



Communication

Fruit Composition of Eggplant Lines with Introgressions from the Wild Relative *S. incanum*: Interest for Breeding and Safety for Consumption

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Abstract: The wild species *Solanum incanum* has been used as a donor parent for the development of a set of eggplant introgression lines (ILs), which are of interest for breeding for stress tolerances and relevant morpho-agronomic traits but could also be useful for breeding for fruit quality, due to the generally higher content in health-promoting compounds of *S. incanum*. The use of eggplant ILs with introgressions from *S. incanum* requires ensuring that glycoalkaloids levels are below safety limits. We evaluated 25 fruit composition traits, including proximate composition, sugars, acids, phenolics, glycoalkaloids, and minerals in a set of 16 eggplant ILs with *S. incanum*, both parents and the F₁, grown under two environments (open field and greenhouse). The results demonstrated that the parents were significantly different regarding most fruit composition traits. Large variation was found among the 16 ILs for all traits analyzed and a strong influence of the environment accounted for the variation of 17 out of the 25 traits evaluated. Although the *S. incanum* parent produced fruits with high levels of glycoalkaloids, the 16 ILs showed mean values of total glycoalkaloids below the currently accepted safety limit for human consumption (200 mg kg⁻¹ fresh weight). Overall, the ILs produced fruits that are safe for consumption, with nutritional and functional quality similar to the recipient parent. Furthermore, six putative QTLs were detected spread over chromosomes 3 for crude protein, 5 for malic and total acids, and 7 for chlorogenic acid and solamargine, and potential candidate genes were spotted for most of them, which provide new relevant information for eggplant breeding.

Keywords: *Solanum melongena*; nutritional quality; bioactive compounds; QTLs; pre-breeding materials; glycoalkaloids



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1. Introduction

Eggplant (*Solanum melongena* L.) fruits represent an important source of dietary fiber, minerals, and antioxidants [1]. Their functional properties are linked to an outstanding content in phenolic compounds, mainly anthocyanins in the peel and chlorogenic acid in the flesh [2,3]. Several nutritional and bioactive compounds have been identified and quantified in eggplant and its wild relatives, revealing the interest of crop wild relatives for improving eggplant fruit composition [4–6]. However, the utilization of crop wild relatives in breeding is challenging [7].

Introgression lines (ILs) are useful resources for breeding, as they are elite materials with a mostly cultivated genetic background, and can be directly incorporated by breeders

in their breeding pipelines [7]. Furthermore, ILs are powerful tools for the elucidation of complex genetic traits, as they have the advantage over other mapping populations such as F_2 , double haploids, or RILs of minimizing the linkage drag [8,9]. So far, only one collection of eggplant ILs covering a significant proportion of the donor genome is available in eggplant [10]. This ILs set was developed using the wild relative *S. incanum* L. as a donor parent and it has been characterized for morphological and agronomic traits, including a detailed characterization of fruit shape [11,12]. These latter studies revealed the interest of this set of ILs for the genetic improvement of eggplant for important morpho-agronomic traits.

Solanum incanum is a wild species of interest for eggplant breeding due to its tolerance to drought and several diseases [13,14], but could also be of interest for breeding for composition traits. In this way, higher levels of antioxidant activity, total phenolics, and chlorogenic acid have been reported in *S. incanum* compared with *S. melongena* [5,6,15]. Also, because eggplant wild relatives often have high concentrations of glycoalkaloids [4,16], frequently above 200 mg kg^{-1} of fresh weight which is the internationally accepted safety limit [17], the use of ILs in breeding requires ensuring their safety in terms of glycoalkaloids content.

In this work, we performed a detailed evaluation of 25 composition traits, including proximate composition, sugars, acids, phenolics, glycoalkaloids, and minerals in the set of eggplant ILs with *S. incanum*, both parents and the hybrid in two environments (open field and screenhouse). The results will provide information on the interest of *S. incanum* and their derived introgression lines for eggplant breeding for composition traits as well as on their consumption safety. Thanks to a previous high-throughput genotyping of the ILs set [10], the detection of stable QTLs for the traits evaluated will be possible, providing relevant information for eggplant breeding for fruit quality traits.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

A total of 16 introgression lines (ILs) from the set of ILs developed in the eggplant background (*S. melongena*; accession AN-S-26) carrying fragments of the genome of a wild relative (*S. incanum*; accession MM577) [10] were used for fruit composition evaluation. Details about the genetic and phenotypic characteristics of the parents and the ILs selected are available in Gramazio et al. [10] and Mangino et al. [11,12].

Five plants of each of the two parents (AN-S-26 and MM577), the F_1 hybrid, and each of the 16 ILs were grown in a randomized block design under each of two environments (open field and screenhouse), and were distributed in five blocks per environment; i.e., five plants per genotype were tested in the open field ($n = 5$) and five plants per genotype in the screenhouse ($n = 5$). Each plant was considered a replicate. The two environments were located in the campus of the Universitat Politècnica de València (GPS coordinates: latitude, $39^\circ 28' 55''$ N; longitude, $0^\circ 20' 11''$ W; 7 m a.s.l.) (Figure 1). The same standard crop management practices and drip fertigation were applied to both environments. In addition, manual weeding and phytosanitary treatments were performed when necessary.

