



Characterization and QTL identification in eggplant introgression lines under two N fertilization levels

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ABSTRACT

Lowering nitrogen inputs is a major goal for sustainable agriculture. In the present study, a set of 10 *Solanum melongena* introgression lines (ILs) developed using *Solanum incanum* as the exotic donor parent were grown under two nitrogen fertilization doses supplied with the irrigation system: 1) 8.25 mmol · L⁻¹ NH₄NO₃, corresponding to the high nitrogen treatment (HN), and 2) no external nitrogen supply, corresponding to the low nitrogen treatment (LN). Twenty traits, including plant growth and yield parameters, fruit size and morphology, nitrogen and carbon content in leaf and fruit, and phenolics content in fruit, were evaluated. The aim was to select of potential materials for eggplant breeding under low N inputs, as well as to identify and locate putative QTLs associated with the traits evaluated. No significant differences were observed between the soil characteristics of the HN and LN treatments, except for nitrogen and iron content, which was slightly lower in the HN, probably as a consequence of higher nutrient removal from soil by plants in the latter group. Analysis of variance showed that lowering nitrogen inputs did not significantly affect the final yield, fruit morphology, size and phenolics content. Most agronomic traits were highly and positively correlated with each other under both treatments, as well as total phenolics with chlorogenic acid content. The assessment of the differences between each IL and the recipient parent resulted in the identification of 36 QTLs associated with most of the traits—12 were specific to the HN, 17 specific to the LN, and 7 were stable across treatments. The introgressed fragment of *S. incanum* generally had a negative effect on the trait, except for QTLs for fruit dry matter, for fruit length on chromosome 10 under the HN, and for fruit pedicel length on chromosome 9 under the LN. The increase over AN-S-26 of the allele of *S. incanum* for the QTLs detected ranged between -73.98% and 26.03% in HN and -73.67% and 34.43% in LN. These findings provide useful tools for the utilization of *S. incanum* in eggplant breeding under lower nitrogen fertilization.

Keywords: *Solanum melongena*; *Solanum incanum*; Wild relatives; N fertilization; Abiotic stress; QTL

1. Introduction

The high yields achieved through the development of modern plant varieties during the second half of the 20th century have strongly relied on the application of inorganic nitrogen

(N)-enriched fertilizers (Lammerts Van Bueren et al., 2011). However, there is a growing awareness of the harmful environmental effects of over-fertilization (Stevens, 2019; Xu et al., 2021), which has led to a shift in production policies and new challenges in breeding programs towards a more sustainable agriculture

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(Fess et al., 2011; Zhang et al., 2015). Therefore, one of the main objectives in the development of new varieties for a sustainable agriculture is their adaptation to low input conditions and greater efficiency in the use of nutrients and water. Since N is an essential nutrient for plant growth and development, it is necessary to investigate the effects on plant performance of lowering N inputs and to screen the existing diversity for potential materials with high nitrogen use efficiency (NUE). In this way, N rate has been reported to significantly affect plant height, leaf area, and shoot dry weight in wheat seedlings (Wang et al., 2019), yield and fruit load in processing tomato (Elia and Conversa, 2012), plant growth and chlorophyll content in a local variety of pepper (Stagnari et al., 2021), grain quality in rice (Zhou et al., 2018), content in flavor-related compounds (Wang et al., 2007), and antioxidants (vitamin C, carotenoids, phenolics) in different varietal groups of tomato (Hernández et al., 2020).

Crop wild relatives (CWRs) constitute a valuable source of variation for tolerance to abiotic stresses including low inputs, since they naturally grow in areas with harsh environmental conditions (Dempewolf et al., 2017; Prohens et al., 2017). CWRs can also be an advantageous material for the improvement of other traits such as nutritional content or the introduction of new characteristics of fruit such as shapes, sizes, and colors (Leiva-Brondo et al., 2012; Ebert, 2020). In addition, using CWRs in breeding broadens the genetic base of the cultivated species, making new genetic diversity potentially available to the breeders. However, the direct use of CWRs in breeding pipelines is often difficult (Dempewolf et al., 2017). In this way, interspecific introgression lines (ILs) have the advantage of carrying a single fragment of a wild genome on a mostly cultivated genetic background, facilitating their use in breeding (Prohens et al., 2017; Pratap et al., 2021). In addition, ILs are a powerful tool for the detection of Quantitative Trait Loci (QTLs) associated with complex traits.

Eggplant (*Solanum melongena* L.) is the fifth most economically important vegetable worldwide (FAOSTAT, 2019). However, there is little information on its diversity for nitrogen use efficiency and performance under reduced N-inputs (Mauceri et al., 2019, 2021; Villanueva et al., 2021). Furthermore, genetic research and development of genomic tools in eggplant have lagged behind other important Solanaceae such as tomato (Gramazio et al., 2018, 2021). Nevertheless, in recent years, the well-established synteny between the eggplant and tomato genomes (Gramazio et al., 2014; Rinaldi et al., 2016; Barchi et al., 2019) and the availability of recently developed intra and interspecific mapping populations with associated genetic linkage maps (Gramazio et al., 2014, 2017; García-Forteza et al., 2019), as well as of several high-quality eggplant genome assemblies (Wei et al., 2020; Barchi et al., 2021; Li et al., 2021), have dramatically increased genomic studies on eggplant. Since then, an array of QTLs, as well as candidate genes, for agronomic (Doganlar et al., 2002; Portis et al., 2014; Miyatake et al., 2016; Mangino et al., 2020), fruit development (Miyatake et al., 2012), morphological (Doganlar et al., 2002; Frary et al., 2014; Toppino et al., 2016; Mangino et al., 2020, 2021; Villanueva et al., 2021) and quality traits (Gramazio et al., 2014; Docimo et al., 2016; Toppino et al., 2016; Barchi et al., 2019; Villanueva et al., 2021; Rosa-Martínez et al., 2022), have been identified in eggplant in several studies using these tools.

Among eggplant CWRs (Knapp et al., 2013), *Solanum incanum* L. is a cross-compatible CWR of eggplant from desertic and semi-desertic areas in East Africa and the Middle East (Knapp and Vorontsova, 2016), which has tolerance to drought and resistance to biotic stresses such as *Fusarium* and bacterial wilts (Knapp et al., 2013; Mishra et al., 2021). Given that it naturally grows in areas with poor soils, we hypothesize that it could also be a source of variation for resilience to low N inputs. In addition, it has been reported to exhibit a high content of bioactive phenolics that are of interest for human health (Prohens et al., 2013; Kaur et al., 2014). A set of ILs carrying fragments of the *S. incanum* genome in an *S. melongena* cultivated genetic background has been developed recently (Gramazio et al., 2017). This IL collection has already been proven to be of interest in eggplant breeding for important agronomic and fruit morphology traits (Mangino et al., 2020, 2021). In addition, it has been characterized in terms of fruit quality in a recent study (Rosa-Martínez et al., 2022), which demonstrated the safety for consumption of their fruits, since they accumulated levels of glycoalkaloids below the internationally accepted limit (200 mg · kg⁻¹ of fresh weight) (OECD, 2020).

