480.1	SNP	L535W	MM557	Deleterious	0.00	Deleterious	-3.260	SMI_12.6
		SNP	C215F	MM557	Deleterious	0.00	Neutral	1.106	

SlSUN32	SMEL_012g380740.1	SNP	S296A	MM557	Deleterious	0.00	Neutral	0.912	-
SlOFP10	SMEL_005g229890.1	SNP	E162V	AN-S-26	Neutral	0.70	Deleterious	-5.967	-
SlOFP22	SMEL_010g357940.1	SNP	E240G	MM557	Neutral	0.43	Deleterious	-3.350	-
		SNP	M253I	MM557	Neutral	1.00	Deleterious	-3.500	
		SNP	Y274N	MM557	Neutral	0.85	Deleterious	-8.900	
SlYABBY2b	SMEL_012g395510.1	SNP	H169R	AN-S-26	Neutral	0.38	Deleterious	-6.206	SMI_12.6
YABBY1a	SMEL_001g131520.1	SNP	H9Q	MM557	Deleterious	0.00	Neutral	0.150	SMI_1.1

5. Discussion

Fruit shape is a relevant morphological trait for eggplant breeding, and, like fruit size and color, is quantitatively inherited (Page et al., 2019). Despite its importance, compared to tomato, little information is available about the genetic basis of fruit shape in eggplant. QTLs and genes controlling traits associated to eggplant fruit shape have been previously detected by linkage mapping approach in interspecific and intraspecific F2 populations (Doganlar et al., 2002; Frary et al. 2014; Portis et al., 2014; Wei et al., 2020) and through genome-wide association studies (Portis et al., 2015). The present study is the first combining the use of an experimental introgression line population and a phenomics tool to enhance the precision in the detection of genomic regions controlling this quantitative trait.

As expected, many differences in fruit shape were found between the two parents, the cultivated *S. melongena* AN-S-26 and the wild relative *S. incanum* MM557, confirming that diversification from small rounded to more elongated fruit occurred during eggplant domestication process (Wang et al., 2008). Although the hybrid set fruits only in the open field, it displayed negative heterosis for most fruit shape descriptors, so that they are skewed towards that of the wild parent. In this regard, Kaushik et al. (2016) observed that, in the case of crosses involving cultivated eggplants and wild species, generally, the hybrid fruit is phenotypically closer to its wild parent than to the cultivated one, probably due to the overall dominance of wild traits over the domesticated ones (Lester, 1989; Page et al., 2019). The negative heterotic values of the interspecific hybrid over the mid-parent for fruit shape traits are the opposite of the positive heterotic values observed for vigour-related traits in Mangino et al. (2020).

Significant differences found in fruit shape between ILs and the recipient parent suggested the existence of a relevant effect of the introgressions on this trait, even in presence of small wild donor fragments. This is in contrast with previously reported for morphological traits, for most of which the ILs showed minimal phenotypic differences compared to the recipient parent, even in the presence of large *S. incanum* introgressions (Mangino et al., 2020). This discrepancy can be explained due to the quantitative nature of both vigour-related traits of Mangino et al. (2020) and shape-related traits of this study. However, while for fruit shape, major genes have been described that affect substantially the phenotype, like *SUN*, *OVATE* or *FAS* in tomato (Liu et al., 2002; Xiao et al., 2008; Xu et al., 2015), no major genes have been reported so far that impact dramatically the plant vigour or morphology traits like those assessed in Mangino et al. (2020). Thus, it is likely that introgressions, even small, that carry some major genes, or with medium effects, on fruit shape can have a significative impact on fruit phenotype and exhibit significant differences with the recipient parent. Similarly, the same introgressions can carry genes that are involved in vigour or morphology traits, but their effects are too low to be considered as significant. Analysis of variance showed wide variations among fruit shape morphometric traits for the genotype factor contribution, environment, or G × E interaction effects. Although the contribution of the environment and the G × E interaction was significant for almost all descriptors, their effects on fruit shape were relatively low. In fact, the contribution of genotype factor, in general, was the largest for many descriptors, indicating that the variation observed in fruit shape is mainly genetically regulated, as previously reported in tomato (El-Gabri et al., 2014; Monforte et al., 2014; Figàs et al., 2018). However, the fact that the contribution of the residual effect was the largest for many descriptors, might indicate that the influence of the environment was greater than genetic variability between the two parents for these specific traits. This is probably caused because for these traits there is little genetic variation among the ILs for these traits. The use of larger sample sizes might help to discern if some genetic variation exists among the ILs.

As found in previous studies in eggplant (Hurtado et al., 2013), tomato (Figas et al., 2015; Mohan et al., 2016) and pepper (Tripodi and Greco, 2018; Colonna et al., 2019), many Tomato Analyzer descriptors are interrelated, since they measure very similar shape characters. Here, nine clusters of highly correlated descriptors were observed, suggesting that, although Tomato Analyzer software can provide a good characterization of eggplant fruit shape (Prohens et al., 2012; Hurtado et al., 2013; Plazas et al., 2014; Kaushik et al., 2016, 2018), with fewer traits assessed we could obtain similar comprehensive information on this trait in eggplant.

In total, we identified 41 stable QTLs related to fruit shape, increasing the number of known QTLs in eggplant, particularly for fruit shape. In agreement with previous studies based on intraspecific population between eggplant lines ‘305E40’ and ‘67/3’ (Portis et al., 2014) and GWAS analysis (Portis et al., 2015), we detected four QTLs controlling fruit width, among which two (*wmh3.6* and *mw3.6*) were on chromosome 3 (SMI_3.6) and the two other (*wmh10.1* and *mw10.1*) on chromosome 10 (SMI_10.1). We found that *wmh3.6*/*wmh10.1* and *mw3.6*/*mw10.1* increased Width_MH between 26.3% and 49.0% and Max_Width between 23.9 and 48.7%, respectively, suggesting that *S. incanum* harbours QTLs which would make the fruit wider. QTLs controlling fruit shape index in eggplant have been identified spread over many chromosomes (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014; Portis et al., 2015; Wei et al., 2020). Using an F2 between *S. melongena* ‘MM738’ and *S. linneanum* ‘MM195’, Doganlar et al. (2002) identified two fruit-shape index related QTLs on chromosome 2 and 7 which explained between 34% to 36% of the variation for this trait. Portis et al. (2014) detected five major QTLs affecting fruit shape index on chromosomes 1, 3, 7, 11 and 12, and, subsequently, additional QTLs were detected on chromosome 5 and 10 (Portis et al., 2015). Moreover, a more recent study used an F2 population between *S. melongena* ‘1836’ and *S. linnaeanum* ‘1809’ reported four fruit shape index QTLs on chromosome 1 and 3 (Wei et al., 2020). In the present study, for each of the three Fruit shape index descriptors (F_Shape_E_I, F_Shape_E_II and C_F_Shape) we detected four stable QTLs spread over chromosomes 2, 3, 4 and 7, confirming the QTLs locations of previous studies. Moreover, probably thanks to the higher precision of the phenomic analysis made with Tomato Analyser, we could also confirm a site-specific and minor fruit shape index QTL identified on chromosome 4 by Portis et al. (2014), suggesting the importance of high-resolution morphometric tools and advanced introgressed materials in the detection of minor QTLs for the explanation of the phenotypic variation.

