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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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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

1. Introduction

Eggplant (*Solanum melongena* L., Solanaceae; 2n = 2x = 24) ranks fifth among all vegetables and second, after tomato, among Solanaceae vegetables in global production [1]. However, despite its importance, still few genetic and genomic studies have been performed in eggplant [2,3]. In fact, even though the first draft of the genome was released in 2014, indicating a genome size of 1.13 Gb [4], just last year the first high-quality chromosome-anchored reference genome with an estimated genome size of 1.21 Gb [5] and the first resequencing study [6] 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 [7]. 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 [8–10]. 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 [11–15]. Only recently, the first introgression line (IL) population, using *S. incanum* L. as a donor parent, has been developed in eggplant [16]. *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 [17,18]. The interest in this species lies in its drought tolerance [19,20], high content of bioactive phenolic compounds [21–24], and resistance to some diseases [25,26]. As a result, *S. incanum* has been used for a plethora of breeding and genetic studies [6,24,27–31].

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/) [32], were launched to unlock the potential of the unexploited eggplant crop wild relatives (CWRs). In this respect, conventional breeding and new approaches, like "introgressiomics" [33], 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 [34–36], provide a promising opportunity to efficiently incorporate exotic natural variation in the modern breeding programs [33,35]. Due to their high homozygosity, ILs are a stable resource that can be used for a multitude of genetic studies [35]. 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) [37–40]. As reported by Zamir [35], 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 [35,41–43]. 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 [39,44]. However, up to now, IL populations have been developed mainly in major cultivated crops [45–47] and regarding Solanaceae almost exclusively in tomato [48–50].

The advantage of using IL populations for QTL identification has been demonstrated in many studies. For example, the *S. pennellii* IL population [34] allowed so far the identification of almost 3000 putative QTLs for different traits, like morphology, stress tolerance, yield, fruit colour, and bioactive compounds [37,51–54]. 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 [55–60]. 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 [61].

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 [16]. 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.

2. Materials and Methods

2.1. Plant Material

From the IL population of *Solanum incanum* (MM577) developed in the *S. melongena* (AN-S-26) background [16], 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).
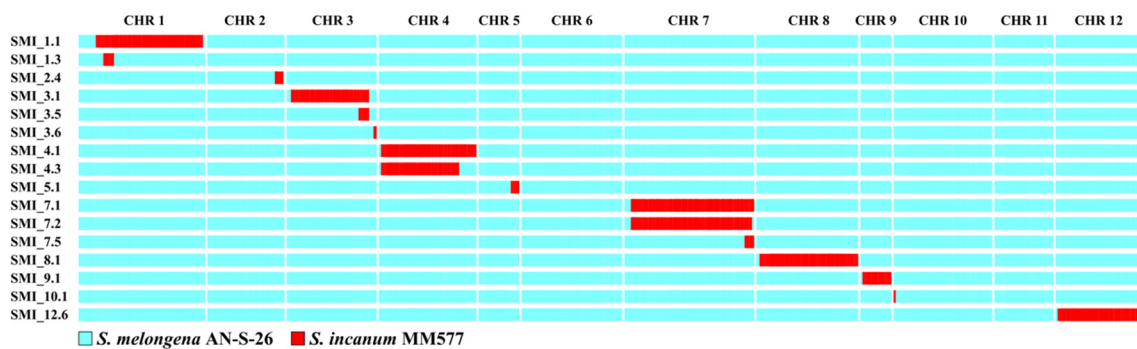

Figure 1. Graphical genotypes of the selected introgression lines (ILs) for QTLs identification: The rows indicate IL codes, and the columns indicate the chromosomes. Homozygous introgressions of *S. incanum* MM577 are depicted in red, while the genetic background of the recipient parent (*S. melongena* 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 [5]: For further details about the marker-assisted selection of the introgressions, refer to Gramazio et al. [16].

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		
SMI_3.5	3	0.6	8	78–86	8.3	82	84.5
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		
SMI_7.2	7	10.9	125	14–135	85.2	125	88.0
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	

Abbreviations: ILs: Introgression lines; Chr.: Chromosome.

2.2. Field Cultivation and Phenotypic Evaluation of the Traits

Seeds were germinated in Petri dishes, following the protocol developed by Ranil et al. [62], 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 [63] (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.

2.3. 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:

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

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 [64]:

For one environment (open field or screenhouse):

$$H^2 = V_G/(V_G + V_E) \tag{2}$$

For both environments (open field and screenhouse):

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

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

2.4. 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:

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

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

3. Results

3.1. 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 ***	9000 ***
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 ($p \geq 0.05$); na = data not available.

3.2. 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 ($p \geq 0.05$).

3.3. 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.

4. Discussion

IL populations have demonstrated to be a useful and powerful genetic resource for the identification of QTLs in several crops [65]. The present work provides a first phenotypic evaluation involving 17 agronomic traits of the first set of eggplant ILs [16]. 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 [66,67]. In agreement with previous works [14,23,68], 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 [27]. 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* [14,69,70], where the interspecific hybrids are pricklier than any of the parents. Other studies using segregating populations of *S. linneanum* [71] and *S. incanum* [29] 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 eggplant [68] as well as in other related crops such as tomato [39,72].

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 [73]. 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 [74], 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. [75] 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 [8,9,71], here, we detected the first QTL that influences stem diameter (*sd5.2*), an important trait related to vigour and for grafting [76,77]. 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 [39]. 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 [78,79], suggesting that a new stable locus controlling stigma length was detected.

On the other hand, Doganlar et al. [71] and Frary et al. [8] 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 [29]. 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 [37–40]. 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 [80] 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 [81–83], this trait was found to be controlled primarily by only three loci, which were identified by positional cloning in chromosomes 2, 3, and 11 [72,84,85]. Putative orthologous loci were detected in eggplant using interspecific populations [8,71,80] and GWAS analysis [9]. 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.

5. 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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