In the present work, a set of eggplant lines with introgressions of *S. incanum* (Gramazio et al., 2017), along with their *S. melongena* recipient parent (AN-S-26), were evaluated for morpho-agronomic and composition traits under two N fertigation conditions. The aim was to provide insight into the performance of the ILs to identify potential materials for eggplant breeding under low N inputs. In addition, the combination of phenotyping data with the available genotyping (Gramazio et al., 2017) of the ILs allowed the detection of QTLs associated with traits of interest for eggplant breeding.

2. Materials and methods

2.1. Plant material

A set of ten introgression lines (ILs) of *S. melongena* (AN-S-26) with introgressed fragments of the wild relative *S. incanum* (MM577) were used for the present study (Gramazio et al., 2017). Each IL carried a single fragment of one donor chromosome, corresponding to chr. 1 to 5, 7 to 10, and 12. Altogether, the set of ILs covered 64.6% of the *S. incanum* genome, with introgressions ranging from 1.9% for chr. 10–90.1% for chr. 7 (Fig. 1). Detailed information about the ILs population and the corresponding parents can be found in Gramazio et al. (2017) and Mangino et al. (2020, 2021).

2.2. Cultivation conditions

Plants were grown in an open-air field located in the campus of Universitat Politècnica de València (latitude, 39°28'55"N; longitude, 0°20'11"W; 7 m a.s.l.) during the late summer season of 2019 (August 21st to November 28th). Within the field, the plants were distributed in two plots located 3 m apart, each submitted to different nitrogen (N) fertigation dosages provided with a drip irrigation system.

Physicochemical analysis of the soil was performed before cultivation following the same procedure described in Rosa-Martínez et al. (2021). Intake water was also analyzed. Based on soil and water analyses and the nutrient requirements for

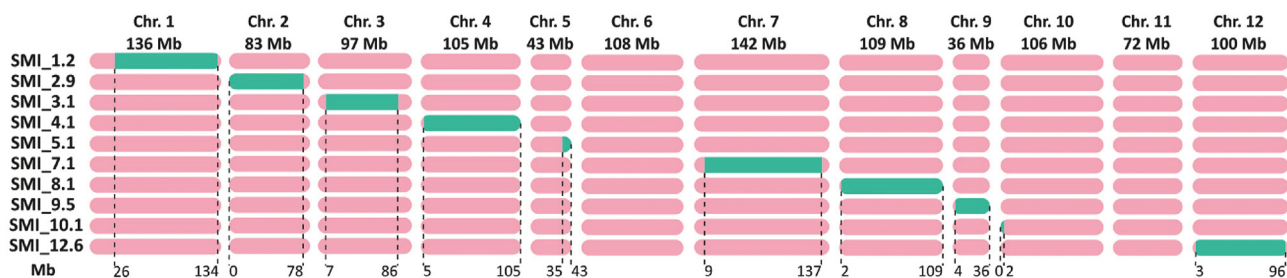


Fig. 1 Graphical genotypes of the eggplant introgression lines (ILs) with *S. incanum* introgressions evaluated in the present work
Each row corresponds to the genotype of each IL. The first column indicates the IL codes and the top row indicates the eggplant chromosomes and respective length (Mb). Homozygous introgressions of *S. incanum* (MM577) are colored in green, while the genetic background of *S. melongena* (AN-S-26) is colored in pink. The start and end position of the introgressed fragment for each chromosome is displayed at the bottom of the figure.

eggplant cultivation (Baixauli and Aguilar, 2002), one plot was submitted to a fertigation solution of $\text{mmol} \cdot \text{L}^{-1} \text{NH}_4^+$ and $8.25 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$ in the form of ammonium nitrate (Antonio Tarazona S.L., Valencia, Spain) plus $2.3 \text{ mmol} \cdot \text{L}^{-1} \text{K}_2\text{SO}_4$ (Antonio Tarazona S.L., Valencia, Spain), which were added to the irrigation system. This was considered as the high N treatment (HN) in the present work. The other plot was submitted to a N-reduced fertigation solution prepared by adding $2.3 \text{ mmol} \cdot \text{L}^{-1} \text{K}_2\text{SO}_4$ to the irrigation system, thus without providing any N other than that already supplied by the intake water (Table 1). This was considered as the low N treatment (LN). In addition, both HN and LN solutions were supplemented with $0.025 \text{ L} \cdot \text{m}^{-3}$ of a microelements Welgro Hydroponic fertilizer mix (Química Massó S.A., Barcelona, Spain), which contained copper (Cu-EDTA; 0.17% p/v), iron (Fe-DTPA; 3.00% p/v), boron (BO_3^{3-} ; 0.65% p/v), manganese (Mn-EDTA; 1.87% p/v), zinc (Zn-EDTA; 1.25% p/v), and molybdenum (MoO_4^{2-} ; 0.15% p/v). The pH of both irrigation solutions was finally adjusted to 5.5–5.8 with 20% HCl. Another chemical analysis of the irrigation water flowing out of the drip irrigation emitters was performed for each of the two N treatments after the fertigation solutions were prepared. The compositional characteristics of the intake water and the final HN and LN treatment fertigation solutions are displayed in Table 1. The intake water was slightly basic, had low content of nitrates, ammonium, phosphates, carbonates, and potassium; moderate content of sulphates and magnesium; and high content of calcium (Table 1). After preparing the fertigation solutions, in addition to the expected difference in nitrate and ammonium content between the HN and LN solutions, the electrical conductivity increased, mainly for HN, as well as the content of sulphates, magnesium and potassium, while bicarbonates were reduced (Table 1). Another soil physicochemical analysis was carried out after the cultivation period in each of the two field plots (HN and LN) separately.