In addition, we have reported novel QTLs associated with Tomato Analyzer descriptors (Blockiness, Homogeneity, Asymmetry and Internal Eccentricity) that have not been assessed before in eggplant. Regarding D_Blockiness and P_Blockiness, we detected four QTLs in our lines being two of them syntenic to tomato (*pfb1* and *dfb8*) (Brewer et al., 2007; Gonzalo and van der Knaap, 2008). As well, we described novel QTLs on chromosomes 2, 3, 4 and 7 for F_Shape_I, which describes the shape of the internal ellipse drawn around the seed area, and on chromosomes 1, 4, 8, 9, 11 and 12 for Eccentricity area index (Ec_area), which explains the ratio of the ellipse area over total fruit area; some of them are syntenic to those identified in tomato on chromosomes 2 and 8 (Gonzalo et al., 2009). We found that QTLs affecting ellipsoid or circular fruit shape were located on chromosomes 2 (*cir2*), 3 (*cir3*), 4 (*eli4* and *cir4*) and 7 (*eli7* and *cir7*), while rectangular fruit shape resulted affected by QTLs located on chromosomes 1 (*rec1*), 5 (*rec5*), 10 (*rec10*) and 12 (*rec12*). In tomato, long and oxheart fruit shape are often associated with mutations in *SUN* gene, mapped on chromosome 7, while obovoid, rectangular, ellipsoid, heart and pear fruit shape are often associated with mutations in the *OVATE* gene, mapped on chromosome 2 (Rodríguez et al. 2011). Our results suggest that rectangular fruit shape and ellipsoid/circular fruit shape are controlled by QTLs mapped to different chromosomes in eggplant. Moreover, of the six QTLs controlling ellipsoid and a circular shape in eggplant, one (*cir2*) and two (*eli7* and *cir7*) are syntenic to those of tomato on chromosome 2 and 7, respectively. We detected a QTL influencing obovoid fruit shape located on chromosome 8 (*obv8*). Similarly, a major QTL (*fs8.1*) controlling fruit shape in tomato by promoting the growth along the proximal-distal axis has been detected on chromosome 8 (Grandillo et al., 1996; Ku et al., 2000). Synteny between tomato and eggplant for the genomic region harboring *fs8.1* have been previously described by Portis et al. (2015). These evidences indicate the conservation of this QTL among some *Solanum* crops bearing fruits and suggest that in eggplant it could be involved in the regulation of obovoid fruit shape.

In general, we observed that QTLs detected for significantly correlated traits (r > 0.9) clustered within the same genomic regions. This might indicate that, what appear to be QTL clusters, probably, could be the result of a single pleiotropic locus, or, that different traits measured with Tomato Analyzer are assessing the same underlying character.

In the genomic regions where stable QTLs were detected, we assessed the presence of mutations in the genes of *SUN*, *OVATE* and *FAS* families from tomato that could be associated with the fruit shape variations in eggplant. Deleterious mutations (i.e., high-impact mutations) have been identified mainly in genes belonging to the *SUN* and *FAS* family, while in *OFP* family, deleterious mutations have been identified for genes mapped outside the QTL regions. In the small QTL regions of the non-overlapping ILs SMI_2.4 and SMI_3.6, we identified deleterious mutations for the tomato syntenic genes *SlSUN7* and *SlSUN11*, which could be proposed as candidate genes involved in the control of fruit shape in eggplant. Although most candidate genes have been identified in QTL regions covering a broad segment of a chromosome, we found some congruences with previous studies. In fact, according to Huang et al. (2013) which proposed *SlSUN22* as the gene underlying the *fs8.1* locus in tomato, in this study *SlSUN22* mapped in the QTL region of chromosome 8 (SMI_8.1) and showed two deleterious mutations (V12I and I297V). These evidences suggest that, although other unknown genes located on the same genomic regions may be involved in the regulation of fruit shape, candidate genes that we have identified could have a great impact on the determination of fruit shape in eggplant. forward in the understanding of this trait of great interest for eggplant breeding.

6. Conclusions

In the present study, we have demonstrated the utility of combining the use of a powerful phenomics tool (Tomato Analyzer) with an experimental population (ILs) for a more precise identification of genomic regions controlling fruit shape in eggplant. This has allowed the detection of many phenotypic variations for fruit shape traits between ILs and recipient parent (*S. melongena*), even in the presence of small introgression from *S. incanum* parent. New stable QTLs for fruit shape traits first identified here in eggplant, as well as QTLs syntenic to those previously reported in tomato and eggplant populations, have been detected. In addition, in genomic regions underlying QTLs, we identified potential candidate genes syntenic to tomato ones belonging to the *SUN* and *YABBY* families that could have a significant effect on the fruit shape variations in eggplant. These findings are of great interest for eggplant breeding and make a relevant contribution to elucidate the genetic basis of fruit shape in this crop. interest for eggplant breeding.

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8. Additional files

Supplementary data S1. Pearson's correlation coefficients among the fruit shape descriptors using data of the ILs and AN-S-26 in both environments. https://ars.els-cdn.com/content/image/1-s2.0-S0304423821001138-mmc1.xlsx

Supplementary data S2. Effect prediction on protein functionality using SIFT (Sorting Intolerant From Tolerant) (Ng and Henikoff, 2001) and PROVEAN (PROtein Variation Effect Analyzer) (Choi et al., 2012) software for the homozygous variants between the two parents of the IL population in eggplant genes identified as putative orthologous of tomato genes controlling fruit shape belonging to the SUN, OVATE and YABBY gene families described in Huang et al. (2013). https://ars.els-cdn.com/content/image/1-s2.0-S0304423821001138-mmc2.xlsx

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Chapter 3: Development of an 8-way multiparent MAGIC population in eggplant

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Author Contributions: S.V., P.G. and J.P. conceived the idea and supervised the manuscript; **G.M.**, A.A., M.P. and P.G. performed the field trials. All authors analyzed the results. **G.M.** and A.A. prepared a first draft of the manuscript and the rest of authors reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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1. Abstract

MAGIC populations facilitate the genetic dissection of complex quantitative traits in plants and are valuable breeding materials. We report the development of the first eggplant MAGIC population (S3MEGGIC; 8-way), constituted by 420 S3 individuals developed from the intercrossing of seven cultivated eggplant (*Solanum melongena*) and one wild relative (*S. incanum*) parents. The S3MEGGIC recombinant population was genotyped with the eggplant 5k probes SPET platform. The 7,724 filtered high-confidence SNPs confirmed a low residual heterozygosity (6.87%) and a lack of genetic structure in the S3MEGGIC population, including no differentiation among subpopulations carrying cultivated or wild cytoplasm. Inference of haplotype blocks of the nuclear genome revealed an unbalanced representation of founder genomes, suggesting cryptic selection in favour or against specific parental genomes. Our new eggplant MAGIC population is the largest recombinant population in eggplant and is a powerful tool for eggplant genetics and breeding studies.

Keywords: Multi parent Advanced Generation Inter Crosses (MAGIC), eggplant (*Solanum melongena* L.), *S. incanum*, interspecific MAGIC founders, SPET (Single Primer Enrichment Technology), Single nucleotide polymorphism (SNP).

2. Introduction

Multi-parent experimental populations are of great interest for the genetic dissection of quantitative traits as well as for the development of new recombinant materials for plant breeding (Huang et al., 2015). Despite their complex management and resources requirement, multi-parent advanced generation inter-cross (MAGIC) populations represent powerful next-generation mapping tools by combining high genetic diversity and recombination with low population structure (Arrones et al., 2020; Scott et al., 2020). MAGIC populations are already available in model species such as Arabidopsis thaliana and in several crops, such as cereals, pulses and vegetables (Kover et al., 2009; Bandillo et al., 2013; Pascual et al., 2015; Huynh et al., 2018), and have demonstrated their power to dissect the structure of complex traits (Dell'Acqua et al., 2015; Stadlmeier et al., 2018).

Although available MAGIC populations have become a useful resource for genetic studies and breeding, most of them have only exploited intraspecific variation. The incorporation of crop wild relatives (CWRs) as founders could be a way of including multiple wild genomic fragments or introgressions into cultivated background genomes (Arrones et al., 2020). Apart from being of great interest for genetic analysis, interspecific MAGIC populations can be useful for broadening the genetic base of crops and provide new variation for breeding multiple traits, including those related to adaption to climate change (Gramazio et al., 2020a). However, so far, the potential of interspecific MAGIC populations for plant breeding has largely remained unexploited (Arrones et al., 2020).

Eggplant (*Solanum melongena* L.) is a major vegetable crop of increasing importance, ranking fifth in global production among vegetables (Faostat, 2019). Despite its economic importance, eggplant has lagged behind other major crops and little efforts have been made to develop immortal experimental populations and genetic and genomic tools (Gramazio et al., 2018, 2019). So far, only one population of recombinant inbred line (RILs) and one set of introgression lines (ILs) are publicly available (Lebeau et al., 2013; Gramazio et al., 2017a), while no multiparent population has been developed so far. Conversely, in other related Solanaceae crops such as tomato, several experimental populations have been developed including MAGIC populations, which have allowed great advances in the genetic dissection of traits of interest (Pascual et al., 2015; Campanelli et al., 2019). For this reason, the development of this type of population would represent a landmark in eggplant breeding.