2.2. Fruit Processing and Chemical Analyses

At least three fruits per replicate were harvested at the commercial ripeness stage, then washed, peeled, and cut into pieces. The peel was frozen in liquid N and subsequently freeze-dried for anthocyanin and chlorophyll quantification. One fraction of the flesh pieces was also freeze-dried and homogenized using a domestic grinder for content determination of sugars, acids, chlorogenic acid, total phenolics, total antioxidant activity, and glycoalkaloids. The other fraction was dried in an oven at 70°C up to constant weight and powdered for subsequent quantification of crude protein and minerals. Dry matter was calculated for each accession as the average of $100 \times [\text{dry weight (dw)}/\text{fresh weight (fw)}]$ and expressed as g kg^{-1} fw. Units of the rest of the traits are expressed on a dw basis.

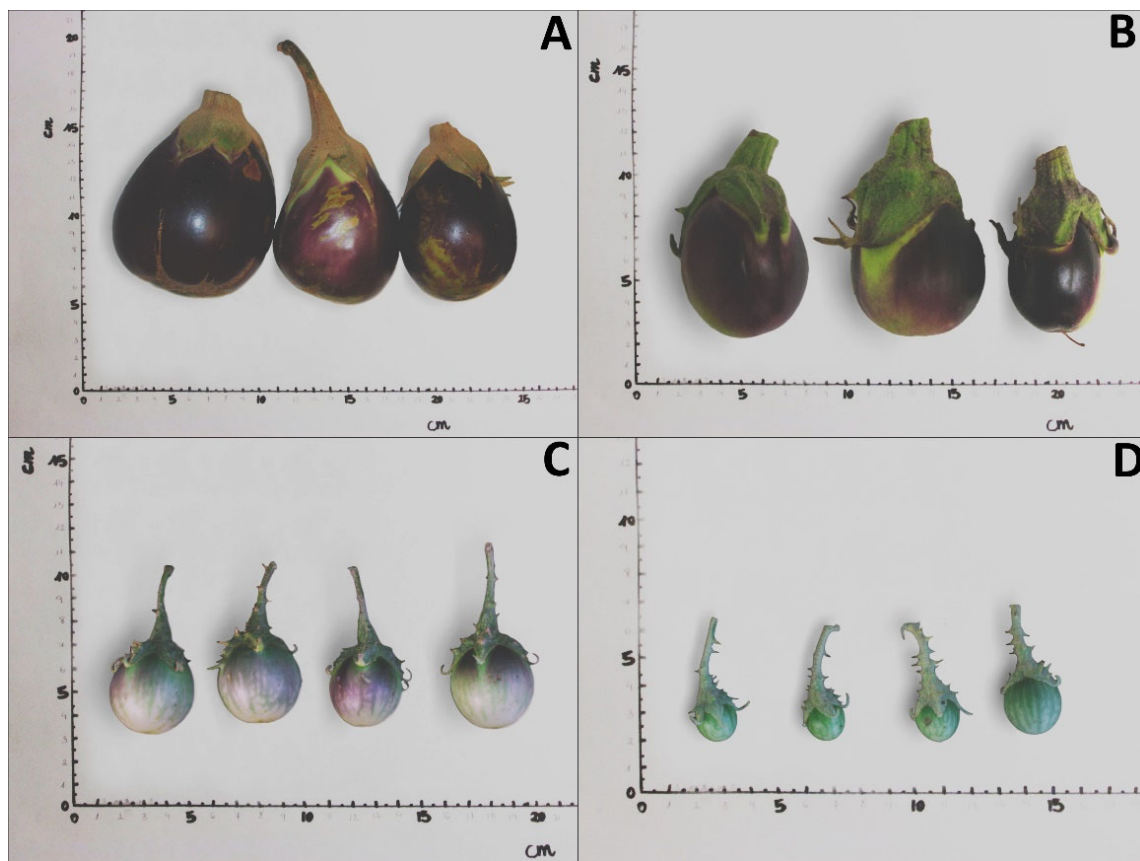


Figure 1. Representative fruits of the parents of the IL population and the hybrid: (A) fruits of the recipient parent AN-S-26 (*S. melongena*) harvested under open field; (B) fruits of AN-S-26 under greenhouse; (C) fruits of the F₁ hybrid under open field; (D) fruits of the donor parent MM577 (*S. incanum*) under an open field. The scale is in cm.