Five plants per IL, along with 15 plants of the recipient parent *S. melongena* AN-S-26, were distributed in each plot following a completely randomized design. Each plant was considered a replicate. The plants in each plot were separated 1.5 m and 0.7 m between and within rows, respectively. Similar crop management practices were applied to both field plots, which included no pruning, manual weeding and phytosanitary treatments against whiteflies and spider mites when necessary. For the drip

Table 1 Irrigation water chemical composition before (intake water) and after preparing the fertilizer solution for each nitrogen (N) treatment (high N treatment –HN solution– and low N treatment –LN solution–)

Water characteristics	Intake water	HN solution	LN solution
pH	7.5	6.2	6.1
Electrical conductivity ($\text{mS} \cdot \text{cm}^{-1}$)	1.6	2.8	1.9
Nitrates ($\text{mmol} \cdot \text{L}^{-1}$)	0.6	10.0	0.8
Ammonium ($\text{mmol} \cdot \text{L}^{-1}$)	0.1	0.2	0.1
Phosphates ($\text{mmol} \cdot \text{L}^{-1}$)	0	0	0
Sulphates ($\text{mmol} \cdot \text{L}^{-1}$)	3.7	6.3	6.6
Carbonates ($\text{mmol} \cdot \text{L}^{-1}$)	0	0	0
Bicarbonates ($\text{mmol} \cdot \text{L}^{-1}$)	6.0	0.8	1.4
Ca^{2+} ($\text{mmol} \cdot \text{L}^{-1}$)	5.4	5.4	5.3
Mg^{2+} ($\text{mmol} \cdot \text{L}^{-1}$)	1.9	2.1	2.6
K^+ ($\text{mmol} \cdot \text{L}^{-1}$)	0.2	3.9	3.8

irrigation, the same irrigation timings were applied in both HN and LN field plots during the entire cultivation period, thus totaling 57.5 L of fertigation solution supplied per plant. Considering this, a total amount of $13.81 \text{ g N} \cdot \text{plant}^{-1}$ and $0.53 \text{ g N} \cdot \text{plant}^{-1}$ was supplied with the irrigation to the HN and the LN field plots, respectively.

2.3. Morpho-agronomic traits evaluated

A total of 13 agronomic and fruit morphology-related traits were evaluated for both high and low N treatments (Table 2). Regarding agronomic traits, the SPAD value was measured per plant using a chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) as the mean of ten readings distributed in five fully expanded leaves. The SPAD measures were taken on the same day for all the plants, 38 days after transplant (September 27th, 2019), during the plant vegetative growth period and fruits were harvested when commercially ripe and weighed. At the end of the cultivation (November 28th, 2019), the maximum height and the stem diameter of each plant were measured using a digital caliper. Subsequently, they were cut at ground level, and immediately, the plant aerial biomass was weighed using a Sauter FK-250 dynamometer (Sauter, Balingen, Germany). Yield was

Table 2 Description of agronomic, fruit and composition traits evaluated in the set of introgression lines (ILs), abbreviations used in tables and figures, and units in which they are expressed in the present work

Trait	Abbreviation	Units
Morpho-agronomic traits		
SPAD	SPAD	–
Plant height	P_Height	Cm
Aerial biomass	P_Biomass	kg fw
Stem diameter	P_Diam	mm
Early yield	E_yield	g fw · plant ⁻¹
Yield	Yield	g fw · plant ⁻¹
Nitrogen Use Efficiency		
Nitrogen Use Efficiency	NUE	–
Total number of fruits per plant	F_Number	–
Fruit mean weight	F_Weight	g fw
Fruit pedicel length	F_PedLength	Mm
Fruit calyx length	F_CaLength	Mm
Fruit length	F_Length	Mm
Fruit width	F_Width	Mm
Composition traits		
Fruit dry matter	F_dm	%
Nitrogen content in leaf	N_Leaf	g · kg ⁻¹ dm
Carbon content in leaf	C_Leaf	g · kg ⁻¹ dm
Nitrogen content in fruit	N_Fruit	g · kg ⁻¹ dm
Carbon content in fruit	C_Fruit	g · kg ⁻¹ dm
Total phenolics content	TPC	g · kg ⁻¹ dm
Chlorogenic acid content	CGA	g · kg ⁻¹ dm

Note: fw, fresh weight; dm, dry matter.

calculated as the total fruit biomass produced per plant, while early yield was calculated as the fruit biomass produced per plant on October 7th, 2019. Nitrogen Use Efficiency (NUE) was calculated per plant as the ratio between fruit yield, expressed on a dry weight basis, and the amount of N supplied for per plant (Mauceri et al., 2019). In addition, the total number of fruits per plant was counted and the fruit mean weight was calculated as the ratio between yield and fruit number. Regarding fruit morphological traits, the pedicel length, calyx length, fruit length and fruit width were measured in three representative commercially mature fruits per plant and the mean was calculated for each trait. Units in which the traits analyzed are expressed in the present work and the abbreviations used in tables and figures are shown in Table 2.

2.4. Leaf and fruit processing and composition analyses

At least five fully expanded leaves per plant were collected on November 7th, 2019, frozen in liquid nitrogen and stored at -80°C for subsequent freeze-drying in a VirTis Genesis lyophilizer (SP Scientific, Warminster, Pennsylvania, USA). In addition, during the harvest period, at least three commercially mature fruits per plant were harvested, cleaned, peeled, and cut into pieces, which were subjected to the same freezing and freeze-drying steps as the leaves. Subsequently, freeze-dried leaves and fruits were homogenized using a domestic grinder. Fruit dry matter was calculated as $100 \times (\text{freeze-dried weight}/\text{fresh weight})$.

Total carbon (C) and nitrogen (N) content was determined in fruit and leaf using 0.5 g of the homogenized materials in a TruSpec CN elemental analyzer (Leco, Michigan, USA). The analysis was based on complete combustion of the sample at 950°C in the presence of oxygen. C and N were measured as CO_2

and N_2 gases, respectively. An infrared detector was used for C determination, while N was detected in a thermal conductivity cell (Gazulla et al., 2012).

Total phenolics content was determined in fruit by spectrophotometry using 0.125 g of homogenized fruit material according to the Folin-Ciocalteu method (Singleton and Rossi, 1965), while chlorogenic acid content was evaluated in 0.1 g of homogenized fruit material by high-performance liquid chromatography (HPLC) using a 1220 Infinity LC System (Agilent 196 Technologies, CA, USA) equipped with a binary pump, an automatic injector and a UV detector. The extraction and analysis procedure followed for each trait were described in detail in Plazas et al. (2014). Units in which the traits analyzed were expressed in the present work and the abbreviations used in tables and figures were shown in Table 2.

2.5. Data analysis

Physicochemical data of the soil before cultivation, after cultivation in the HN field plot, and after cultivation in the LN field plot were subjected to a one-factor analysis of variance (ANOVA) and to a post-hoc Student-Newman-Keuls multiple range test to evaluate significant differences among the three conditions for each trait ($P < 0.05$).

