Performance of a Set of Eggplant (*Solanum melongena*) Lines With Introgessions From Its Wild Relative *S. incanum* Under Open Field and Screenhouse Conditions and Detection of QTLs

Giulio Mangino 1, Mariola Plazas 1, Santiago Vilanova 1, Jaime Prohens 1 and Pietro Gramazio 2,*

1 Institute for the Conservation and Improvement of Valencian Agrodiversity, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain; giuman2@upvnet.upv.es (G.M.); sanvina@upvnet.upv.es (S.V.); jprohens@btc.upv.es (J.P.)

2 Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan

* Correspondence: gramazio.pietro.gn@u.tsukuba.ac.jp

Received: 26 February 2020; Accepted: 25 March 2020; Published: 27 March 2020

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 gene pool 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. IL Size (Mb) | Total chr. IL 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              | 6                       | 7.2                    |
| 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             |                         |                        |
| SMI_4.3     | 4    | 8.8              | 101          | 4–85             | 75.2             | 101                     | 96.1                   |
| 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).

| Descriptor Code | Trait Descriptor | Descriptor Scale/Unit |
|----------------|-----------------|-----------------------|
| 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 \(^a\) |
| 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 \(^a\) |
| CD             | Corolla diameter | cm |
| PL             | Peduncle length | cm |
| SL             | Stigma length | cm |
| FLCP           | Flower calyx prickles | 0–9 \(^a\) |
| 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:

\[
H_{MP} (%) = \left(\frac{F1 - MP}{MP}\right) \times 100
\]

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 x environment interaction (G x 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):

$$H^2 = \frac{V_G}{V_G + V_E}$$  \hspace{1cm} (2)

For both environments (open field and screenhouse):

$$H^2 = \frac{V_G}{V_G + V_E + V_{GxE}}$$  \hspace{1cm} (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:

$$\text{Increase over SM} \, (\%) = \frac{\text{mean (IL)} - \text{mean (SM)}}{\text{mean (SM)}} \times 100$$  \hspace{1cm} (4)

$$\text{Allelic effect} = \frac{\text{mean (IL)}}{\text{mean (SM)}}/2$$  \hspace{1cm} (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 * | $H_{MP}$ (%) * | SM  | SI * | $H_{MP}$ (%) * |
| **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 | 0.00 *** | −66.6 *** | 1.00 | 0.00 *** | −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 *** | 9000 *** |
| **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 different from SM at p values < 0.001, < 0.01, and < 0.05 or not significant (p ≥ 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, FPL, 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 $^a$ | $H^2$ | Environment $^a$ | G × E $^a$ | $H^2$ |
| **Plant** | | | | | | | | |
| PH | 4.65 *** | 0.43 | 9.89 *** | 0.64 | 11.05 *** | 0.64 | 353.63 *** | 2.95 *** | 0.38 |
| SD1 | 2.34 ** | 0.21 | 5.33 *** | 0.46 | 6.06 *** | 0.46 | 0.38 ns | 1.75 * | 0.27 |
| SD5 | 4.44 ** | 0.41 | 2.45 ** | 0.23 | 5.36 *** | 0.23 | 5.47 * | 1.96 * | 0.22 |
| **Leaf** | | | | | | | | |
| LCC | 2.82 ** | 0.27 | 2.52 ** | 0.23 | 4.03 *** | 0.23 | 6.23 * | 1.30 ns | 0.20 |
| **Flower** | | | | | | | | |
| CD | 2.50 ** | 0.23 | 1.47 ns | 0.09 | 2.10 * | 0.10 | 62.12 *** | 1.74 * | 0.03 |
| PL | 3.65 *** | 0.35 | 9.70 *** | 0.63 | 9.50 *** | 0.63 | 0.64 ns | 2.19 ** | 0.36 |
| SL | 4.42 *** | 0.41 | 18.82 *** | 0.78 | 15.60 *** | 0.78 | 6.50 * | 2.81 *** | 0.50 |
| FLCP | 3.20 *** | 0.31 | 6.46 *** | 0.52 | 7.92 *** | 0.52 | 8.07 ** | 2.11 * | 0.31 |
| **Fruit** | | | | | | | | |
| FCL | 6.69 *** | 0.54 | 2.88 ** | 0.30 | 6.86 *** | 0.30 | 0.50 ns | 0.96 ns | 0.38 |
| FPL | 11.86 *** | 0.69 | 8.20 *** | 0.62 | 16.20 *** | 0.62 | 10.16 * | 2.38 ** | 0.53 |
| TY | 2.12 * | 0.18 | 3.86 *** | 0.39 | 2.39 ** | 0.39 | 51.11 *** | 1.76 * | 0.05 |
| FW | 5.44 *** | 0.47 | 2.96 ** | 0.31 | 6.35 *** | 0.31 | 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 significant ($p \geq 0.05$).