Anthocyanins ($\text{mg cm}^{-2} \text{ dw}$) were extracted from the part of the peel with a darker color, and quantified from absorbance values of the extract at 530 nm as cyanidin-3-galactoside equivalents [18]. Total chlorophylls in peel ($\text{mg g}^{-1} \text{ dw}$) were also measured spectrophotometrically, as described in Herraiz et al. [19]. Sugars and organic acids were determined by High-Performance Liquid Chromatography (HPLC) with a 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) and quantified using external standard curves. Fructose (FRU; $\text{mg g}^{-1} \text{ dw}$), glucose (GLU; $\text{mg g}^{-1} \text{ dw}$), and sucrose (SUC; $\text{mg g}^{-1} \text{ dw}$) were then detected by refractive index using a 350 RI detector (Varian, Palo Alto, CA, USA), whereas malic (MAL; $\text{mg g}^{-1} \text{ dw}$) and citric (CIT; $\text{mg g}^{-1} \text{ dw}$) acids were detected by UV at 210 nm. Contents in total sugars ($\text{mg g}^{-1} \text{ dw}$) and total acids ($\text{mg g}^{-1} \text{ dw}$) were calculated from concentrations of individual compounds as $\text{FRU} + \text{GLU} + \text{SUC}$ and $\text{CIT} + \text{MAL}$, respectively. Chlorogenic acid and total phenolics were extracted and measured according to the methods described in Plazas et al. [20]. While chlorogenic acid content ($\text{mg g}^{-1} \text{ dw}$) was determined by reversed-phase (RP) HPLC-UV at 325 nm, total phenolic content ($\text{mg g}^{-1} \text{ dw}$), expressed as chlorogenic acid equivalents, was estimated spectrophotometrically according to the Folin-Ciocalteu procedure optimized to carry out the redox reaction in a 96-well plate. Total antioxidant activity was evaluated following the colorimetric assay of DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity [21], and results were expressed as $\mu\text{mol Trolox equivalents (TE) g}^{-1} \text{ dw}$. The glycoalkaloids solamargine (SM; $\text{mg g}^{-1} \text{ dw}$) and solasonine (SS; $\text{mg g}^{-1} \text{ dw}$) were extracted using 95% ethanol and quantified by RP-HPLC-UV at 205 nm, according to Mennella et al. [4], and total glycoalkaloids ($\text{mg g}^{-1} \text{ dw}$) were calculated as $\text{SM} + \text{SS}$. Crude protein content ($\text{mg g}^{-1} \text{ dw}$) in fruit was estimated as $6.25 \times \text{total N}$, which was

measured following the Kjeldahl method [22]. Also, mineralized samples were obtained for subsequent determination of minerals (Fe, Cu, Zn, Na, Mg, Ca, K, P) following the MAPA procedures [23], as described in Raigón et al. [24], and contents were expressed as mg g^{-1} dw. Detailed information on the methods of fruit composition analysis is provided in Supplementary file S1.

2.3. Data Analysis

For each of the traits analyzed, the mean and its standard error (SE) were calculated for the recipient parent (AN-S-26) in each of the two environments (open field and screenhouse). For the donor parent (MM577) and the F_1 hybrid, no data was obtained in the screenhouse because they did not set fruit under these conditions. Thus, in these cases, mean and SE were calculated for all traits only under open field conditions. The normality of data within each of the two parents and the F_1 was checked with a Shapiro-Wilk test. Statistical differences among the means of the four groups (i.e., AN-S-26 in open field, AN-S-26 in screenhouse, MM577 in open field, and the F_1 in open field) were assessed with an analysis of variance (ANOVA) and a Student-Newman-Keuls posthoc multiple range test at $p < 0.05$ for all traits. Mid-parent heterosis was also calculated for all traits only under open field conditions and its significance was evaluated with a Student's t -test at $p < 0.05$.

Data of the 16 ILs along with AN-S-26 for all traits were subjected to a bifactorial ANOVA for the evaluation of differences among the accessions (G, 17 levels), between environments (E, 2 levels), and for the occurrence of $G \times E$ interactions [25]. The normality of data within each of the ILs was checked with a Shapiro-Wilk test. Furthermore, mean, range values, and phenotypic coefficient of variation (CVP) of the ILs, together with the recipient parent, were calculated under each environment (open field and screenhouse).

A principal component analysis (PCA) was performed using pairwise Euclidean distances among means of the ILs and AN-S-26 for all the traits for each environment, in order to globally evaluate the variation of the ILs compared to the recurrent parent based on the traits evaluated. The *ggplot2* [26] and *stats* packages of the R statistical software v4.0.2 [27,28] were used for this purpose.

Given that each IL harbored only one introgressed fragment from the donor wild parent on a single chromosome within the cultivated genetic background, the existence of a significant difference between the mean of one IL and the cultivated parent was assumed to indicate the presence of a QTL for a particular trait within the introgressed fragment. In order to detect significant QTLs, the mean of the replicates for each trait, IL, and the environment was compared with the recipient parent AN-S-26 using a Dunnett's test at $p < 0.05$ [29], as described by Mangino et al. [12]. A stable QTL was reported when the difference between the IL and the recipient parent AN-S-26 was consistently significant in both environments. For each putative QTL detected, the relative increase over the recipient parent and the allelic effect was calculated in each of the environments.

3. Results and Discussion

The ANOVA performed among the recipient parent AN-S-26 in each of the two environments, the donor parent MM577 and the F_1 revealed significant differences for all traits evaluated except sucrose, Fe, Mg, and K (Table 1). The results demonstrated that the parents were considerably different regarding fruit composition. In this way, significant differences were observed for 18 out of the 25 traits evaluated. Among these, on average, fruits of AN-S-26 accumulated more anthocyanins and had 3-fold higher chlorophyll content in the peel. As well, AN-S-26 had half less dry matter content, accumulated 3.5-fold more total sugars, reflected only in glucose and fructose but not in sucrose, and showed 1.9-fold lower organic acid content than MM577 (Table 1). In addition, although for both parents the malic acid was the major organic acid, the proportion of citric acid to the total acids was much lower in AN-S-26 (6.2%) than in MM577 (35.8%). Regarding minerals, AN-S-26 accumulated, on average, 1.7- and 1.9-fold lower Na and Ca, respectively, than MM577 but higher Cu, Zn, and P by 2.3-, 1.5- and 2.7-fold, respectively. As for major

secondary metabolites in fruit flesh, AN-S-26 showed lower mean values of chlorogenic acid (CGA) content by 1.2-fold (Table 1), suggesting that there is scope for improving the content of this compound in cultivated eggplant using the wild species *S. incanum*.