Regarding the morpho-agronomic and compositional data of the set of selected ILs and the recipient parent (*S. melongena* AN-S-26), a bifactorial ANOVA was performed for every trait evaluated in order to assess the effect of genotype (G), N treatment (N) and $G \times N$ interaction (Gomez and Gomez, 1984). The mean value and its standard error (SE) were calculated for every trait analyzed from the genotypes means for each N treatment. The mean of each genotype was calculated as the mean of the data from the five and 15 replicates used for each IL and the recurrent parent, respectively, in each N treatment. In addition, the paired difference between the average value of each trait under HN and LN was calculated only for the traits which showed a significant effect of the N treatment.

Mean values per genotype of each of the traits evaluated under each of the N treatments were subjected to a principal component analysis (PCA) using the package stats (R Core Team, 2016) of the R software (R Core Team, 2013). Pairwise Euclidean distances were calculated for the analysis among genotypes in each N treatment using standardized data ($\mu = 0$; $\sigma = 1$) for each trait. The aim was to globally evaluate the variation of the ILs compared to the recipient parent based on the traits evaluated. PCA score plot was drawn using R package ggplot2 (Wickham, 2016). In addition, Pearson's correlation coefficients were calculated between means of all traits and their significance was evaluated at $P < 0.01$, using the Statgraphics Centurion XVIII software (StatPoint Technologies, Warrenton, Virginia, USA).

2.6. QTL detection and candidate gene identification

Detection of significant QTLs was carried out in both N treatments separately for all traits by means of a Dunnett's test at $P < 0.05$, which compares the mean value of each IL with the mean value of the recipient parent AN-S-26. The R package multcomp (Hothorn et al., 2021) was used for the analysis. For

Table 3 Mean values and standard error (SE) of the soil chemical composition before transplant and after the cultivation period in each nitrogen (N) treatment separately

Soil characteristics	Before cultivation Mean ± SE	After the cultivation period	
		HN	LN
		Mean ± SE	Mean ± SE
pH in water	8.1 ± 0.1	8.3 ± 0.1	8.2 ± 0.0
pH in KCl	7.6 ± 0.0a	7.9 ± 0.0b	7.9 ± 0.1b
Electrical conductivity (µS · cm ⁻¹)	213.2 ± 26.7a	332.2 ± 45.1b	283.8 ± 13.7ab
Total N (g · kg ⁻¹ dm)	0.7 ± 0.0b	0.6 ± 0.0a	0.6 ± 0.0b
Carbonates (g · kg ⁻¹ dm)	271.6 ± 12.5	274.4 ± 2.1	279.0 ± 2.5
Organic matter (g · kg ⁻¹ dm)	14.6 ± 2.2	13.7 ± 0.4	12.6 ± 0.2
C:N ratio	12.5 ± 1.9	14.4 ± 0.7	11.7 ± 0.3
P (mg · kg ⁻¹ dm)	121.1 ± 10.1	113.6 ± 4.6	122.4 ± 4.9
K (mg · kg ⁻¹ dm)	176.1 ± 12.8b	113.9 ± 12.8a	112.5 ± 7.5a
Ca ²⁺ (cmol · kg ⁻¹ dm)	159.6 ± 2.1a	242.1 ± 8.4b	239.4 ± 4.7b
Mg ²⁺ (cmol · kg ⁻¹ dm)	4.8 ± 0.1a	5.8 ± 0.2b	5.6 ± 0.1b
Fe (mg · kg ⁻¹ dm)	5.3 ± 0.1c	3.5 ± 0.1a	3.9 ± 0.2b
Zn (mg · kg ⁻¹ dm)	9.0 ± 0.5	8.7 ± 0.1	9.4 ± 0.2
Cu (mg · kg ⁻¹ dm)	5.9 ± 0.1b	4.8 ± 0.3a	5.2 ± 0.2a

Note: dm: dry matter; For each trait, means with different letters are significantly different at $P < 0.05$ according to the Student-Newman-Keuls.

each putative QTL detected, the relative increase over AN-S-26 and the allelic effect were calculated.

In order to identify potential candidate genes within each QTL region, a search throughout the '67/3' eggplant reference genome assembly (V3 version) (Barchi et al., 2019) was conducted, using

the Sol Genomics Network database (<http://www.solgenomics.net>). In addition, syntenic regions of the tomato genome were accessed to identify candidate genes co-localizing with the eggplant QTLs, using the comparative map viewer tool of the Sol Genomics Network database (https://maps.solgenomics.net/Map/view_comp).

3. Results

3.1. Soil characteristics

Soil texture for both fields was sandy, with a composition of 86% sand, 11% clay, and 3% silt (Soil Science Division Staff, 2017). The analysis of variance of the soil characteristics revealed no significant differences among the three soil stages (before cultivation, after cultivation in the HN field plot and after cultivation in the LN field plot) for pH in water, C:N ratio and content of carbonates, organic matter, phosphorus, and zinc (Table 3). Significant differences were found between soils before and after cultivation for pH in KCl and in calcium, magnesium, potassium, and copper content. For the first three traits (pH in KCl, calcium and magnesium content), the soil after cultivation for both HN and LN showed higher values by 1.04-fold, 1.5-fold, and 1.2-fold, respectively. Contrarily, content of potassium and copper was significantly higher in the soil before cultivation by 1.5-fold and 1.2-fold. The electrical conductivity increased after the cultivation period in both HN and LN, although it was only significant in the HN plot soil (1.6-fold increase). A slightly but significantly lower average N content ($0.56 \text{ g} \cdot \text{kg}^{-1} \text{ dm}$) was found in the HN plot soil after cultivation compared to both the LN plot soil after cultivation ($0.63 \text{ g} \cdot \text{kg}^{-1} \text{ dm}$) and the field soil before cultivation ($0.69 \text{ g} \cdot \text{kg}^{-1} \text{ dm}$). Lastly, average Fe content decreased significantly in soil after the cultivation period in both HN and LN, the

Table 4 F-ratio values for genotype (G), nitrogen treatment (N), and genotype per N treatment interaction (G × N) of each trait evaluated in the present study, obtained from the bifactorial ANOVA