Here we report on the first eggplant MAGIC population derived from an interspecific cross of seven accessions of *S. melongena* and one of the wild relative *S. incanum* (Gramazio et al., 2019). It represents the largest experimental population described so far in eggplant, with a similar population size to MAGIC populations in other solanaceous crops. The population has been genotyped by applying the Single Primer Enrichment Technology (SPET) to explore its genetic architecture and the contribution of founders to the final population.

3. Materials and methods

MAGIC population construction

The eggplant MAGIC population has been developed by intermating seven cultivated eggplants, i.e., MM1597 (A), DH ECAVI (B), AN-S-26 (D), H15 (E), A0416 (F), IVIA-371 (G) and ASI-S-1 (H), and the *S. incanum* accession MM577 (C) (Figure 1). The wild relative founder was chosen for its tolerance to some biotic and abiotic stresses, mainly drought (Knapp et al., 2013), and for showing high phenolic content (Prohens et al., 2013). The performance of the founders was comprehensively characterized in previous morphoagronomic and genetic diversity studies (Hurtado et al., 2014; Gramazio et al., 2017b; Kaushik et al., 2018) and their genomes have been resequenced (Gramazio et al., 2019). The latter study highlighting that in the founder parents the residual heterozygosity was less than 0.06%.

In order to develop the eggplant S3MEGGIC (S3 Magic EGGplant InCanum) collection, founder lines have been inter-crossed by following a simple “funnel” approach (Wang et al., 2017; Arrones et al., 2020) (Figure 1). The eight founders (A-H) have been pairwise inter-crossed to produce two-way or simple F1 hybrids (AB, CD, EF, GH), which were subsequently inter-crossed in pairs (AB × CD and EF × GH) to obtain two four-way or double hybrids (ABCD and EFGH). In order to achieve a complete admixture of all founder genomes and to avoid assortative mating, the double hybrids were intercrossed following a chain pollination scheme, with each individual being used once as a female parent and as a male parent (Díez et al., 2002) (Figure 2a).

All the eight-way or quadruple hybrids obtained (S0 generation) presented all the eight genomes randomly shuffled and only differed for the cytoplasm inherited from the maternal parent. The S0 progenies obtained using the double hybrid ABCD as female parent carried the cytoplasm of the wild *S. incanum* MM577, while those derived using the double hybrid EFGH as female parent carried the cytoplasm of the cultivated *S. melongena* ASI-S-1. Subsequently, the S0 progenies were selfed for three generations by single seed descent (SSD) to obtain the S3 segregating individuals that were genotyped in this study. To ensure the continuity of the S0 progenies and to accelerate the self-fertilization process, four plants of each S0 progeny were germinated, selecting for the next generation (S1) only the first two that set viable seed (Figure 2b). From each of the two S0 selected plants, two S1 plants were germinated and only the first one setting fruit was selected for the S2 generation. The same was done for the S3 generation, so that for each progeny two plants were germinated but only one was used for originating the next generation. Differently, when in the S3 progenies two individuals displayed some phenotypic differences, both were included in the S3MEGGIC population.

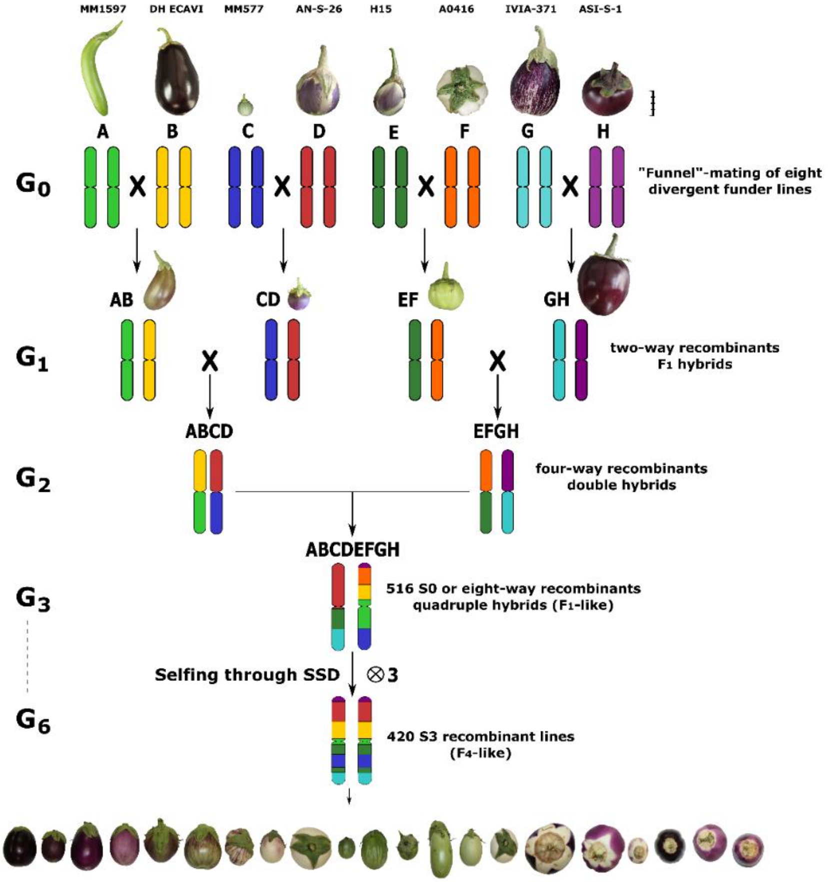

Figure 1. The funnel breeding design used, across the six generations (G1 to G6), to develop the 420 S3 individuals of the S3MEGGIC population. The eight parents, coded from A to H and each with a different color to represent their genomic background, are represented above at a scale based on the real fruit size. Scale bar represents 5 cm. The four two-way hybrids obtained in the G1 generation (AB, CD, EF and GH) are also represented at the same scale as the founders.

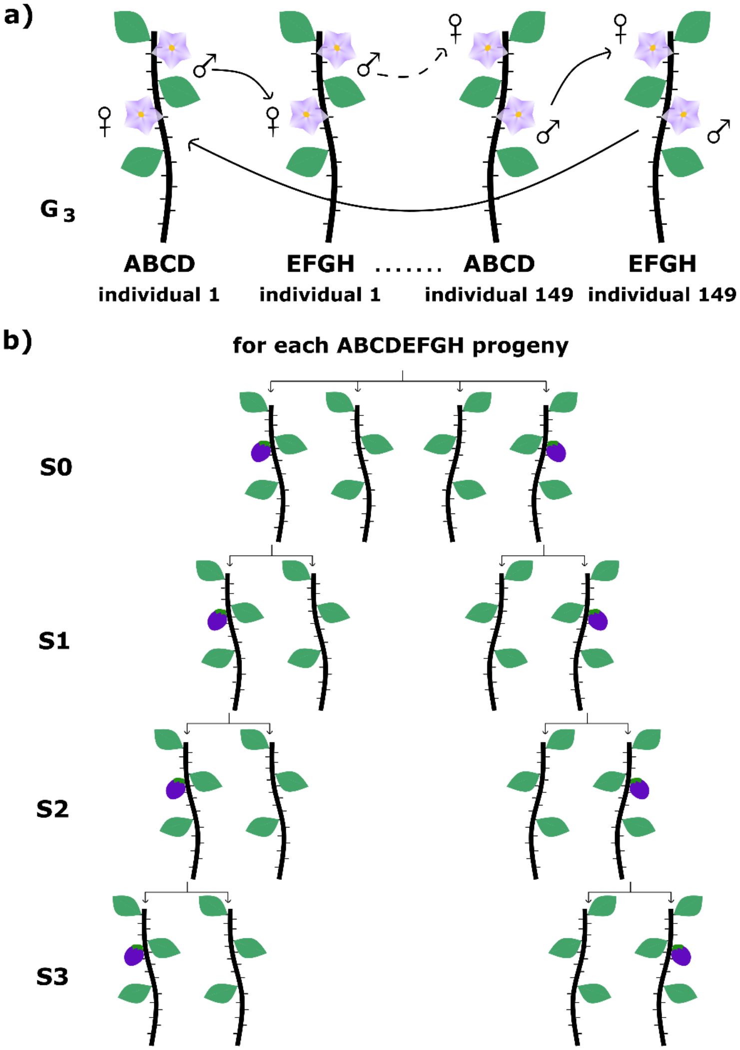

Figure 2. **(a)** Chain pollination scheme of the four-way hybrids followed to obtain the eight-way hybrids. **(b)** For each S0 progeny, four plants were germinated, selecting for the next generation (S1) only the first two setting fruits with viable seed. For subsequent generations, two plants were germinated and only the first one setting fruit was selected for the next generation.