CGA is known to be the predominant phenolic acid and antioxidant in eggplant and *S. incanum* [5] and the current interest for this molecule resides in its health-promoting properties such as free radical scavenger, anti-inflammatory, and anti-microbial, among others [1]. In agreement with our data, *S. incanum* has shown contents in CGA above those of cultivated varieties [5,6]. However, no differences were detected for total antioxidant activity and total phenolics between the two parents (Table 1). This could be due to the presence of other compounds in AN-S-26 with greater antioxidant capacity even at low concentrations [30]. The largest differences between the parents were found for total and individual glycoalkaloids. In this way, fruits of AN-S-26 had much lower contents of solamargine and solasonine with an average of 10.7-fold less total glycoalkaloids compared to the wild parent MM577 (Table 1). Thus, total glycoalkaloid content for the latter was, on average, above the safety limit for human consumption [17]. These findings are in agreement with other works on *S. incanum* as well as on other eggplant wild relatives [4,16,31], and underline the potential problem of using eggplant wild species for breeding due to the linkage drag of undesirable traits [7,32]. The differences found for fruit composition between the cultivated and the wild parents show the result of selection events for more palatable non-toxic fruits during domestication [33]. In this way, changes in the regulation of invertase and other enzymes activity related to carbohydrate metabolism could explain the differences found for taste-related compounds [34]. Similarly, the early selection of five major loci during tomato domestication has been demonstrated to be responsible for the dramatic reduction of glycoalkaloids accumulation in fruits [35]. Also, leaving aside the selection for bioactive compounds and stress tolerance during domestication likely resulted in the elimination of alleles that contribute to the high content in phenolics [36].

Table 1. Mean values and standard error (SE) of each trait were analyzed for the recipient parent (*S. melongena*; AN-S-26; n = 5) under open field (OF) and greenhouse (SH), and for the wild parent (*S. incanum*; MM577; n = 5) and the F₁ (n = 5) under OF. Units are expressed on a dry weight (dw) basis except for dry matter. The hybrid mid-parent heterosis (HMP) calculated under OF with its significance is also shown.

Trait (Units)	<i>S. melongena</i> AN-S-26 (n = 5)		<i>S. incanum</i> MM577 (n = 5)	F ₁ (n = 5)	
	Mean ± SE SH	Mean ± SE OF	Mean ± SE OF	Mean ± SE OF	H _{MP} (%) OF ^b
Dry matter (g kg ⁻¹ fw) ^a	86.0 a ± 3.0	90.0 a ± 2.7	178.2 c ± 8.8	126.8 b ± 7.0	−5.3
Anthocyanins in peel (mg cm ⁻² dw)	0.030 b ± 0.003	0.038 b ± 0.009	0.000 a ± 0.000	0.021 b ± 0.004	41.5
Total chlorophylls in peel (mg g ⁻¹ dw)	0.47 b ± 0.06	0.49 b ± 0.10	0.16 a ± 0.04	0.43 b ± 0.04	26.2
Fructose (mg g ⁻¹ dw)	117.2 b ± 2.8	116.9 b ± 7.7	35.2 a ± 4.2	32.7 a ± 2.9	−55.9 ***
Glucose (mg g ⁻¹ dw)	124.4 c ± 4.9	114.4 c ± 5.8	22.1 a ± 2.7	81.3 b ± 2.9	20.6
Sucrose (mg g ⁻¹ dw)	14.0 ± 2.5	17.9 ± 3.1	12.7 ± 3.2	16.2 ± 3.2	7.7
Total sugars (mg g ⁻¹ dw)	255.5 c ± 7.7	249.2 c ± 14.8	70.0 a ± 7.8	130.1 b ± 7.5	−17.0
Malic acid (mg g ⁻¹ dw)	12.34 a ± 1.47	14.55 a ± 2.12	19.29 b ± 0.47	20.42 b ± 0.88	21.6 **
Citric acid (mg g ⁻¹ dw)	1.29 a ± 0.22	0.96 a ± 0.13	10.78 b ± 2.50	4.86 b ± 0.91	5.7
Total acids (mg g ⁻¹ dw)	13.63 a ± 1.28	15.52 a ± 2.12	30.07 b ± 2.39	25.28 b ± 1.10	13.5
Protein (mg g ⁻¹ dw)	91.9 a ± 3.0	107.3 b ± 5.8	116.5 b ± 2.5	108.0 b ± 4.0	−2.5
Total phenolic content (mg g ⁻¹ dw)	22.31 ab ± 1.72	20.78 ab ± 0.89	24.64 b ± 1.57	18.19 a ± 0.84	−18.6
Total antioxidant activity (μmol ET g ⁻¹ dw)	240.6 a ± 4.8	307.6 b ± 9.4	314.2 b ± 4.5	301.2 b ± 3.9	−3.0
Chlorogenic acid (mg g ⁻¹ dw)	6.27 a ± 0.14	6.70 a ± 0.14	7.69 b ± 0.15	6.32 a ± 0.16	−12.0 *

Table 1. Cont.