Trait	Genotype (G)	N treatment (N)	G × N	Mean ± SE HN	Mean ± SE LN	Paired HN-LN ^a
Morpho-agronomic traits						
SPAD	2.3*	51.7***	0.9 ^{ns}	55.9 ± 0.7	51.1 ± 0.6	4.8
P_Height (cm)	5.5***	19.5***	0.6 ^{ns}	74.4 ± 2.4	64.7 ± 2.6	9.7
P_Biomass (kg fw)	4.1***	25.1***	0.3 ^{ns}	0.5 ± 0.2	0.4 ± 0.0	0.2
P_Diam (mm)	2.7**	1.6 ^{ns}	0.4 ^{ns}	16.5 ± 0.5	15.8 ± 0.6	
E_yield (g fw · plant ⁻¹)	4.7***	5.0*	0.6 ^{ns}	376.2 ± 42.4	299.0 ± 39.2	77.3
Yield (g fw · plant ⁻¹)	4.9***	3.5 ^{ns}	0.9 ^{ns}	1 007.4 ± 90.3	894.4 ± 91.3	
NUE	2.2*	237.5***	1.9*	6.2 ± 0.6	137.3 ± 12.5	-131.1
F_Number	2.8**	5.3*	1.1 ^{ns}	13.3 ± 0.9	11.1 ± 1.0	2.2
F_Weight (g fw)	5.2***	0.5 ^{ns}	0.8 ^{ns}	75.4 ± 3.2	78.0 ± 5.6	
F_PedLength (mm)	8.5***	0.1 ^{ns}	1.5 ^{ns}	53.0 ± 1.9	53.4 ± 2.5	
F_CaLength (mm)	3.2**	0 ^{ns}	1.0 ^{ns}	51.3 ± 1.4	51.5 ± 1.7	
F_Length (mm)	4.2***	0.1 ^{ns}	1.0 ^{ns}	77.1 ± 2.3	77.7 ± 2.6	
F_Width (mm)	5.7***	2.3 ^{ns}	1.1 ^{ns}	54.5 ± 1.7	56.9 ± 2.0	
F_dm (%)	8.5***	5.8*	1.2 ^{ns}	8.7 ± 0.2	8.2 ± 0.3	0.4
Composition traits						
N_Leaf (g · kg ⁻¹ dm)	3.0**	48.4***	1.5 ^{ns}	55.0 ± 0.6	49.4 ± 1.0	5.6
C_Leaf (g · kg ⁻¹ dm)	3.9***	7.0**	3.8***	433.6 ± 1.9	429.0 ± 2.8	4.6
N_Fruit (g · kg ⁻¹ dm)	4.5***	21.4***	1.0 ^{ns}	31.9 ± 0.7	28.5 ± 0.9	3.5
C_Fruit (g · kg ⁻¹ dm)	1.0 ^{ns}	0.1 ^{ns}	0.9 ^{ns}	420.2 ± 1.3	420.4 ± 0.8	
TPC (g · kg ⁻¹ dm)	5.3***	0.4 ^{ns}	1.1 ^{ns}	24.5 ± 1.2	23.9 ± 1.1	
CGA (g · kg ⁻¹ dm)	7.9***	0.1 ^{ns}	1.8 ^{ns}	16.2 ± 1.0	16.0 ± 1.2	

Note: ^aCalculated only for traits with significant difference between HN and LN; fw: fresh weight; dm: dry matter. Mean values ± standard error (SE) for each trait and N treatment are also shown. Only for the traits with a significant effect of the N treatment, the paired difference of HN (high N treatment)–LN (low N treatment) was calculated, based on the genotype means. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.



Fig. 2 Characteristics of fruits from *Solanum melongena* (AN-S-26) and introgression lines of the latter with *S. incanum* (MM577). Fruits of the *S. incanum* donor parent are also included.

reduction being more pronounced in the case of HN plot soil (1.6-fold decrease).

3.2. Characterization of the ILs and the recipient parent under high and low N treatments

According to the bi-factorial ANOVA performed for the set of ten ILs plus the recipient parent, significant differences among genotypes were found for all traits evaluated except for C content in fruit (C_Fruit) (Table 4). Representative fruits produced by each IL and the recipient parent AN-S-26 are in Fig. 2. In addition, the ANOVA revealed a significant effect of the N treatment over ten out of the twenty traits evaluated (Table 4). Except for NUE, the other nine traits presented higher values under high N treatment (HN). In this way, the agronomic and vegetative traits SPAD, plant height (P_Height), plant biomass (P_Biomass), early yield (E_yield), and fruit number (F_Number) were reduced by 8.6%,

13.0%, 33.3%, 20.5%, and 16.5%, respectively, under the low N treatment (LN). Furthermore, the composition traits fruit dry matter (F_dm), N content in leaf (N_Leaf), C content in leaf (C_Leaf), and N content in fruit (N_Fruit) were also reduced under LN by 4.7%, 10.2%, 1.1%, and 10.7%, respectively (Table 4). Among the traits that showed significant differences between N treatments, the F-ratio of the N treatment factor was higher than the genotype factor in all cases except for F_dm (Table 4). Only NUE and C_Leaf showed a significant genotype \times N treatment interaction, although the F-ratio of this factor was lower than the F-ratio of the genotype or N treatment effects.

3.3. Multivariate principal components analysis

The two first components (PCs) of the principal components analysis (PCA) for all the traits evaluated accounted for 37.2% and 18.6% of the variation. However, as NUE was highly correlated

Table 5 Correlation coefficients between all traits evaluated except nitrogen use efficiency (NUE) and the two first principal components (PC1 and PC2) of the PCA

Traits	PC1	PC2
Morpho-agronomic traits		
SPAD	-0.0818	0.1769
P_Height	-0.1991	0.4172
P_Biomass	-0.2894	0.0566
P_Diam	-0.3236	0.0276
E_yield	-0.2887	0.0574
Yield	-0.3384	0.0271
F_Number	-0.2786	0.1510
F_Weight	-0.2739	-0.1904
F_PedLength	-0.2881	0.0225
F_CaLength	-0.2518	0.0656
F_Length	-0.3004	-0.0915
F_Width	-0.1989	-0.2510
Composition traits		
F_dm	0.2482	0.0029
N_Leaf	-0.2031	0.0545
C_Leaf	-0.0897	-0.1544
N_Fruit	-0.0361	0.4599
C_Fruit	0.0781	0.0822
TPC	0.1020	0.4467
CGA	0.0994	0.4548

Note: Those correlations with absolute values ≥ 0.2 are highlighted in bold.