Cultivation conditions

Seeds were germinated in Petri dishes, following the protocol developed by Ranil et al. (2015), and subsequently transferred to seedling trays in a climatic chamber under photoperiod and temperature regime of 16 h light (25 °C) and 8 h dark (18 °C). After acclimatization, plantlets were transplanted to 15 L pots and grown in a pollinator-free benched glasshouse of the UPV, Valencia, Spain (GPS coordinates: latitude, 39° 28′ 55″ N; longitude, 0° 20′ 11″ W; 7 m above sea level). Plants were spaced 1.2 m between rows and 1.0 m within the row, fertirrigated using a drip irrigation system and trained with vertical strings. Pruning was done manually to regulate vegetative growth and flowering. Phytosanitary treatments were performed when necessary. In order to shorten generation time of subsequent generations (S0-S3), plantlets were transplanted to individual thermoformed pots (1.3 L capacity) in a pollinator-free glasshouse and selfings were stimulated by mechanical vibration.

High-throughput genotyping

Young leaf tissue was sampled for 420 S3 individuals, the eight founders and the four two-way hybrids. Genomic DNA was extracted using the SILEX extraction method (Vilanova et al., 2020) and checked for quality and integrity by agarose electrophoresis and Nanodrop ratios (260/280 and 260/230), while its concentration was estimated with Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, United States). After dilution, the samples were sent to IGA Technology Services (IGATech, Udine, Italy) for library preparation and sequencing with NextSeq500 sequencer (150 paired-end) for high-throughput genotyping using the Single Primer Enrichment Technology (SPET) technology using the 5k probes eggplant SPET platform (Barchi et al., 2019a). The latter comprises 5,093 probes and was developed by filtering out the most informative and reliable polymorphisms (3,372 of them in CDSs and 1,721 in introns and UTRs regions) from the set of over 12 million SNPs identified among the MAGIC founders (Gramazio et al., 2019).

Raw reads were demultiplexed and the adapters removed using standard Illumina pipeline and Cutadapt (Martin, 2011) while trimming was performed by ERNE (Del Fabbro et al., 2013). Clean reads were mapped onto the eggplant reference genome "67/3" (Barchi et al. 2019b) using BWA-MEM (Li, 2013) with default parameters and only uniquely aligned reads were selected for the variant calling performed with GATK 4.0 (DePristo et al., 2011) following the best practice recommended by the Broad Institute (http://www.broadinstitute.org).

The SNPs identified by SPET were filtered using the Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) software (ver. 5.0, Bradbury et al., 2007) in order to retain the most reliable ones (minor allele frequency > 0.01, missing data < 10%

and maximum marker heterozygosity < 70%). In addition, a LD k-nearest neighbour genotype imputation method (LD KNNi) was performed to fill missing calls or genotyping gaps.

Population structure, heterozygosity, haplotype blocks inferring

A principal component analysis (PCA) was performed to assess the population structure of S3MEGGIC using the R package vcfR (Knaus and Grünwald, 2017) and the function glPCA of the Adegenet package (Jombart, 2008). Finally, the PCA was graphically plotted with ggplot2 (Wickham, 2016). An Analysis of Molecular Variance (AMOVA) was performed to estimate population differentiation according to the cytoplasm (cultivated vs. wild) of the individuals of the S3MEGGIC population by using the function poppr.amova of the poppr R package (Kamvar et al., 2014). The residual heterozygosity and its distribution were evaluated with TASSEL software (ver. 5.0, Bradbury et al., 2007). Parental contribution to S3MEGGIC individuals and haplotype blocks were estimated by using R-package HaploBlocker (Pook et al., 2019).

4. Results

MAGIC population construction

Seven accessions of eggplant and one of the wild relative *S. incanum* were selected as founder parents (A-H) for the construction of the eggplant MAGIC population (S3MEGGIC). Following a funnel breeding scheme (Figure 1) a total of 420 individuals of the MAGIC populations were obtained. First, founders were pairwise inter-crossed to produce four two-way hybrids (AB, CD, EF, and GH), which were subsequently inter-crossed in pairs to obtain four-way hybrids (ABCD and EFGH). One-hundred and forty-nine individuals of each of the two four-way hybrids were inter-crossed using a chain pollination scheme (Figure 2). Out of the theoretical maximum of 298 eight-way hybrid progenies (S0), seeds were obtained for 209 of them, of which 116 carried the *S. melongena* ASI-S-1 cytoplasm and 93 the *S. incanum* MM577 cytoplasm. Two plants per S0 progeny were used to advance the population reaching 402 S1 progenies. These S1 progenies were advanced through single seed descend (SSD) to obtain 391 S2 and 305 S3 MAGIC progenies. The final S3MEGGIC population was constituted by 420 S3 individuals, of which 348 individuals carried the cultivated cytoplasm and 72 the wild cytoplasm.

SPET genotyping

The genotyping of the 420 S3 MAGIC individuals, the eight founders and the four two-way hybrids by the eggplant SPET platform yielded 22,146 SNPs. After filtering, 7,724 high-confidence SNPs were retained for the subsequent analysis and the low percentage of missing calls (0.53%) was imputed. Filtered SNPs were distributed across the entire eggplant genome, although the distribution of SNPs varied within and among chromosomes (Table 1, Figure 3). Chromosome 9 had the highest average marker density after SNP filtering with approximately 173.07 SNPs per Mb, while chromosome 7 the lowest with an average of 76.85 SNPs per Mb. Generally, most of the SNPs were located in regions with high gene density and decayed around the centromere (Figure 3). S3 MAGIC individuals exhibited a heterozygosity average of 6.87%, with only 15 individuals (3.57%) with a proportion of residual heterozygosity higher than 20% (Figure 4).

Table 1. Statistics of the genotyping using the eggplant SPET platform of the 420 S3MEGGIC population individuals using the '67/3' eggplant reference genome (Barchi et al., 2019b).

Chr	Markers	Filtered markers	Markers (%)	Filtered markers (%)	Chr length (Mb)	Marker density (Mb)	Filtered marker density
1	3,234	1,205	14.60	15.60	13.66	236.82	88.24
2	1,119	424	5.05	5.49	8.34	134.21	50.85
3	2,004	758	9.05	9.81	9.71	206.41	78.07
4	1,567	588	7.08	7.61	10.57	148.23	55.62
5	1,438	571	6.49	7.39	4.39	327.80	130.16
6	2,108	794	9.52	10.28	10.90	193.38	72.84
7	2,483	328	11.21	4.25	14.24	174.34	23.03
8	1,392	550	6.29	7.12	10.96	126.98	50.17
9	1,841	625	8.31	8.09	3.61	509.80	173.07
10	2,069	798	9.34	10.33	10.67	193.86	74.77
11	1,260	453	5.69	5.86	7.23	174.23	62.64
12	1,631	630	7.36	8.16	10.05	162.33	62.70
Total	22,146	7,724	100.00	100.00	114.33		
Average						215.70	76.85

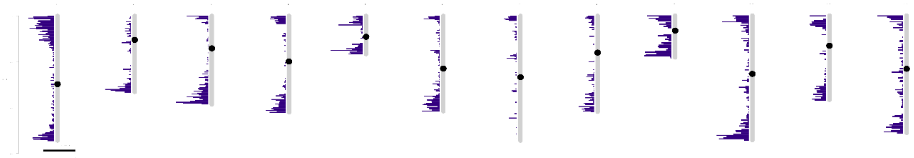

Figure 3. The distribution of the molecular markers across the chromosomes used for the genotyping.