### 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 fwo2) 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 fwo2) 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 fwo2 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 |
| SDS   | 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 ²                  | SMI_3.1 |
|       | Screenhouse | flcp3 | 3 | 7–86       | 180.0                | 0.9 ²                  | SMI_3.1 |
|       | Open field  | flcp5 | 5 | 35–43      | 300.0                | 1.5 ³                  | SMI_5.1 |
|       | Screenhouse | flcp5 | 5 | 35–43      | 180.0                | 0.9 ³                  | 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 | fpl12 | 12 | 3–96       | −41.6                | −1.51 (cm)             | SMI_12.6 |
| FW    | Open field  | fwo2 | 2    | 75–81       | −39.5                | −14.45 (g)             | SMI_2.4 |
|       | Screenhouse | fwo2 | 2    | 75–81       | −39.1                | −12.64 (g)             | SMI_2.4 |

*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. linnaeanum* [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 (*pl8*) 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 (fwp2), 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 fwp2) 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 to ultimately identify the gene/s accounting for the QTL effect.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/4/467/s1, Table 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.

Author Contributions: Conceptualization, J.P. and P.G.; data curation, G.M.; formal analysis, G.M., J.P., and P.G.; investigation, G.M.; methodology, G.M., M.P., S.V., J.P., and P.G.; project administration, J.P., and P.G.; resources, S.V., J.P., and P.G.; supervision, M.P., S.V., J.P., and P.G.; validation, G.M.; visualization, G.M., M.P., S.V., J.P., and P.G.; writing—original draft preparation, G.M.; writing—review and editing, G.M., M.P., S.V., J.P., and P.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was undertaken as part of the initiative “Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives”, which is supported by the Government of Norway. The project is managed by the Global Crop Diversity Trust with the Millennium Seed Bank of the Royal Botanic Gardens, Kew and implemented in partnership with national and international gene banks and plant breeding institutes around the world. For further information, see the project website: http://www.cwrdiversity.org/. Funding was also received from Spanish Ministerio de Economía, Industria y Competitividad and Fondo Europeo de Desarrollo Regional (grant AGL2015-64755-R from MINECO/FEDER); from Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (grant RTI-2018-094592-B-100 from MCIU/AEI/FEDER, UE); from European Union’s Horizon 2020 Research and Innovation Programme under grant agreement No. 677379 (G2P-SOL project: Linking genetic resources, genomes and phenotypes of Solanaceous crops); and from Vicerrectorado de Investigación, Innovación y Transferencia de la Universitat Politècnica de València (Ayuda a Primeros Proyectos de Investigació, PAID-06-18). Pietro Gramazio is grateful to Japan Society for the Promotion of Science for a postdoctoral grant (P19105, FY2019 JSPS Postdoctoral Fellowship for Research in Japan (Standard)).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. FAOSTAT. Available online: http://www.fao.org/faostat/ (accessed on 12 September 2019).
2. Gebhardt, C. The historical role of species from the Solanaceae plant family in genetic research. Theor. Appl. Genet. 2016, 129, 2281–2294. [CrossRef] [PubMed]
3. Gramazio, P.; Vilanova, S.; Prohens, J. Resequencing. In The Eggplant Genome; Chapman, M., Ed.; Springer: Basel, Switzerland, 2019; pp. 81–89.
4. Hirakawa, H.; Shirasawa, K.; Miyatake, K.; Nunome, T.; Negoro, S.; Ohyama, A.; Yamaguchi, H.; Sato, S.; Isobe, S.; Tabata, S.; et al. Draft genome sequence of eggplant (*Solanum melongena* L.): The representative solanum species indigenous to the old world. *DNA Res.* 2014, 21, 649–660. [CrossRef]