(0.459) with the second component and basically separated the HN and LN treatments (Fig. S1), a new PCA was performed excluding NUE. In this new PCA excluding NUE, the first two components accounted for 56.0% of the total variation observed, with PC1 and PC2 accounting for 39.2% and 16.8% of the variation, respectively. Among the traits with the highest correlation coefficient (≥ 0.2), those related to yield and fruit morphology, except for SPAD, P_Height, and F_Width, were negatively correlated to

PC1, while fruit dry matter (F_dm) was positively correlated to the same component (Table 5). On the other hand, the composition traits N in fruit (N_Fruit), total phenolics (TPC), and chlorogenic acid (CGA), as well as P_Height, were highly positively correlated to PC2, whereas F_Width was negatively correlated to PC2.

The distribution of the genotypes in the PCA score plot showed a trend towards higher PC1 and lower PC2 values for genotypes subjected to the low N treatment compared to those of the high N treatment (Fig. 3). This change was associated with a greater dry matter of the fruit, C content in leaf, and fruit width and weight; but lower yields, fruit number, plant height and biomass, stem diameter, and N content in fruit. The exception to this trend was the IL SMI_12.6, which for the LN treatment had PC1 values slightly lesser than those of the HN treatment (Fig. 3). The most distant ILs from the recipient parent in the score plot, and therefore most different in terms of the traits represented in the analysis, were SMI_2.9 and SMI_12.6 in the case of HN; and SMI_2.9, SMI_3.1, and SMI_4.1 for LN (Fig. 3). Some of the ILs performed similarly under both N treatments, as they appear in close proximity according to PC1 and PC2. This is the case for SMI_5.1, SMI_12.6, and SMI_9.5 (Fig. 3).

3.4. Analysis of correlations

Significant correlations between traits at $P < 0.01$ were found for 16 out of the 20 traits evaluated considering the HN and the LN treatments (Table 6). High positive ($r > 0.70$) correlations of similar values were found among most agronomic traits, as well as between total phenolics (TPC) and chlorogenic acid content (CGA), for both LN and HN treatments (Table 6). Among these, the correlation coefficient I between NUE and yield was the highest (0.98 for HN and 0.97 for LN). In addition, yield and NUE

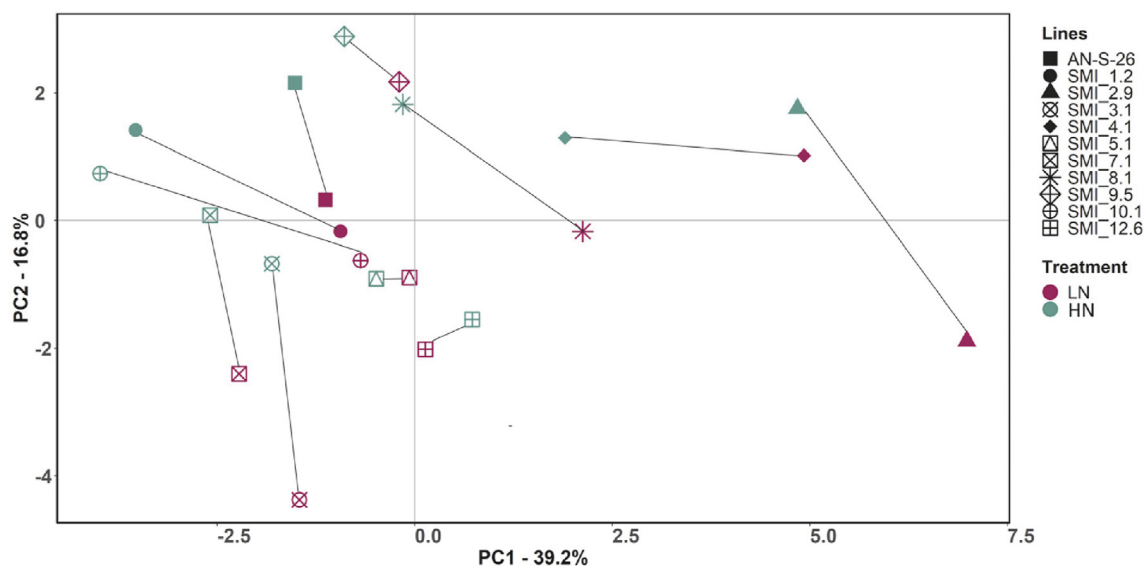


Fig. 3 PCA score plot based on the two principal components (PC1 and PC2) of the analysis performed for all traits except nitrogen use efficiency (NUE)

PC1 and PC2 accounted for 39.2% and 16.8% of the total variation, respectively. The introgression lines (SMI_codes) and the *S. melongena* recipient parent (AN-S-26) are represented by different symbol and different color according to the nitrogen (N) treatment in which they were grown, as displayed in the figure legend (HN for the high N treatment, and LN for the low N treatment). Black lines connect the same genotype under each of the two N treatments.

Table 6 Pearson linear correlation coefficients (r) between accession mean values for all traits evaluated in the present work