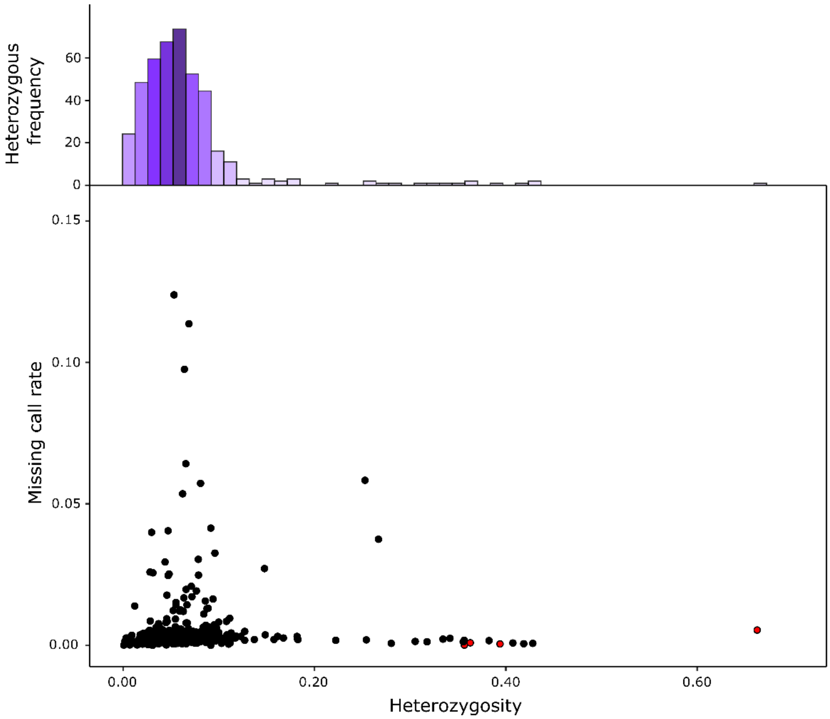

Figure 4. Heterozygosity and proportion of missing data from the S3MEGGIC population. The middle graph reports residual heterozygosity of S3 individuals as black dots compared against two-way hybrids represented as red dots. The top histogram represents the observed heterozygosity which is skewed to the left (mean 6.87%; mode 5.02%).

Population structure

Population stratification performed by PCA indicated the absence of subgroups in the S3 individuals assessed since no clear clustering was observed (Figure 5). The first two principal components (PCs) explained respectively 5.15% (PC1) and 3.30% (PC2) and the first 10 PCs explain altogether only 25.14% of the total variation, revealing the absence of genetic structure in the S3MEGGIC population. No differentiated clusters among individuals carrying the wild (*S. incanum* MM577) or the cultivated (*S. melongena* ASI-S-1) cytoplasm were observed. An Analysis of Molecular Variance (AMOVA) was also performed revealing that only 0.29% of the total sums of squares is accounted for the molecular variation among the *S. melongena* and *S. incanum* cytoplasm groups resulting in a very small phi-value of 0.0019, which indicates a low level of differentiation supporting that no population structure exists.

The genome mosaics reconstruction of the S3 MAGIC individuals in terms of the eight founder haplotypes showed different haplotype block proportions depending on the genomic position for all chromosomes (Figure 6). The estimated contribution of some founders to the overall S3MEGGIC population differed from the expected value of 12.5%. Two of the founder genomes (A0416 and IVIA-371) had a high representation in the genome of the S3 individuals (32.6% and 23.6%, respectively) while two others (AN-S-26 and H15) had a small representation (0.3% in both cases). The wild founder *S. incanum* had an average haplotype representation of 5.8%.

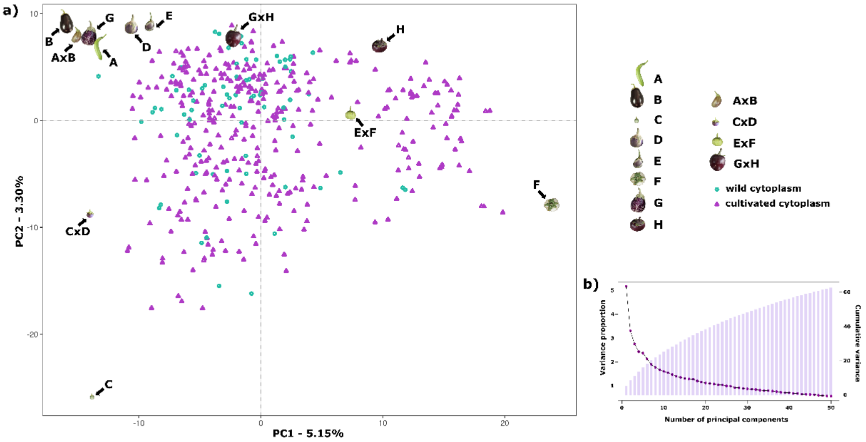

Figure 5. Results of a Principal Component Analysis (PCA) on the S3MEGGIC population. **(a)** PCA of the first two PCs including S3 individuals, founder lines and two-way hybrids. S3 individuals with wild cytoplasm are represented with blue dots while those ones with cultivated cytoplasm are represented with purple triangles. **(b)** Scree plot of the PCs (x-axis) and their contribution to variance (left y-axis); bar blot of the PCs (x-axis) and the cumulative proportion of variance explained (right y-axis).

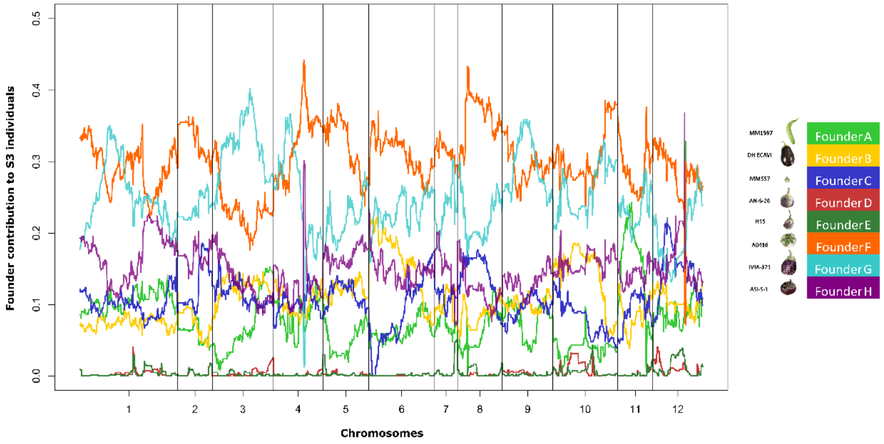

Figure 6. Genome-wide founder haplotype blocks assignment across the entire S3MEGGIC population. In the x-axis is represented the 12 eggplant chromosomes and in the y-axis the average percentage of founders contribution for the 420 S3 individuals. In the legend the color code associated with each founder as in Figure 1.

5. Discussion

MAGIC populations are outstanding genetic materials for identifying gene-trait associations with high resolution (Arrones et al., 2020; Scott et al., 2020). The introduction of multiple founders with increased genetic and phenotypic diversity together with the multiple rounds of inter-crossing and selfing increases the number of accumulated recombinant events and, thus, improves mapping accuracy (Scott et al., 2020). By introducing a wild relative as a founder parent, the genetic variability in the population increases, which is a key point for QTL identification (Gramazio et al., 2020a). Here we present the first eggplant MAGIC of which one of the founders was one accession of the wild close relative *S. incanum.*

Large population sizes are essential to increase power and mapping resolution in MAGIC populations (Collard et al., 2005; Valdar et al., 2006; Jaganathan et al., 2020). Following a simple "funnel" scheme design, the population was kept as large as possible to gather a large number of recombination events. However, a sharp reduction in the number of progenies was observed at the S0 generation, which might be related to the use as a founder of the wild species *S. incanum* (C) as a female parent to obtain the simple (CD) and the double (ABCD) hybrids. This interspecific crossing dragged the maternal cytoplasmic background of the wild parent, which might have caused partial sterility and bias in subsequent generations. Some studies confirmed a strong effect of wild *Solanum* cytoplasm in the reduction in pollen fertility of the alloplasmic lines (Isshiki et al., 2020, Khan et al., 2020). However, the PCA highlighted the absence of population structure, also confirmed by the lack of genetically differentiated cytoplasmic groups.