5. Barchi, L.; Pietrella, M.; Venturini, L.; Minio, A.; Toppino, L.; Acquadro, A.; Andolfo, G.; Aprea, G.; Avanzato, C.; Bassolino, L.; et al. A chromosome-anchored eggplant genome sequence reveals key events in Solanaceae evolution. *Sci. Rep.* 2019, 9, 1–13. [CrossRef]

6. Gramazio, P.; Yan, H.; Hasing, T.; Vilanova, S.; Prohens, J.; Bombarely, A. Whole-Genome resequencing of seven eggplant (*Solanum melongena*) and one wild relative (*S. incanum*) accessions provides new insights and breeding tools for eggplant enhancement. *Front. Plant Sci.* 2019, 10, 1220. [CrossRef] [PubMed]

7. Gramazio, P.; Prohens, J.; Plazas, M.; Mangino, G.; Herraiz, F.J.; Garcia-Forotea, E.; Vilanova, S. Genomic tools for the enhancement of vegetable crops: A case in eggplant. *Not. Bot. Horti Agrobot. Cluj-Napoca* 2018, 46, 1–13. [CrossRef]

8. Frary, A.; Daunay, M.C.; Huvenaars, K.; Mank, R.; Doganlar, S. QTL hotspots in eggplant (*Solanum melongena*) detected with a high resolution map and CIM analysis. *Euphytica* 2014, 197, 211–228. [CrossRef]

9. Portis, E.; Cericola, F.; Barchi, L.; Toppino, L.; Acciarri, N.; Pulcini, L.; Sala, T.; Lanteri, S.; Rotino, G.L. Association mapping for fruit, plant and leaf morphology traits in eggplant. *PLoS ONE* 2015, 10, e0135200. [CrossRef] [PubMed]

10. Barchi, L.; Portis, E.; Toppino, L.; Rotino, G.L. Molecular Mapping, QTL Identification, and GWA Analysis. In The Eggplant Genome; Chapman, M., Ed.; Springer: Basel, Switzerland, 2019; pp. 41–54.

11. Toppino, L.; Valè, G.; Rotino, G.L. Inheritance of *Fusarium* wilt resistance introgressed from *Solanum aethiopicum* Gilo and *Aculentum* groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. *Mol. Breed.* 2008, 22, 237–250. [CrossRef]

12. Liu, J.; Zheng, Z.; Zhou, X.; Feng, C.; Zhuang, Y. Improving the resistance of eggplant (*Solanum melongena*) to *Verticillium* wilt using wild species *Solanum linnaeanum*. *Euphytica* 2015, 201, 463–469. [CrossRef]

13. Kouassi, B.; Prohens, J.; Gramazio, P.; Kouassi, A.B.; Vilanova, S.; Galán-Ávila, A.; Herraiz, F.J.; Kouassi, A.; Seguí-Simarro, J.M.; Plazas, M. Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*). *Sci. Hortic.* 2016, 213, 199–207. [CrossRef]

14. Plazas, M.; Vilanova, S.; Gramazio, P.; Rodriguez-Burruezo, A.; Fita, A.; Herraiz, F.J.; Ranil, R.; Fonseka, R.; Niran, L.; Fonseka, H.; et al. Interspecific hybridization between eggplant and wild relatives from different gene pools. *J. Am. Soc. Hortic. Sci.* 2016, 141, 34–44. [CrossRef]