	SPAD	P_Height	P_Biomass	P_Diam	E_yield	Yield	NUE	F_Number	F_Weight	F_PedLength	F_CaLength	F_Length	F_Width	F_dm	N-Leaf	C-Leaf	N-Fruit	C-Fruit	TPC	CGA
SPAD	1.00	-0.24	0.04	-0.23	0.18	-0.06	-0.04	-0.20	0.09	0.24	0.31	0.45	0.18	0.43	0.16	-0.02	0.10	-0.48	-0.41	-0.25
P_Height	-0.28	1.00	0.15	0.51	0.48	0.46	0.32	0.37	0.41	0.62	0.41	0.22	-0.09	-0.72	0.28	0.26	0.70	0.01	0.38	0.45
P_Biomass	0.32	0.26	1.00	0.75	0.57	0.79	0.75	0.73	0.69	0.65	0.57	0.70	0.61	-0.60	0.80	0.36	-0.25	0.23	-0.43	-0.51
P_Diam	0.21	0.42	0.89	1.00	0.70	0.75	0.68	0.76	0.58	0.65	0.47	0.60	0.30	-0.79	0.78	0.67	0.07	0.04	-0.33	-0.19
E_yield	-0.02	0.39	0.48	0.69	1.00	0.83	0.81	0.64	0.77	0.79	0.51	0.73	0.47	-0.42	0.42	0.63	0.20	-0.19	-0.44	-0.30
Yield	-0.04	0.45	0.74	0.84	0.86	1.00	0.98	0.90	0.75	0.77	0.67	0.75	0.43	-0.56	0.54	0.44	0.01	-0.02	-0.23	-0.26
NUE	-0.07	0.36	0.72	0.79	0.89	0.97	1.00	0.91	0.69	0.66	0.67	0.77	0.47	-0.40	0.43	0.45	-0.13	-0.04	-0.29	-0.31
F_Number	-0.09	0.55	0.47	0.55	0.80	0.86	0.85	1.00	0.43	0.50	0.62	0.73	0.24	-0.53	0.52	0.42	-0.19	0.06	-0.20	-0.21
F_Weight	0.07	0.19	0.71	0.82	0.49	0.67	0.56	0.24	1.00	0.85	0.52	0.46	0.68	-0.50	0.44	0.45	0.17	0.09	-0.25	-0.29
F_PedLength	-0.10	0.55	0.46	0.57	0.49	0.71	0.52	0.55	0.70	1.00	0.62	0.65	0.44	-0.62	0.62	0.31	0.50	-0.17	-0.11	-0.06
F_CaLength	-0.56	0.67	0.48	0.55	0.41	0.68	0.63	0.56	0.45	0.60	1.00	0.73	0.35	-0.25	0.48	0.43	0.04	-0.08	-0.07	0.07
F_Length	-0.21	0.48	0.60	0.65	0.56	0.79	0.68	0.64	0.64	0.74	0.74	1.00	0.38	-0.26	0.63	0.34	-0.03	-0.29	-0.49	-0.36
F_Width	-0.15	0.24	0.65	0.73	0.18	0.46	0.37	0.07	0.83	0.43	0.56	0.56	1.00	-0.09	0.17	0.26	-0.17	0.43	-0.28	-0.31
F_dm	0.06	-0.64	-0.65	-0.69	-0.39	-0.71	-0.54	-0.60	-0.68	-0.85	-0.69	-0.88	-0.63	1.00	-0.68	-0.25	-0.34	-0.26	-0.10	-0.03
N-Leaf	0.23	-0.21	0.54	0.50	-0.06	0.24	0.18	0.00	0.52	0.10	0.11	0.32	0.74	-0.40	1.00	0.36	0.03	-0.12	-0.42	-0.34
C-Leaf	-0.09	-0.39	-0.04	-0.12	-0.42	-0.26	-0.29	-0.30	0.06	-0.23	-0.21	-0.08	0.39	-0.02	0.74	1.00	-0.16	-0.01	-0.48	-0.17
N-Fruit	0.42	0.43	-0.05	-0.07	-0.31	-0.24	-0.31	0.03	-0.34	-0.04	-0.12	-0.18	-0.23	-0.14	-0.06	-0.12	1.00	-0.28	0.49	0.61
C-Fruit	0.26	-0.12	-0.23	-0.15	-0.12	-0.36	-0.25	-0.40	-0.17	-0.42	-0.32	-0.55	-0.27	0.54	-0.40	-0.45	0.21	1.00	0.30	0.07
TPC	-0.05	0.54	-0.15	-0.08	-0.15	-0.21	-0.17	-0.10	-0.34	-0.19	0.19	-0.23	-0.19	0.10	-0.44	-0.48	0.65	0.63	1.00	0.87
CGA	-0.22	0.53	-0.40	-0.23	-0.03	-0.14	-0.12	0.12	-0.46	-0.06	0.22	-0.16	-0.42	0.12	-0.64	-0.61	0.54	0.48	0.86	1.00

Note: Correlations under the high N treatment (HN) are shown above the diagonal; correlations under the low N treatment (LN) below the diagonal. Only significant correlations at $P < 0.01$ are displayed. The color scale from red to green represents the scale of r values from 1.0 (the highest positive correlation) to -1.0 (the highest negative correlation).

were positively correlated with early yield (E_yield) and the total number of fruits (F_Number) under both N treatments, as well as yield with traits related to plant and fruit sizes (P_Biomass, P_Diam and F_Length) (Table 6). More significant positive correlations were found for each N treatment separately. Plant size-related traits (P_Biomass and P_Diam) were correlated with N content in leaf (N_Leaf) under the HN treatment. For the same HN treatment, yield-related traits (E_yield and Yield) were correlated to fruit weight (F_Weight) and fruit pedicel length (F_PedLength) (Table 6). NUE was correlated with P_Biomass and F_Length only under HN, while it was correlated with P_Diam only under the LN treatment (Table 6). Furthermore, correlations with high positive r were found among traits related to fruit size for LN (i.e., F_Weight with F_Width or F_Length with F_PedLength and F_CaLength). Additionally, N and C content in leaf (N_Leaf, C_Leaf) were correlated to each other (Table 6). Finally, only three highly negative (< -0.75) correlations were found among traits, which corresponded to fruit dry matter (F_dm) with P_Diam under the HN treatment, and F_dm with F_PedLength and F_Length under the LN treatment (Table 6).

3.5. Detection of putative QTLs

The results of the Dunnett's test for the comparison of each IL with the *S. melongena* parent (AN-S-26) allowed the detection of 19 putative QTLs for 10 traits evaluated under the high N (HN) treatment, as well as 24 putative QTLs for 15 traits under the low N (LN) treatment (Table 7).

Under the HN treatment, the total number of QTLs detected were scattered over six of the ten chromosomes represented in

the IL population. The highest number of QTLs under this N treatment was identified on chromosome (chr.) 12 (Table 7). In this way, one QTL for each of the following traits: plant height (P_Height), fruit pedicel length (F_PedLength), fruit N content (N_Fruit), total phenolics (TPC), and chlorogenic acid content (CGA) colocalized on the introgressed fragment of chr. 12 (3–96 Mb), all of them accounting for a significant decrease of the trait mean value compared to AN-S-26 (Table 7). Additionally, eight QTLs specific to the HN treatment were identified associated with both morpho-agronomic and composition traits. In this regard, QTLs were identified for P_Height on chr. 3 and chr. 5, for CGA on chr. 5 and chr. 7, and one QTL was identified for each of the following traits: early yield (E_yield) and F_PedLength, which colocalized on chr. 2; fruit length (F_Length) on chr. 10; N content in leaf (N_Leaf) on chr. 4; and C content in leaf (C_Leaf) on chr. 5 (Table 7). Except for the QTL for F_Length, the wild introgression had a reducing effect on the final phenotype compared to the recipient parent (Table 7).