The genotyping of the S3MEGGIC population was carried out with the 5k probes eggplant SPET platform with a well-distributed marker density along all chromosomes (Barchi et al., 2019a). This genotyping strategy has already been used in the analysis of biparental populations (Herrero et al., 2020), and here we have verified that its use can be extended to multiparent populations. The genotyping revealed a low heterozygosity in the S3MEGGIC individuals, similar to the expected value for a F5-like biparental inter-cross generation (6.25%). The contributions of each of the founder parents to the S3MEGGIC population revealed that some parents had higher representation than others. Apart from drift effects, several biological reasons could potentially explain cryptic selection processes that may explain this unbalanced representation of the genomes (Rockman and Kruglyak, 2008; Thépot et al., 2015). Among them, seed dormancy, delayed germination, precocity, reduced fertility, parthenocarpy associated to some genomes have already been reported in eggplant and other crops (Barchi et al., 2010; Khan et al., 2015; Prohens et al., 2017). The rather limited contribution of the wild species *S. incanum* to the final S3 MAGIC individuals may have been caused by selection pressure, as progenies bred from crosses involving two different species tend to suffer from reduced fertility and show segregation distortion (Lefebvre et al., 2002; Barchi et al., 2010). In addition, *S. incanum* has a recalcitrant germination and a very erratic flowering and fruit set, which strongly depends on environmental conditions (Gisbert et al., 2011; Mangino et al., 2020, 2021). Other reasons for the segregation distortion could be the inability of the current genotyping density to efficiently distinguish between founders that are genetically closer like AN-S-26 and H15 genotypes. This phenomenon has already been observed in previous MAGIC populations (Dell'Acqua et al., 2015). A deeper genotyping resulting in a better haplotype reconstruction might shed light on the mechanisms that have led to the unbalanced representation of the founder genomes in the S3MEGGIC population.

6. Conclusions

In conclusion, the S3MEGGIC population represents a landmark breeding material and tool of great value which will allow the study and fine mapping of complex traits due to: i) the highly phenotypically diverse founders; ii) the large population size being the largest eggplant experimental population up to now; iii) the high degree of homozygosity of the final individuals which constitute a population of fixed "immortal" lines nearly homozygous at each locus; and, iv) the tailored genotyping SPET platform used for the genetic analysis of the population, which has been developed from the WGS of the founders and allows the comparison with materials genotyped with the same setup (Gramazio et al., 2020b).

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GENERAL DISCUSSION

Agriculture is the most vulnerable sector to climate change, as its activities directly depend on climatic conditions (Praveen and Sharma, 2019; Skendzic et al., 2019; Malhi et al., 2021). Reducing environmental impact and increasing crop resilience without affecting productivity are the main objectives of modern agriculture to face climate change effects (Raza et al., 2019; Agrimonti et al., 2020). One strategy to mitigate the impact of climate change on agricultural systems consists in the development of new varieties adapted to the emerging biotic and abiotic stresses by using crop wild relatives (CWRs) (Dempewolf et al., 2014, 2017; Prohens et al., 2017; Killian et al., 2021). This approach in plant breeding demonstrated to have a positive economic impact on many crops as well as a great potential to help sustainably increase agricultural production (Tyack and Dempewolf, 2015).

For these reasons, the development of this doctoral thesis represents an important advance for eggplant breeding, since it reported on the evaluation of a set of introgression lines carrying genome fragments of its wild relative *S. incanum* and the development of the first interspecific eggplant MAGIC population. This work not only entails a significant increase in the genetic diversity of eggplant, but also has great implications for the elucidation of the genetic basis of traits of interest in this crop.

1. Characterization and QTL identification of morpho-agronomic traits in a set of eggplant interspecific introgression lines.

In the first chapter of this doctoral thesis, we have conducted a phenotypic evaluation of a set of 16 ILs derived from *S. melongena* × *S. incanum* (Gramazio et al., 2017). The performance of the ILs was tested in two environments (open field and greenhouse) through the analysis of 17 agronomic traits. The cultivated and wild parents as well as the F1 hybrid were included in the evaluation, and mid-parent heterosis were estimated. In this way, the effects of the environment on the investigated traits as well as the occurrence of genotype × environment were assessed, and stable QTLs were identified.

Significant difference in fruit size and prickliness-related traits found between cultivated and wild parents demonstrated that domestication process involved changes in these traits (Wang et al., 2008; Page et al., 2019). We observed that the hybrid was heterotic for traits related to vigour and prickles, and this is a common phenomenon occurring in interspecific hybrids between eggplant and its wild relatives (Lester, 1986; Prohens et al., 2012, 2013; Plazas et al., 2016; Kaushik et al., 2016). As observed in a previous study (Kaushik et al., 2016), we found a great similarity in fruit size between the hybrid and the donor parent, although it recovers quickly in a few backcross generations (Prohens et al., 2013). Despite significant G x E interaction was detected for most traits, the ILs generally exhibited few phenotypic differences with the recipient parent, indicating that large introgression fragments had minimal effect on relevant agronomic traits, as previously reported in tomato (Schouten, et al., 2019).

In total, we detected ten stable QTLs scattered over seven chromosomes, of which two were for plant-related traits and four for both flower-related and fruit-related traits. In general, *S. incanum* introgressions improved the performance of most traits related to plant and flower and decreased that of fruit-related traits. Seven detected QTLs were new. Three of them were the first QTLs reported in eggplant for stem diameter (*sd5*), peduncle length (*pl1*), and stigma length (*sl8*). The other four, which were associated with plant height (*ph8*), flower calyx prickles (*flcp3* and *flcp5*), and fruit pedicel length (*fpl12*), did not colocalized with any previous identified QTLs in eggplant populations (Doganlar et al., 2002; Frary et al., 2003, 2014; Portis et al., 2014; Wei et al., 2020), demonstrating the power of ILs in the detection of QTLs with minor effect or whose effect could be masked by large effect loci (Eshed and Zamir, 1995; Eduardo et al., 2005; Keurentjes et al., 2007; Gur and Zamir, 2015; Yin et al., 2015). Our results revealed that the QTLs for fruit pedicel length *fpl4* and *fpl8* located on chromosome 4 and 8, respectively, were syntenic to other loci previously detected in an intraspecific population of eggplant derived from 305E40 × 67/3 cross (Portis et al., 2014). Moreover, we found a QTL involved in the control of fruit weight, a trait of great importance in solanaceous crops. The genetic base of the fruit weight has been extensively studied in tomato using interspecific as well as intraspecific populations. Although hundreds of QTLs have been mapped (Grandillo et al., 1999; Illa-Berenguer et al., 2015; Cambiasso et al., 2019), only three loci identified by positional cloning in chromosomes 2, 3, and 11 (Frary et al., 2000; Chakrabarti et al., 2013; Mu et al., 2017) are responsible for most of the fruit weight variation in this crop. Putative orthologous loci were detected in eggplant using interspecific populations (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014) and GWAS analysis (Portis et al., 2015), although none of them have been cloned yet. In our study, the identified QTL controlling fruit weight was located on chromosome 2 (*fw2*), evidencing the conservation of these important loci among Solanaceae and suggesting that variation in the phenotype of this trait in eggplant is controlled by few genes with major effects.

One of the advantages of the IL populations is that allow not only to attribute the phenotypic variation between lines in the library to a specific introgressed segment (Tanksley and Nelson, 1996; Zamir et al., 2001; Lippman et al., 2007; Calafiore et al., 2019; Boopathi, 2020), but also to delimitate a QTL to a smaller region on the genome using a series of overlapping ILs (Paterson et al., 1990). In this study, each of the QTLs *flcp3* and *fpl4* was mapped in one of two overlapping ILs (SMI_3.1 and SMI_3.2 for the former, and SMI_4.1 and SMI_4.2 for the latter). In this way, we have provided useful information for reducing the chromosomal regions where these QTLs are located.

2. Phenomic analysis and identification of QTLs and candidate genes for fruit shape using a set of eggplant interspecific introgression lines.

In view of the promising results obtained by testing the performance of the *S. incanum* ILs set for morpho-agronomic traits, we have used a similar approach to investigate on the genetic base of eggplant fruit shape, a trait with a significant impact on commercial use and economic value of this crop.

Although fruit shape characterization is generally based on simple traits measured manually, and on the identification of shape patterns that could be matched with qualitative descriptors (IBPGR, 1990; IPGRI, 1996; Scott, 2010; UPOV, 2017), the development of the modern phenomic tool Tomato Analyzer (Brewer et al., 2006; Rodriguez et al., 2010a, 2010b) has allowed a more precise and comprehensive characterization of fruit morphology not only for tomato (Brewer et al., 2007; Gonzalo and van der Knapp, 2008; Gonzalo et al., 2009; Rodriguez et al., 2011, 2013; Figàs et al., 2015; Marefatzadeh-Khameneh et al., 2021), but also for other vegetables crops including pepper (Naegele et al., 2016; Tripodi and Greco, 2018; Pereira-Diaz et al., 2020; Nankar et al., 2020; García-González and Silvar, 2020; González-Lopez et al., 2021), and melon (Diaz et al., 2017; Oren et al., 2020).