15. Garcia-Forotea, E.; Gramazio, P.; Vilanova, S.; Fita, A.; Mangino, G.; Villanueva, G.; Arrones, A.; Knapp, S.; Prohens, J.; Plazas, M. First successful backcrossing towards eggplant (*Solanum melongena*) of a New World species, the silverleaf nightshade (*S. elaegnifolium*), and characterization of interspecific hybrids and backcrosses. *Sci. Hortic.* 2019, 246, 563–573. [CrossRef]

16. Gramazio, P.; Prohens, J.; Plazas, M.; Mangino, G.; Herraiz, F.J.; Vilanova, S. Development and genetic characterization of advanced backcross materials and an introgression line population of *Solanum incanum* in a *S. melongena* background. *Front. Plant Sci.* 2017, 8, 1477. [CrossRef] [PubMed]

17. Syfert, M.M.; Castañeda-Álvarez, N.P.; Khoury, C.K.; Särkinen, T.; Sosa, C.C.; Achicanoy, H.A.; Bernau, V.; Prohens, J.; Daunay, M.C.; Knapp, S. Crop wild relatives of the brinjal eggplant (*Solanum melongena*): Poorly represented in genebanks and many species at risk of extinction. *Am. J. Bot.* 2016, 103, 635–651. [CrossRef]

18. Vorontsova, M.S.; Knapp, S. A Revision of the “Spiny Solanums,” *Solanum subgenus Leptostemonum* (*Solanaceae*), *in Africa and Madagascar*; American Society of Plant Taxonomists: Ann Arbor, MI, USA, 2016; pp. 1–432.

19. Daunay, M.C. Eggplant. In *Vegetables II*; Springer: New York, NY, USA, 2008; pp. 163–220.

20. Knapp, S.; Vorontsova, M.S.; Prohens, J. Wild Relatives of the Eggplant (*Solanum melongena* L.: *Solanaceae*): New understanding of species names in a complex group. *PLoS ONE* 2013, 8, e57039. [CrossRef]

21. Stommel, J.R.; Whitaker, B.D. Phenolic acid content and composition of eggplant fruit in a germplasm core subset. *J. Am. Soc. Hortic. Sci.* 2003, 128, 704–710. [CrossRef]

22. Ma, C.; Dastmalchi, K.; Whitaker, B.D.; Kennelly, E.J. Two new antioxidant malonated caffeoylquinic acid isomers in fruits of wild eggplant relatives. *J. Agric. Food Chem.* 2011, 59, 9645–9651. [CrossRef]
23. Prohens, J.; Whitaker, B.D.; Plazas, M.; Vilanova, S.; Hurtado, M.; Blasco, M.; Gramazio, P.; Stommel, J.R. Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, Solanum melongena, and its wild ancestor (S. incanum). Ann. Appl. Biol. 2013, 162, 242–257. [CrossRef]

24. Meyer, R.S.; Whitaker, B.D.; Little, D.P.; Wu, S.B.; Kennelly, E.J.; Long, C.L.; Litt, A. Parallel reductions in phenolic constituents resulting from the domestication of eggplant. Phytochemistry 2015, 115, 194–206. [CrossRef]

25. Rotino, G.L.; Sala, T.; Toppino, L. Alien Gene Transfer in Crop Plants, Volume 2; Pratap, A., Kumar, J., Eds.; Springer: New York, NY, USA, 2014; pp. 381–409.

26. Taher, D.; Solberg, S.O.; Prohens, J.; Chou, Y.; Rakha, M.; Wu, T. World Vegetable Center Eggplant Collection: Origin, composition, seed dissemination and utilization in breeding. Front. Plant Sci. 2017, 8, 1484. [CrossRef]

27. Gisbert, C.; Prohens, J.; Raigón, M.D.; Stommel, J.R.; Nuez, F. Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. Sci. Hortic. (Amsterdam). 2011, 128, 14–22. [CrossRef]

28. Salas, P.; Prohens, J.; Segui-Simarro, J.M. Evaluation of androgenic competence through anther culture in common eggplant and related species. Euphytica 2011, 182, 261–274. [CrossRef]