Trait (Units)	<i>S. melongena</i> AN-S-26 (n = 5)		<i>S. incanum</i> MM577 (n = 5)	<i>F</i> ₁ (n = 5)	
	Mean ± SE SH	Mean ± SE OF	Mean ± SE OF	Mean ± SE OF	H _{MP} (%) OF ^b
Solasonine (mg g ^{−1} dw)	0.32 a ± 0.06	0.33 a ± 0.03	5.37 c ± 1.33	3.25 b ± 0.33	28.9
Solamargine (mg g ^{−1} dw)	0.50 a ± 0.08	0.83 a ± 0.10	6.47 b ± 2.91	4.27 b ± 1.59	35.3
Total glycoalkaloids (mg g ^{−1} dw)	0.82 a ± 0.10	1.15 a ± 0.09	12.35 b ± 3.58	7.52 b ± 1.69	28.6
Fe (mg g ^{−1} dw)	0.017 ± 0.005	0.016 ± 0.001	0.024 ± 0.000	0.025 ± 0.002	38.6
Cu (mg g ^{−1} dw)	0.010 a ± 0.000	0.018 b ± 0.002	0.008 a ± 0.000	0.018 b ± 0.002	54.2
Zn (mg g ^{−1} dw)	0.023 b ± 0.001	0.027 b ± 0.001	0.018 a ± 0.001	0.023 b ± 0.001	2.7
Na (mg g ^{−1} dw)	0.23 a ± 0.05	0.37 b ± 0.02	0.64 c ± 0.04	0.17 a ± 0.04	−59.6
Mg (mg g ^{−1} dw)	2.21 ± 0.11	2.48 ± 0.30	2.65 ± 0.08	2.58 ± 0.26	10.5
Ca (mg g ^{−1} dw)	3.01 b ± 0.64	1.54 a ± 0.04	2.90 b ± 0.18	3.17 b ± 0.16	46.2
K (mg g ^{−1} dw)	26.00 ± 0.81	30.72 ± 1.63	30.21 ± 3.42	26.75 ± 0.71	−6.0
P (mg g ^{−1} dw)	2.68 a ± 0.63	4.54 b ± 0.42	1.67 a ± 0.16	4.96 b ± 0.10	66.6

^a For each trait, where significant differences have been found, means separated by different letters are significant according to the Student-Newman-Keuls test at a significance level of $p < 0.05$. ^b ***, **, * indicate significant at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

Mean values of fruit composition traits for *F*₁ were intermediate between the two parents for contents in dry matter, glucose, total sugars, and solasonine. On the other hand, fruits of *F*₁ showed anthocyanins in peel like the recipient parent AN-S-26 (Table 1). The genetic dominance of the presence over non-presence of anthocyanins in peel in inter-specific hybrids has already been reported in other works [5,37]. Similarly, *F*₁ fruits were phenotypically more similar to AN-S-26 for average contents in total chlorophylls, total phenolics, CGA, Cu, Zn, and P; and similar to the wild parent MM577 for average contents in fructose, organic acids, solamargine, total glycoalkaloids and Ca. The mid-parent heterosis only was significantly positive for content in malic acid and negative for fructose and CGA (Table 1). Our results differed from those of Prohens et al. [5], who found the inter-specific hybrid showing intermediate values of phenolics content. However, these authors evaluated groups of phenolics conjugates instead of CGA individually. Besides, values of heterosis for biochemical compounds have been reported to be highly variable, strongly dependent on the environment [38]. On the other hand, our results are in agreement with previous studies that evaluated CGA and/or total phenolic content in different inter- and intraspecific hybrids [32,39,40], which showed lower CGA content than the mid-parent value or even lower than the parent with the lowest value.

The results of the ANOVA performed to evaluate the significant effects of the genotype (G), environment (E), and G × E among the 16 ILs and AN-S-26 are shown in Table 2. Significant differences among genotypes were observed for all traits evaluated except total antioxidant activity (TAA), Fe, Mg and Ca. A significant environment (E) effect was detected for eleven traits, with average values of dry matter, total chlorophylls, protein, TAA, CGA, Cu, and K being higher under open field (OF) conditions, and of citric acid, total acids, Fe and Ca being higher under greenhouse (SH) conditions. For those 11 traits, F-ratio values for E were much greater than those of the G factor, with TAA showing the highest value. Combining significant E and G × E interaction effects, a strong influence of the environment accounted for the variation of 17 out of 25 traits evaluated, which makes harder the identification of stable QTLs, but could represent an advantage for selection and breeding for specific environmental conditions [41]. Significant seasonal [42], environmental [38], and cultivation practices [24,43] effects have also been reported within and among cultivated varieties for several fruit composition traits. Relative ranges of variation (maximum mean value/minimum mean value) were higher under SH, except for malic and total acids, solamargine, Fe, Cu, and Zn. The lowest phenotypic coefficient of variation (CVP) was observed for total antioxidant activity under both OF and SH (4.1%)

and 3.6%, respectively), while the highest CVP was observed for solasonine and citric acid content under OF (93.4% and 76.2%, respectively) and SH (126.3% and 179.7%, respectively) (Table 2). Despite the large variation found within the ILs set, the wild parent MM577 had glycoalkaloids levels significantly higher than those of the ILs and recipient parent, and mean values of total glycoalkaloids for each of the 16 ILs were below the internationally accepted safety limit for human consumption [17] (Table 2). This is in agreement with the previous characterization of glycoalkaloids in a set of advanced backcrosses derived from three eggplant allied species [32]. This is a result of special interest in the development and release of new eggplant varieties using the set of ILs of *S. incanum*, since glycoalkaloids are the main undesirable compounds that can accumulate in eggplant and related species in high doses such as to cause harm to human health [16,32]. Another relevant undesirable trait in eggplant are steroidal saponins, which are not considered lethally toxic but may cause gastrointestinal irritation, and, like glycoalkaloids, produce the bitter taste of the fruit [44]. However, the accumulation of glycoalkaloids at high concentrations is more of a concern when releasing new varieties to the market since they are very stable to cooking processes, in contrast to saponins [45].