For fleshy-fruited plant species, tomato has served as a model system for studying the genetic base of a multitude of traits including fruit shape (Grandillo et al., 1999; Kimura and Sinha, 2008; Gonzalo et al., 2009; Gebhardt, 2016; Kim et al., 2017; Anwar et al., 2019). This explains the detection of numerous QTLs involved in genetic regulation of this trait (Eshed and Zamir, 1995; Grandillo and Tanksley, 1996; Tanksley et al., 1996; Fulton et al., 1997; Bernacchi et al., 1998; Lippman and Tanksley, 2001; Monforte et al., 2001; van der Knapp and Tanksley, 2003; Frary et al., 2004; Yates et al., 2004; Haggard et al., 2013; Barrantes et al., 2016; Celik et al., 2017; Di Giacomo et al., 2020) as well the identification by positional cloning of three major genes responsible for most of the fruit shape variation in tomato (Liu et al., 2002; Xiao et al., 2008; Xu et al., 2015).

Compared to tomato, research aimed to dissect the genetic base of fruit shape in eggplant has lagged behind. QTL mapping analysis in few biparental populations (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014; Wei et al., 2020) and GWAS studies (Portis et al., 2015) have allowed the identification of some major QTLs associated to simple eggplant fruit shape traits. Although phenomic studies utilizing Tomato Analyzer have been performed using different eggplant germplasm materials (Prohens et al., 2012; Hurtado et al., 2013; Plazas et al., 2014; Kaushik et al., 2016, 2018), they only provided descriptive information on the diversity within collections of materials and segregating populations.

In this scenario, we have proposed the first study combining the use of an experimental introgression line population and a phenomics tool in order to enhance the precision in the detection of genomic regions controlling this quantitative trait and identify potential candidate genes in the introgressed fragment. We have evaluated the two parents, hybrids, and IL set for fruit shape in two environments (open field and screenhouse) performing a detailed phenotypic analysis of the fruits by using 32 descriptors of the Tomato Analyzer.

Large differences in fruit morphology have been observed between the ILs parents. The hybrids displayed negative values of heterosis for many fruit shape traits, being phenotypically closer to its wild parent than to the cultivated one. This is a common phenomenon occurring in interspecific hybrid of eggplant (Kaushick et al. 2018), probably due to the overall dominance of wild traits over the domesticated ones (Lester, 1989; Page et al., 2019). We have found significant differences for most fruit shape descriptors between ILs and the recipient parent. This means that *S. incanum* introgressions had a significant effect on fruit shape traits, even in the case of small wild fragments. This result is in contrast with that reported for morphological traits in the first chapter of this doctoral thesis, for which ILs and recipient parent showed minimal phenotypic difference, even in the presence of large *S. incanum* introgressions. We believe that the discrepancy of these results lies in the quantitative nature of the vigour-related and shape-related traits analyzed in our two studies, since the same introgressions, even small, can carry major QTLs/genes affecting more or less significantly the phenotype of these traits depending on the magnitude of their effect. Despite the contribution of the environment and the G × E interaction was significant for almost all descriptors, we found that the variations in fruit shape is mainly genetically regulated, as previously reported for tomato (El-Gabri et al., 2014; Monforte et al., 2014; Figàs et al., 2018).

The use of Tomato analyzer software has allowed to perform a more precise characterization of eggplant fruit shape, since the evaluation of 32 descriptors have provided quantitative data on many fruit traits that generally are not scored when manually measured morphological descriptors are used. As previously reported in tomato (Figàs et al., 2015; Mohan et al., 2016; Marefatzadeh-Khameneh et al., 2021) and pepper (Tripodi and Greco, 2018; Colonna et al., 2019; Nankar et al., 2020; García-González and Silvar, 2020; González-López et al., 2021), the detection of several clusters of highly correlated traits indicates that many Tomato Analyzer descriptors measures similar fruit shape characteristics, and, probably, an evaluation of few of them could provide a similar comprehensive information on these traits in eggplant.

With the identification of 41 QTLs, we have increased the number of known QTLs for fruit shape in eggplant. Sixteen of these, four (*wmh3.6*, *wmh10.1*, *mw3.6*, and *mw10.1*) controlling the two fruit width descriptors (Width Mid-Heigt and Maximum Width), and twelve (*fseI2*, *fseI3*, *fseI4*, *fseI7*, *fseII2*, *fseII3*, *fseII4*, *fseII7*, *cfs2*, *cfs3*, *cfs4*, and *cfs7*) controlling the three fruit shape index descriptors (Fruit Shape Index External I, Fruit Shape Index External II, and Curved Fruit Shape

Index) were syntenic to other reported in several intraspecific and interspecific eggplant populations (Doganlar et al., 2002; Frary et al., 2014; Portis et al., 2014, 2015; Wei et al., 2020). Twenty-five QTLs were new, and were associated with Tomato Analyzer descriptors (Blockiness, Homogeneity, Asymmetry and Internal Eccentricity) that have not been assessed before in eggplant. Of these, two QTLs affecting proximal and distal blockiness (*pfb1* and *dfb8*, respectively) were syntenic to tomato (Brewer et al., 2007; Gonzalo and van der Knaap, 2008). Evidence of synteny to other previously detected in tomato using three segregating F2 populations (Gonzalo et al., 2009) have been found for two QTLs affecting Fruit Shape Index Internal (*fsi2*) and Eccentricity Area Index (*eca8*). In agreement with Portis et al., (2015), a QTL influencing obovoid fruit shape have been mapped on chromosome 8 (*obv8*). This result suggested a synteny with tomato for a QTL controlling fruit shape by promoting the growth along the longitudinal axis (*fs8.1*), indicating a conservation of this locus among some Solanaceous crops. Ten QTLs identified for Homogeneity descriptors (Circular, Ellipsoid, and Rectangular) have been mapped on the chromosome 1 (*rec1*), 2 (*cir2*), 3 (*cir3*), 4 (*cir4* and *eli4*), 5 (*rec5*), 7 (*cir7* and *eli7*), 10 (*rec10*) and 12 (*rec12*). In tomato, long and oxheart fruit shape are often associated with mutations in *SUN* gene, mapped on chromosome 7, while obovoid, rectangular, ellipsoid, heart and pear fruit shape are often associated with mutations in the *OVATE* gene, mapped on chromosome 2 (Rodriguez et al., 2011). Despite the evidence of synteny with tomato for three QTLs controlling Circular (*cir2* and *cir7*) and Ellipsoid (*eli7*), our results have indicated that ellipsoid/circular fruit shape and rectangular fruit shape are controlled by QTLs mapped to different chromosomes in eggplant.

Finally, in the genomic regions where stable QTLs have been identified, we have reported the presence of deleterious mutations in genes of *SUN*, *OVATE* and *FAS* families described in tomato (Huang et al., 2013), which could be associated to variation in fruit shape in eggplant. Except for the two candidate genes syntenic to tomato genes *SlSUN7* and *SlSUN11*, which have been mapped in the small QTL regions of the nonoverlapping ILs SMI_2.4 and SMI_3.6, respectively, all the others have been identified in QTL regions covering a broad segment of a chromosome, where probably other unknown genes involved in the regulation of fruit shape are located. Despite this, congruences with the study of Huang et al., (2013) which we have observed for the tomato syntenic gene *SlSUN22*, suggested that candidate genes that we have proposed could have a great impact on the determination of fruit shape in eggplant.

In conclusion, we have demonstrated that IL populations are extremely useful for the genetic dissection of traits of interest for eggplant. In addition, our results indicate that the IL set used in this work constitutes a valuable resource that can be directly introduced into breeding pipelines, as they do not display undesirable wild traits.

3. Development of the first eggplant MAGIC population derived from an interspecific cross of seven accession of *S. melongena* and one of wild relative *S. incanum*.