29. Gramazio, P.; Prohens, J.; Plazas, M.; Andjar, I.; Herraiz, F.J.; Castillo, E.; Knapp, S.; Meyer, R.S.; Vilanova, S. Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant. BMC Plant Biol. 2014, 14, 350. [CrossRef]

30. Gramazio, P.; Blanca, J.; Ziarso, P.; Herraiz, F.J.; Plazas, M.; Prohens, J.; Vilanova, S. Transcriptome analysis and molecular marker discovery in Solanum incanum and S. aethiopicum, two close relatives of the common eggplant (Solanum melongena) with interest for breeding. BMC Genomics 2016, 17, 300. [CrossRef]

31. Gramazio, P.; Prohens, J.; Borrós, D.; Plazas, M.; Herraiz, F.J.; Vilanova, S. Comparison of transcriptome-derived simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers for genetic fingerprinting, diversity evaluation, and establishment of relationships in eggplants. Euphytica 2017, 213, 264. [CrossRef]

32. Dempewolf, H.; Eastwood, R.J.; Guarino, L.; Khoury, C.K.; Müller, J.V.; Toll, J. Adapting agriculture to climate change: A global initiative to collect, conserve, and use crop wild relatives. Agrocol. Sustain. Food Syst. 2014, 38, 369–377. [CrossRef]

33. Prohens, J.; Gramazio, P.; Plazas, M.; Dempewolf, H.; Kilian, B.; Diez, M.J.; Fita, A.; Herraiz, F.J.; Rodriguez-Burruezo, A.; Soler, S.; et al. Introgressomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. Euphytica 2017, 213, 158. [CrossRef]

34. Eshed, Y.; Zamir, D. A genomic library of Lycopersicon pennelli in L. esculentum: A tool for fine mapping of genes. Euphytica 1994, 79, 175–179. [CrossRef]

35. Zamir, D. Improving plant breeding with exotic genetic libraries. Nat. Rev. Genet. 2001, 2, 983–989. [CrossRef]

36. Eduardo, I.; Arús, P.; Monforte, A.J. Development of a genomic library of near isogenic lines (NILs) in melon (Cucumis melo L.) from the exotic accession PI161375. Theor. Appl. Genet. 2005, 112, 139–148. [CrossRef]

37. Eshed, Y.; Zamir, D. An introgression line population of Lycopersicon pennelli in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 1995, 141, 1147–1162.

38. Alonso-Blanco, C.; Koornneef, M.; van Ooijen, J.W. QTL analysis. In Arabidopsis Protocols. Methods in Molecular Biology; Salinas, J., Sanchez-Serrano, J.J., Eds.; Humana Press, 2006; Volume 323, pp. 79–99. [CrossRef]

39. Gur, A.; Zamir, D. Mendelizing all components of a pyramid of three yield QTL in tomato. Front. Plant Sci. 2015, 6, 1096. [CrossRef]

40. Yin, X.; Struik, P.C.; Gu, J.; Wang, H. Crop Systems Biology: Narrowing the Gaps Between Crop Modelling and Genetics; Yin, X., Struik, P., Eds.; Springer: Basel, Switzerland, 2015; pp. 193–218.

41. Tanksley, S.D.; Nelson, J.C. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 1996, 92, 191–203. [CrossRef]

42. Ashikari, M.; Matsuoka, M. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends Plant Sci. 2006, 11, 344–350. [CrossRef]

43. Calafiore, R.; Albieri, A.; Ruggieri, V.; Oliveri, F.; Rigano, M.M.; Barone, A. Phenotypic and molecular selection of a superior Solanum pennelli introgression sub-line suitable for improving quality traits of cultivated tomatoes. Front. Plant Sci. 2019, 10, 190. [CrossRef]
Agronomy 2020, 10, 467

44. Eshed, Y.; Zamir, D. Less-than-additive epistatic interactions of quantitative trait loci in tomato. *Genetics* 1996, 143, 1807–1817.