Table 2. F-ratio values for genotype (G; 16 ILs plus the recipient parent AN-S-26), environment (E; open field and screenhouse), and genotype per environment interaction ($G \times E$), obtained from the bifactorial ANOVA, and significance of each effect for each trait. Mean, range, and phenotypic coefficient of variation (CV_P) of the ILs, together with the recipient parent, under both open field and screenhouse are also shown. Units are expressed on a dry weight (dw) basis except for dry matter.

Trait (Units)	Factors ^a			Open Field			Screenhouse		
	G	E	$G \times E$	Mean	Range	CV_P	Mean	Range	CV_P
Dry matter (g kg ⁻¹ fw)	3.40 ***	13.11 **	1.21	82.5	75.0–97.1	11.5	77.3	67.6–87.9	11.1
Anthocyanins in peel (mg cm ⁻² dw)	4.23 ***	2.48	1.87 *	0.034	0.020–0.060	56.6	0.028	0.006–0.077	47.5
Total chlorophylls in peel (mg g ⁻¹ dw)	6.45 ***	13.68 **	0.79	0.38	0.16–0.64	51.8	0.21	0.07–0.47	41.9
Fructose (mg g ⁻¹ dw)	7.84 ***	3.63	3.30 ***	124.4	97.9–146.0	15.3	118.2	80.8–170.8	19.4
Glucose (mg g ⁻¹ dw)	6.26 ***	3.34	1.61	115.4	85.4–147.8	19.5	120.1	77.5–153.9	22.5
Sucrose (mg g ⁻¹ dw)	3.89 ***	0.95	1.13	22.1	9.6–29.1	42.1	24.5	13.4–50.0	55.3
Total sugars (mg g ⁻¹ dw)	6.59 ***	0.00	1.86 *	262.0	211.0–318.2	15.5	262.1	199.0–343.6	16.9
Malic acid (mg g ⁻¹ dw)	9.31 ***	3.21	1.32	15.60	9.72–28.30	44.4	17.68	10.70–30.45	47.5
Citric acid (mg g ⁻¹ dw)	3.61 ***	3.75 ***	21.30 **	1.43	0.88–4.00	76.2	2.68	0.86–9.19	179.7
Total acids (mg g ⁻¹ dw)	9.49 ***	8.93 *	1.67	16.93	10.80–29.57	41.7	20.53	13.03–34.76	46.5
Protein (mg g ⁻¹ dw)	5.52 ***	32.93 ***	1.18	113.1	97.4–130.6	11.5	102.2	82.1–117.8	9.8
Total phenolic content (mg g ⁻¹ dw)	6.12 ***	0.36	0.90	20.14	15.57–28.10	21.1	19.74	10.99–29.04	27.0
Total antioxidant activity (µmol ET g ⁻¹ dw)	1.34	887.40 ***	0.39	298.0	289.0–308.5	4.1	215.2	203.9–240.6	3.6
Chlorogenic acid (mg g ⁻¹ dw)	4.25 ***	24.56 **	1.15	5.70	4.72–6.70	15.9	4.75	3.68–6.27	16.2
Solasonine (mg g ⁻¹ dw)	5.07 ***	4.22	1.73	0.35	0.11–0.98	93.4	0.57	0.08–1.12	126.3
Solamargine (mg g ⁻¹ dw)	5.03 ***	3.49	1.99 *	0.46	0.03–0.83	65.7	0.30	0.05–0.52	45.0
Total glycoalkaloids (mg g ⁻¹ dw)	3.50 ***	0.14	2.23 **	0.77	0.46–1.38	50.7	0.83	0.27–1.40	69.0
Fe (mg g ⁻¹ dw)	0.90	8.62 *	1.51	0.016	0.013–0.019	18.5	0.018	0.014–0.023	32.3
Cu (mg g ⁻¹ dw)	2.79 ***	41.91 ***	2.04 *	0.019	0.012–0.025	23.4	0.010	0.007–0.013	16.4
Zn (mg g ⁻¹ dw)	2.77 ***	1.52	2.33 **	0.024	0.018–0.029	17.6	0.025	0.022–0.030	12.9
Na (mg g ⁻¹ dw)	1.98 *	1.55	1.34	0.28	0.20–0.37	29.9	0.33	0.22–0.59	69.9
Mg (mg g ⁻¹ dw)	0.67	0.00	0.82	2.38	1.94–2.76	23.6	2.38	1.95–2.92	22.3
Ca (mg g ⁻¹ dw)	0.86	28.72 ***	1.01	1.70	1.40–1.99	19.3	2.74	1.99–3.29	48.1
K (mg g ⁻¹ dw)	2.07 *	27.86 ***	1.31	31.35	28.02–36.31	11.5	26.53	22.77–29.74	8.0
P (mg g ⁻¹ dw)	1.82 *	5.03	1.11	4.65	3.21–5.42	20.1	3.31	2.48–5.09	26.2

^a ***, **, * indicate significant differences at $p < 0.001$, < 0.01 or < 0.001 , respectively.