Multi-parent experimental populations are of great interest for developing new recombinant materials for plant breeding (Huang et al., 2015). In particular, MAGIC populations, which combine high genetic/phenotypic diversity of multiple founders together with high recombination rate and low population structure, have demonstrated to be powerful mapping tools for dissecting the structure of complex traits (Dell'Acqua et al., 2015; Stadlmeier et al., 2018; Arrones et al., 2020; Scott et al., 2020). Moreover, the inclusion of one or more wild relative accessions in the founder parents increases the genetic diversity of a MAGIC population, opening new perspectives for the exploitation of this variability. That was why we have developed the first eggplant MAGIC population (S3MEGGIC) by intercrossing seven cultivated eggplant (*Solanum melongena*) and one wild relative (*S. incanum*) parents.

Since large population size are essential for mapping QTLs with a high resolution (Collard et al., 2005; Valdar et al., 2006; Jaganathan et al., 2020), our aim was to get a population with as many individuals as possible. However, we have observed a reduction in progenies number at the S0 generation likely due to the incompatibility between the cytoplasm of *S. incanum*, which have been used as a female founder and parent to obtain the simple (CD) and the double (ABCD) hybrids, and the nuclear genome of *S. melongena*. Although the absence of population structure has been confirmed by PCA analysis and by the lack of genetically differentiated cytoplasmic groups, we believe that these interspecific crossing have dragged maternal cytoplasmic background of the wild parent, which could have generated partial sterility and bias in subsequent generations. Evidence of a strong effect of wild Solanum cytoplasm in the reduction in pollen fertility of the alloplasmic lines have been described in previous studies (Khan et al., 2020; Isshiki et al., 2021). Despite this drawback, with a final population size of 420 individuals, we have developed the largest experimental population described, so far, in eggplant.

We have performed the genotyping of the S3MEGGIC population with the 5k probes eggplant SPET platform (Barchi et al., 2019), extending the use of this technology to multiparent populations. Combining targeted analysis of SNPs with method simpleness, SPET represents a valid alternative to GBS and micro arrays (Scheben et al., 2017). With this approach, we have estimated the contribution of the founders to the final S3MEGGIC population and explored its genetic architecture. Low residual heterozygosity (6.87%) has been found in the

S3MEGGIC individuals. Lack of population structure as well as low level of differentiation among subpopulations carrying a cultivated or wild cytoplasm have been observed.

On the other hand, we have detected an unbalanced representation of the founder genomes, having some of them (A0416 and IVIA-371) a higher representation than others. This phenomenon could be a consequence of several biological processes, including seed dormancy, delayed germination, precocity, reduced fertility, and parthenocarpy, which have been previously associated to some genomes in eggplant (Barchi et al., 2010; Khan et al., 2015; Prohens et al., 2017). We believe that the low representation of the wild species *S. incanum* (5.8%) in the genome of the S3 MAGIC individuals may be the result of a selection pressure, since progenies derived from interspecific crosses suffer from low fertility and high segregation distortion (Lefebvre et al., 2002; Barchi et al., 2010). Furthermore, besides a recalcitrant germination, within the framework of this thesis we have observed that *S. incanum* also presents very irregular flowering and fruit set, which were strongly affected by environmental conditions. Furthermore, the inability of the current genotyping density to provide an efficient distinction between genetically closer parents (i.e. AN-S-26 and H15), might have contributed to founders contribution distortion, as previously reported in maize MAGIC population (Dell'Acqua et al., 2015).

In conclusion, the MAGIC population that we have developed represents a landmark breeding material for future genetics and breeding studies in eggplant. For sure, thanks to its genomic hallmarks, which include (i) large population size, (ii) high degree of phenotypic diversity of the founders, (iii) high degree of homozygosity of the final individuals, and (iv) genotyping carried out with the platform developed starting from whole-genome sequencing data of the founders, the S3MEGGIC will be very useful for fine-mapping of complex traits.

4. Concluding remarks and future perspectives

The material and information developed in this doctoral thesis provide a basis for future genomic studies in eggplant. Following the 'introgressiomics' approach, we have developed advanced materials that have greatly broaden the genetic diversity of eggplant, a crop which presents serious problem of genetic erosion. The evaluation of our experimental populations has demonstrated their potential as pre-breeding materials. Additionally, advances in the knowledge of the genetic basis of agronomic traits of interest in eggplant have been made, reducing the gap with other important Solanaceae, such as tomato.

An important next step would consist in breaking the large fixed ILs into smaller fragments (subILs) in order to fine map the detected QTLs and ultimately identify the gene/s accounting for the QTL effect. In addition, the use of the ILs from *S.*

melongena x *S. incanum* could be of great interest for further mapping studies. In fact, this IL collection has been recently characterized for morpho-agronomic and fruit composition traits, reveling several QTLs involved in their control (Rosa-Martinez et al., 2021). Other research strategies include the development of new genetic tools by introgressing wild genes in the cultivated eggplant background. Currently, three other IL populations with species belonging to primary (*S. insanum*), secundary (*S. dasyphyllum*) and tertiary (*S. elaeagnifolium*) eggplant genepools are being developed (Garcia-Fortea, 2019, 2021), and advanced backcross of one of them have already been evaluated under high and low N fertilization conditions (Villanueva et al., 2021). These promising materials will be evaluated for traits involved in tolerance and resistance to stresses related to climate change.

With respect to S3MEGGIC population, our purpose is to make two more rounds of single seed descend for obtaining a highly homozygous S5 generation eggplant MAGIC population. Subsequently, a deeper genotyping of this more advanced population will be performed in order to provide a more accurate reconstruction of haplotypic blocks and to shed light on the mechanisms that have led to the unbalanced representation of the founder genomes in the S3MEGGIC population. Meanwhile, the S3MEGGIC population will be tested in genome-wide association studies (GWAS) for the identification of candidate genes for traits of interest, which could be later edited and validated by CRISPR/Cas9 system.

Altogether, the information, knowledge, and tools provided by this doctoral thesis has important implication for eggplant breeding and will be of great relevance for the development of a new generation of eggplant varieties adapted to climate change.

GENERAL CONCLUSIONS

1. We have tested the performance of a set of 16 eggplant introgression lines with genomic fragments of *S. incanum*, demonstrating its potential as pre-breeding material. This is the first time that a set of eggplant ILs has been evaluated.

2. The first study combining the use of a phenomic tool (Tomato Analyzer), and an experimental mapping population (ILs) has been performed in eggplant. This approach has provided a more comprehensive and accurate characterization of fruit morphology and enhanced the precision in the detection of genomic regions controlling fruit shape traits.

3. Contrasting effects of the *S. incanum* introgressions on the phenotype of the morpho-agronomic and fruit shape traits of the ILs have been found with respect to recipient parent. These discrepancies lie in the quantitative nature of the traits that we have assessed.

4. Fifty-one stable QTL has been identified in the set of eggplant ILs. Twenty QTLs were syntenic to those previously reported in tomato and eggplant populations. Some of the thirty-one novel QTLs were associated to morpho-agronomic and fruit shape traits that have not been assessed before in eggplant. The use of overlapping ILs have allowed to further reduce the genomic regions that control traits of great importance for eggplant breeding, such as prickliness and fruit pedicel length. In addition, eleven *SUN* and *YABBY* genes have been proposed as potential candidate genes controlling eggplant fruit shape variations.

5. The first eggplant MAGIC population (S3MEGGIC) has been developed from the intercrossing of seven accession of cultivated eggplant and one accession of *S. incanum* using a funnel breeding scheme followed by three round of selfing. With a population size of 420 S3 individuals, the final S3MEGGIC population represents the largest recombinant population developed, so far, in eggplant.

6. The genotyping of the S3 individuals with the eggplant 5k probes Single Primer Enrichment Technology (SPET) platform, which yielded 7,724 SNP, has revealed low residual heterozygosity and a lack of genetic structure, with no genetic differentiation among subpopulations carrying a cultivated or wild cytoplasm. However, we have observed an unbalanced representation of founder genomes, which suggested a cryptic selection of specific parental genomes.

7. Thanks to its genomic hallmarks, which include the high phenotypical diversity of the parents, large population size, and high degree of

homozygosity of the S3 individuals, the S3MEGGIC population constitutes a powerful mapping resource for dissecting complex quantitative traits and detecting QTLs with high precision.

8. The development of this doctoral thesis has important implications for eggplant breeding, since it allowed the development of advanced materials of great interest. We consider that the information gathered in this work provides novel and highly relevant insights on the genetics of important agronomic traits in eggplant and represents a step forward towards the development of new eggplant varieties adapted to challenges posed by climate change scenario.

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