45. Jena, K.K.; Kochert, G.; Khush, G.S. RFLP analysis of rice (*Oryza sativa* L.) introgression lines. *Theor. Appl. Genet.* 1992, 84, 608–616. [CrossRef]

46. Pestsova, E.G.; Börner, A.; Röder, M.S. Development of a Set of *Triticum Aestivum-Aegilops Tauschii* Introgression Lines. *Hereditas* 2004, 135, 139–143. [CrossRef]

47. Szalma, S.J.; Hostert, B.M.; LeDeaux, J.R.; Stuber, C.W.; Holland, J.B. QTL mapping with near-isogenic lines in maize. *Theor. Appl. Genet.* 2007, 114, 1211–1228. [CrossRef]

48. Eshed, Y.; Abu-Abied, M.; Saranga, Y.; Zamir, D. *Lycopersicon esculentum* lines containing small overlapping introgressions from *L. pennellii*. *Theor. Appl. Genet.* 1992, 83, 1027–1034. [CrossRef]

49. Monforte, A.J.; Tanksley, S.D. Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: A tool for gene mapping and gene discovery. *Genome* 2000, 43, 803–813. [CrossRef]

50. Chetelat, R.T.; Qin, X.; Tan, M.; Burkart-Waco, D.; Moritama, Y.; Hox, W.; Wills, T.; Pertuzé, R. Introgression lines of *Solanum sisymbriifolium*, a wild nightshade of the Atacama Desert, in the genome of cultivated tomato. *Plant J.* 2019, 100, 836–850. [CrossRef]

51. Schauer, N.; Semel, Y.; Roessner, U.; Gur, A.; Balbo, I.; Carrari, F.; Pleban, T.; Perez-Melis, A.; Bruedigam, C.; Kopka, J.; et al. Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nat. Biotechnol.* 2006, 24, 447–454. [CrossRef] [PubMed]

52. Rigano, M.M.; Raiola, A.; Tenore, G.C.; Monti, D.M.; Del Giudice, R.; Frusciante, L.; Barone, A. Quantitative trait loci pyramiding can improve the nutritional potential of tomato (*Solanum lycopersicum*) fruits. *J. Agric. Food Chem.* 2014, 62, 11519–11527. [CrossRef] [PubMed]

53. Alseekh, S.; Tohge, T.; Wendenberg, R.; Scossa, F.; Omranian, N.; Li, J.; Kleesssen, S.; Giavalisco, P.; Pleban, T.; Mueller-Roeber, B.; et al. Identification and mode of inheritance of quantitative trait loci for secondary metabolite abundance in tomato. *Plant Cell* 2015, 27, 485–512. [CrossRef]

54. Krause, K.; Johnsen, H.R.; Pielach, A.; Lund, L.; Fischer, K.; Rose, J.K.C. Identification of tomato introgression lines with enhanced susceptibility or resistance to infection by parasitic giant dodder (*Cuscuta reflexa*). *Physiol. Plant.* 2018, 162, 205–218. [CrossRef] [PubMed]

55. Salvi, S.; Cornetti, S.; Bellotti, M.; Carraro, N.; Sanguineti, M.C.; Castelletti, S.; Tuberosa, R. Genetic dissection of maize phenology using an intraspecific introgression library. *BMC Plant Biol.* 2011, 11, 4. [CrossRef]

56. Ma, X.; Fu, Y.; Zhao, X.; Jiang, L.; Zhu, Z.; Gu, P.; Xu, W.; Su, Z.; Sun, C.; Tan, L. Genomic structure analysis of a set of *Oryza nivara* introgression lines and identification of yield-associated QTLs using whole-genome resequencing. *Sci. Rep.* 2016, 6, 27425. [CrossRef]

57. Qiu, X.; Chen, K.; Lv, W.; Ou, X.; Zhu, Y.; Xing, D.; Yang, L.; Fan, F.; Yang, J.; Xu, J.; et al. Examining two sets of introgression lines reveals background-independent and stably expressed QTL that improve grain appearance quality in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 2017, 130, 951–967. [CrossRef]