The PCA also reflected the strong environment effect that influenced the fruit composition of the ILs and AN-S-26 (Figure 2). The two first principal components (PCs) of the PCA accounted for 44.5% of the total variation observed. Contents in Ca, total acids, and solasonine were the traits displaying the highest positive correlation ($r > 0.2$) with PC1, while total antioxidant activity, CGA, K, Cu, P, protein, and total chlorophylls were the traits with the highest absolute negative correlation with PC1. On the other hand, dry matter, citric acid, and Fe were positively correlated with PC2, while malic acid and sugars except for sucrose displayed the highest absolute negative values for the correlation with PC2. All the accessions under OF, except SMI_7.1, clustered together with negative

values of PC1, whereas all accessions under SH were grouped together with positive values of PC2. In addition, minimal overlapping between the 95% significance ellipses of each of the two environments was observed in the PCA score plot, and the more widespread distribution of accessions under SH indicated a larger variability under those conditions. It is noteworthy that ILs SMI_7.1 and SMI_7.2, which overlap for most of the wild genome fragments they contain, plotted close to each other in both environments in the PCA score plot. Furthermore, ILs SMI_12.6, SMI_5.1, SMI_7.1, and SMI_7.2 were the farthest and thus the most different from AN-S-26 under both environments (Figure 2).

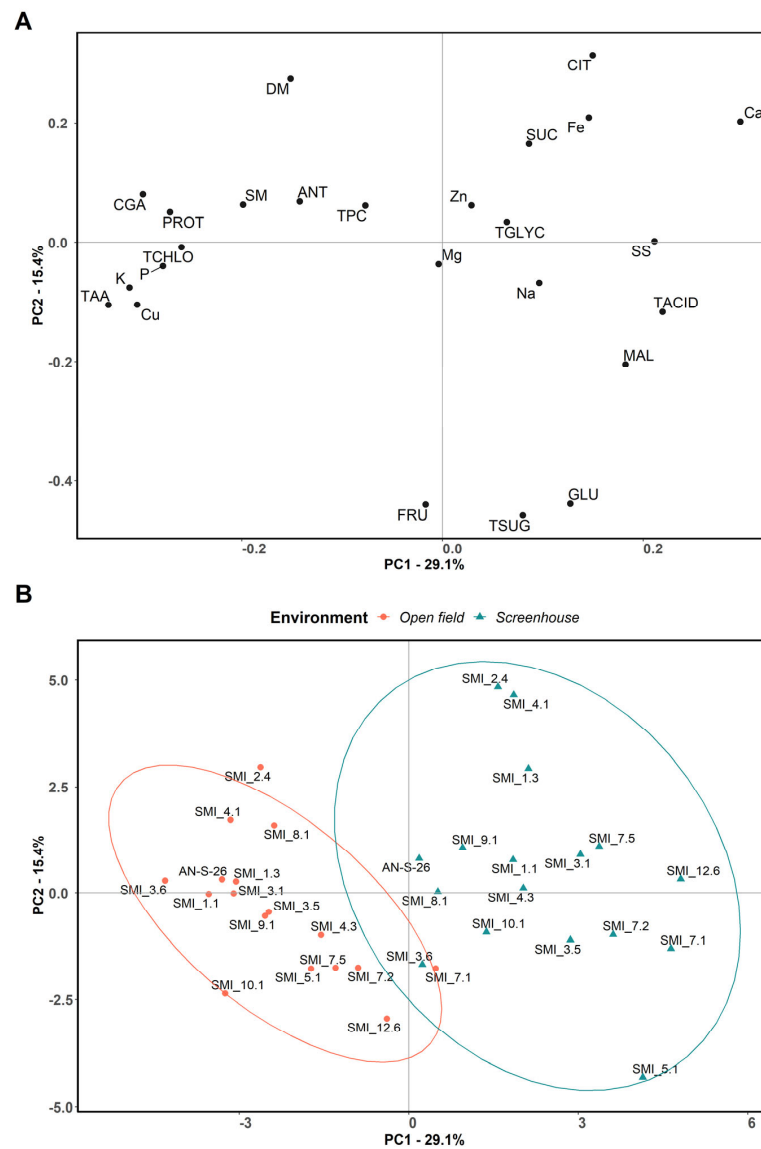


Figure 2. PCA loading plot (A) and score plot (B) based on the two first principal components for 16 introgression lines with *S. incanum* (SMI names) and the recipient *S. melongena* parent (AN-S-26) for 25 composition traits. First and second components account for 29.1% and 15.4% of the total variation, respectively. The accessions are represented by different symbols and colors according to the environment in which they were grown: orange circle for open field, and blue triangle for screenhouse. Ellipses grouped the accessions for each treatment with a 95% confidence level. Acronyms used in the loading plot and their meaning are ANT: anthocyanins; TCHLO: total chlorophylls; FRU: fructose; GLU: glucose; SUC: sucrose; TSUG: total sugars; MAL: malic acid; CIT: citric acid; TACID: total acids; PROT: crude protein; TPC: total phenolics; TAA: total antioxidant activity; CGA: chlorogenic acid; SS: solasonine; SM: solamargine; TGLYC: total glycoalkaloids.