58. De Leon, T.B.; Linscombe, S.; Subudhi, P.K. Identification and validation of QTLs for seedling salinity tolerance in introgression lines of a salt tolerant rice landrace “Pokkali”. *PLoS ONE* 2017, 12, e0175361. [CrossRef]

59. Honsdorf, N.; March, T.J.; Pillen, K. QTL controlling grain filling under terminal drought stress in a set of wild barley introgression lines. *PLoS ONE* 2017, 12, e0185983. [CrossRef] [PubMed]

60. Qin, G.; Nguyen, H.M.; Luu, S.N.; Wang, Y.; Zhang, Z. Construction of introgression lines of *Oryza rufipogon* and evaluation of important agronomic traits. *Theor. Appl. Genet.* 2019, 132, 543–553. [CrossRef] [PubMed]

61. Zhao, X.; Dayigon, V.D.; McNally, K.L.; Hamilton, R.S.; Xie, F.; Reinke, R.F.; Fitzgerald, M.A. Identification of stable QTLs causing chalk in rice grains in nine environments. *Theor. Appl. Genet.* 2016, 129, 141–153. [CrossRef] [PubMed]

62. Ranil, R.H.G.; Niran, H.M.L.; Plazas, M.; Fonseka, R.M.; Fonseka, H.H.; Vilanova, S.; Andujar, I.; Gramazio, P.; Fita, A.; Prohens, J. Improving seed germination of the eggplant rootstock *Solanum torvum* by testing multiple factors using an orthogonal array design. *Sci. Hortic.* 2015, 193, 174–181. [CrossRef]

63. Van Der Weerden, G.M.; Barendse, G.W.M. A web-based searchable database developed for the EGGNET project and applied to the radboud university solanaceae database. In Proceedings of the VI International Solanaceae Conference: Genomics Meets Biodiversity, Madison, WI, USA, 30 June 2007.
Agronomy 2020, 10, 467

64. Wricke, G.; Weber, W.E. Quantitative Genetics and Selection in Plant Breeding; De Gruyter: Berlin, Germany, 1986.

65. Balakrishnan, D.; Surapaneni, M.; Mesapogu, S.; Neelamraju, S. Development and use of chromosome segment substitution lines as a genetic resource for crop improvement. Theor. Appl. Genet. 2019, 132, 1–25. [CrossRef]

66. Wang, J.X.; Gao, T.G.; Knapp, S. Ancient Chinese literature reveals pathways of eggplant domestication. Ann. Bot. 2008, 102, 891–897. [CrossRef]

67. Page, A.; Gibson, J.; Meyer, R.S.; Chapman, M.A. Eggplant Domestication: Pervasive gene flow, feralization, and transcriptomic divergence. Mol. Biol. Evol. 2019, 36, 1359–1372. [CrossRef]

68. Kaushik, P.; Prohens, J.; Vilanova, S.; Gramazio, P.; Plazas, M. Phenotyping of eggplant wild relatives and interspecific hybrids with conventional and phenomics descriptors provides insight for their potential utilization in breeding. Front. Plant Sci. 2016, 7, 677. [CrossRef]

69. Lester, R.N. Taxonomy of scarlet eggplants, Solanum aethiopicum L. In Proceedings of the I International Symposium on Taxonomy of Cultivated Plants, Wageningen, The Netherlands, 1 July 1986.

70. Prohens, J.; Plazas, M.; Raigón, M.D.; Seguí-Simarro, J.M.; Stommel, J.R.; Vilanova, S. Characterization of interspecific hybrids and first backcross generations from crosses between two cultivated eggplants (Solanum melongena and S. aethiopicum Kumba group) and implications for eggplant breeding. Euphytica 2012, 186, 517–538. [CrossRef]

71. Doganlar, S.; Frary, A.; Daunay, M.C.; Lester, R.N.; Tanksley, S.D. Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. Genetics 2002, 161, 1713–1726.