The assessment of the differences among each of the ILs and the recipient parent AN-S-26 resulted in the detection of six stable and novel QTLs in five ILs carrying introgressed fragments of three out of twelve *S. incanum* chromosomes (Table 3). However, in most cases, the wild alleles had a negative effect on the fruit organoleptic and functional quality compared to the cultivated eggplant. One QTL was found for content in malic acid (*ma5*), which mapped in the same position as the QTL identified for total acids (*ac5*) at the end of chromosome 5 (35–43 Mbp) and accounted for a considerable increase of each trait mean value over AN-S-26 (Table 3).

Table 3. List of stable (i.e., detected in both environments) putative QTLs detected in the eggplant IL population. All units are expressed on a dry weight basis (dw).

Trait	QTL	Chr.	Position (Mb)	Open Field			Screenhouse		
				Increase Over AN-S-26 (%)	Allelic Effect (Units)	<i>p</i> -Value	Increase Over AN-S-26 (%)	Allelic Effect (Units)	<i>p</i> -Value
Malic acid	<i>ma5</i>	5	35–43	94.5	6.87 (mg g ^{−1} dw)	<0.001	124.8	7.70 (mg g ^{−1} dw)	0.0048
Total acids	<i>ac5</i>	5	35–43	90.6	7.03 (mg g ^{−1} dw)	<0.001	109.8	7.48 (mg g ^{−1} dw)	0.0121
Protein	<i>pro3</i>	3	93–96	21.8	11.67 (mg g ^{−1} dw)	0.0185	16.9	7.76 (mg g ^{−1} dw)	0.02377
Chlorogenic acid	<i>cga7</i>	7	129–135	−19.2	−0.64 (mg g ^{−1} dw)	0.0031	−33.5	−1.05 (mg g ^{−1} dw)	<0.001
Solamargine	<i>sm7.1</i>	7	129–135	−46.8	−0.18 (mg g ^{−1} dw)	0.0026	−68.5	−0.17 (mg g ^{−1} dw)	<0.001
Solamargine	<i>sm7.2</i>	7	135–139	45.5	0.18 (mg g ^{−1} dw)	0.0054	51.2	0.13 (mg g ^{−1} dw)	0.0113

A search throughout the ‘67/3’ eggplant reference genome assembly (V3 version) [46] using the Sol Genomics Network database (<http://www.solgenomics.net>; accessed on 25 November 2021) identified two potential candidate genes that mapped to the region of the detected QTLs. The genes encode a phosphoenolpyruvate carboxykinase (SMEL_005g236230.1), which catalyzes a reversible reaction involved in gluconeogenesis derived from malic acid, and a peroxisomal acetate/butyrate-CoA ligase (SMEL_005g239840.1) that is probably involved in the activation of exogenous acetate for entry into the glyoxylate cycle. One QTL was detected for crude protein content (*pro3*), at the end of chromosome 3 (93–96 Mbp), which also increased this trait mean value over AN-S-26 (Table 3). Another QTL was detected for CGA content (*cga7*) and its location was narrowed down between 129 to 135 Mbp of chromosome 7. In this case, the introgressed wild allele led to a reduction in CGA (Table 3). An orthologous gene (SMEL_007g290860.1) of the tomato Solyc09g007920, which encodes for phenylalanine ammonia-lyase 1 (*SIPAL1*), a core enzyme in the CGA biosynthesis pathway, was identified within the eggplant genome region of *cga7* and might be a potential candidate for this association. Interestingly, the gene coding the enzyme HQT, which catalyzes the synthesis of CGA from its precursor, quinic acid, was located in the upper part of chromosome 7 in a previous linkage map [47]. Furthermore, we found a cluster of three orthologous genes to AT1G05260.1 (*Arabidopsis thaliana*) encoding a peroxidase, which catalyzes the oxidation of phenolic compounds, that is situated within this region (SMEL_007g288660.1.01, SMEL_007g288680.1.01, SMEL_007g288690.1.01). Lastly, two putative QTLs with opposite effects were detected for solamargine content (*sm7.1* and *sm7.2*). The QTL *sm7.1* was identified between 129 and 135 Mbp on chromosome 7 and led to a decrease of the solamargine average content over AN-S-26, while *sm7.2* was found downstream (135–139 Mbp) and led to an increase of the trait, but of slightly lesser extent than the reducing effect of *sm7.1* (Table 3). The *GAME* (*GLYCOALKALOID METABOLISM*) genes have been widely studied in tomato and potato, and a cluster of

these genes have been located in a region of chromosome 7 in tomato [48]. However, orthologous genes in eggplant were located in the same chromosome but upstream the region of *sm7.1* and *sm7.2* [46]. Among the genes annotated in the eggplant genome within those regions, we were able to identify nine coding for the 72A subfamily of cytochrome P450-like proteins [46]. Some proteins of this subfamily have already been associated with the glycoalkaloid metabolism in tomatoes [48], and may be related to the solamargine QTLs identified in this work.

4. Conclusions

The characterization performed revealed that the set of 16 eggplant introgression lines carrying fragments of the wild relative *S. incanum* genome generally exhibits a nutritional and functional quality similar to that of the recipient parent. This demonstrates the potential of ILs as pre-breeding material and their safety for human consumption since linkage drag of undesirable quality traits such as glycoalkaloids is avoided. The ILs evaluated produce fruits safe for consumption with good quality characteristics, which could be used in the future in breeding programs aimed at improving other interesting traits, such as tolerance to drought and several diseases. In addition, the QTLs detected provide new relevant information for eggplant breeding.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy12020266/s1>, Supplementary file S1: Detailed description of the methods used for fruit chemical composition analyses.

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