72. Frary, A.; Nesbitt, T.C.; Frary, A.; Grandillo, S.; Van Der Knaap, E.; Cong, B.; Liu, J.; Meller, J.; Elber, R.; Alpert, K.B.; et al. fa2.2: A quantitative trait locus key to the evolution of tomato fruit size. Science 2000, 289, 85–88. [CrossRef]

73. Schouten, H.J.; Tikunov, Y.; Verkerke, W.; Finkers, R.; Bovy, A.; Bai, Y.; Visser, R.G.F. Breeding has increased the diversity of cultivated tomato in The Netherlands. Front. Plant Sci. 2019, 10, 1606. [CrossRef]

74. Kearsey, M.J.; Farquhar, A.G.L. QTL analysis in plants; where are we now? Heredity (Edinb.) 1998, 80, 137–142. [CrossRef]

75. Frary, A.; Doganlar, S.; Daunay, M.C.; Tanksley, S.D. QTL analysis of morphological traits in eggplant and implications for conservation of gene function during evolution of solanaceous species. Theor. Appl. Genet. 2003, 107, 359–370. [CrossRef]

76. Fassio, C.; Caution, R.; Pérez-Donoso, A.; Bonomelli, C.; Castro, M. Propagation techniques and grafting modify the morphological traits of roots and biomass allocation in avocado trees. Horttechnology 2016, 26, 63–69. [CrossRef]

77. Alam, M.; Wilkie, J.; Kelly, A.; Hardner, C.; Topp, B. Genetic diversity and variability in graft success in Australian Macadamia rootstocks. In Proceedings of the International Macadamia Research Symposium, Honolulu, HI, USA, 13–14 September 2017.

78. Chen, K.Y.; Tanksley, S.D. High-resolution mapping and functional analysis of se2.1: A major stigma exsertion quantitative trait locus associated with the evolution from allogamy to autogamy in the genus lycopersicon. Genetics 2004, 168, 1563–1573. [CrossRef]

79. Xu, J.; Driedonks, N.; Rutten, M.J.M.; Vriezen, W.H.; de Boer, G.J.; Rieu, I. Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (Solanum lycopersicum). Mol. Breed. 2017, 37. [CrossRef]

80. Portis, E.; Barchi, L.; Toppino, L.; Lanteri, S.; Acciai, N.; Felicioni, N.; Fusari, F.; Barbierato, V.; Cericola, F.; Valé, G.; et al. QTL mapping in eggplant reveals clusters of yield-related loci and orthology with the tomato genome. PLoS ONE 2014, 9, e89499. [CrossRef]

81. Grandillo, S.; Ku, H.M.; Tanksley, S.D. Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor. Appl. Genet. 1999, 99, 978–987. [CrossRef]

82. Illa-Berenguer, E.; Van Houten, J.; Huang, Z.; van der Knaap, E. Rapid and reliable identification of tomato fruit weight and locule number loci by QTL-seq. Theor. Appl. Genet. 2015, 128, 1329–1342. [CrossRef]

83. Cambiasso, V.; Gimenez, M.D.; Pereira da Costa, J.H.; Vazquez, D.V.; Picardi, L.A.; Pratta, G.R.; Rodriguez, G.R. Selected genome regions for fruit weight and shelf life in tomato RILs discernible by markers based on genomic sequence information. Breed. Sci. 2019, 69, 447–454. [CrossRef]
84. Chakrabarti, M.; Zhang, N.; Sauvage, C.; Muños, S.; Blanca, J.; Cañizares, J.; Diez, M.J.; Schneider, R.; Mazourek, M.; McLeod, J.; et al. A cytochrome P450 regulates a domestication trait in cultivated tomato. Proc. Natl. Acad. Sci. USA 2013, 110, 17125–17130. [CrossRef] [PubMed]

85. Mu, Q.; Huang, Z.; Chakrabarti, M.; Illa-Berenguer, E.; Liu, X.; Wang, Y.; Ramos, A.; van der Knaap, E. Fruit weight is controlled by Cell Size Regulator encoding a novel protein that is expressed in maturing tomato fruits. PLoS Genet. 2017, 13, e1006930. [CrossRef]