On the other hand, the QTLs detected under LN were scattered over eight of the ten chromosomes represented in the IL population, with chr. 2 harboring the highest number of QTLs (Table 7). Ten QTLs identified for several traits related to plant growth (P_Height, P_Biom, P_Diam), yield (Yield), and fruit size and morphology (F_Weight, F_PedLength, F_CaLength, F_Length, F_Width) colocalized on chr. 2 (0–78 Mb) and the *S. incanum* alleles accounted for a considerable reduction of the trait mean value compared to AN-S-26 (Table 7). Furthermore, eight identified QTLs were specific to the LN treatment, which were mostly associated with fruit morphology and leaf composition. In this way, four QTLs were linked to F_Width and located on chr. 1, 4, 9

Table 7 List of putative QTLs detected in the set of ILS of *S. melongena* AN-S-26 with introgressions of *S. incanum* MMS77 under both high and low N treatments separately

Trait (units)	Chr.	High Nitrogen (HN)					Low Nitrogen (LN)				
		QTL	Position (Mb)	Increase over AN-S-26 (%)	Allelic effect (units)	P-value	QTL	Position (Mb)	Increase over AN-S-26 (%)	Allelic effect (units)	P-value
P_Height (cm)	2	ph2.HN	0–78	–21.77	–9.29	0.0302	ph2.LN	0–78	–34.95	–13.00	0.0020
	3	ph3.HN	7–86	–22.65	–9.67	0.0093					
	5	ph5.HN	35–43	–25.23	–10.77	0.0028					
	12	ph12.HN	3–96	–21.01	–8.97	0.0194					
P_Biomass (kg fw ^a)	2					pbio2.LN	0–78	–69.23	–0.14	0.0259	
P_Diam (mm)	2					pdiam2.LN	0–78	–36.42	–3.31	0.0036	
E_yield (g fw · plant ^{–1})	2	ey2.HN	0–78	–73.98	–158.33	0.0211					
Yield (g fw · plant ^{–1})	2					y2.LN	0–78	–73.67	–343.07	0.0240	
F_Weight (g fw)	2					fw2.LN	0–78	–47.49	–21.81	0.0032	
F_PedLength (mm)	2	fped2.HN	0–78	–22.82	–6.63	0.0179	fped2.LN	0–78	–23.08	–6.23	0.0144
	4	fped4.HN	5–106	–21.58	–6.27	0.0294	fped4.LN	5–106	–21.93	–5.92	0.0230
	9					fped9.LN	4–36	20.43	5.51	0.0419	
F_CaLength (mm)	2					fcac2.LN	0–78	–27.13	–7.49	0.0079	
	2					fl2.LN	0–78	–20.74	–8.23	0.0480	
	4					fl4.LN	5–706	–25.39	–10.07	0.0077	
F_Width (mm)	10	fl10.HN	0–2	25.82	9.44	0.0224					
	1					fwid1.LN	26–134	–19.95	–6.69	0.0141	
	2					fwid2.LN	0–78	–31.08	–10.42	<1e–04	
	4					fwid4.LN	5–106	–24.92	–8.36	0.0011	
	9					fwid9.LN	4–36	–24.90	–8.35	0.0011	
F_dm (%)	12					fwid12.LN	3–96	–17.32	–5.81	0.0476	
	2	fdm2.HN	0–78	26.03	1.04	0.0014	fdm2.LN	0–78	34.43	1.31	<1e–04
	4	fdm4.HN	5–106	24.91	1.00	0.0382	fdm4.LN	5–106	24.97	0.95	<1e–04
N_Leaf (g · kg ^{–1} dm ^b)	4	leafnit4.HN	5–106	–9.85	–2.73	0.0359					
C_Leaf (g · kg ^{–1} dm)	9					leafnit9.LN	4–36	–15.67	–4.15	0.0189	
	1					leafc1.LN	26–134	–3.94	–8.53	0.0053	
N_Fruit (g · kg ^{–1} dm)	5	leafc5.HN	35–43	–3.68	–8.10	0.0191					
	10					leafc10.LN	0–2	–3.81	–8.23	0.0078	
TPC (g · kg ^{–1} dm)	12	fnit12.HN	3–96	–18.36	–3.24	0.0156	fnit12.LN	3–96	–24.32	–3.81	0.0095
	3					tpc3.LN	7–86	–33.69	–4.66	0.0091	
	12	tpc12.HN	3–96	–38.21	–5.14	0.0018					
CGA (g · kg ^{–1} dm)	3	cga3.HN	7–86	–33.01	–3.36	0.0012	cga3.LN	7–86	–65.98	–6.28	<1e–04
	5	cga5.HN	35–43	–42.77	–4.35	<1e–04					
	7	cga7.HN	9–137	–34.83	–3.54	0.0006					
	12	cga12.HN	3–96	–43.33	–4.40	<1e–04					

Note: QTLs identified under both conditions are in bold font. ^afw: fresh weight; ^bdm: dry matter.

and 12, and one QTL was detected for each of the following traits: F_Length on chr. 4, F_PedLength and N_Leaf, collocated on chr. 9, and C_Leaf on chr. 10 (Table 7). Except for the QTL for F_PedLength, the wild introgression had a reducing effect on the final phenotype compared to the recipient parent (Table 7).

Seven identified QTLs for five traits (P_Height, F_PedLength, F_dm, N_Fruit and CGA) were consistent under both HN and LN treatments, having the same allelic effect. They were detected in four ILS carrying fragments of the *S. incanum* chr. 2, 3, 4, and 12 (Table 7). QTLs for plant height (P_Height) for each HN (*ph2.HN*) and LN (*ph2.LN*) were located on chr. 2. Two QTLs for each N treatment were identified for fruit pedicel length (F_PedLength) on chr. 2 (*fped2.HN*, *fped2.LN*) and 4 (*fped4.HN*, *fped4.LN*). On the same two chromosomes, two QTLs were detected for fruit dry matter (F_dm), both in HN (*fdm2.HN*, *fdm4.HN*) and LN (*fdm2.LN*, *fdm4.LN*). One QTL was identified for N content in fruit (N_Fruit) under both N treatments (*fnit12.HN*, *fnit12.LN*) and mapped on chr. 12. Lastly, one QTL was detected for chlorogenic acid content (CGA) and located on chr. 3 for HN (*cga3.HN*) and LN (*cga3.LN*) (Table 7). For all putative QTLs except F_dm, the introgressed

fragment of *S. incanum* accounted for a considerable reduction of each trait mean value compared to AN-S-26 (Table 7).

3.6. Identification of candidate genes

The search throughout the '67/3' eggplant reference genome assembly (V3 version) (Barchi et al., 2019) identified potential candidate genes that could be associated with some of the putative QTLs detected in our study. Regarding QTLs associated to yield and plant growth parameters, N and C content or fruit dry matter, candidate genes could only be identified in the smallest introgressed fragment of the set of ILS, on chr. 10, since the vast majority of introgressions were too large, thus harboring a massive number of genes. In this way, a gene (SMEL_010g336770.1.01) encoding for a protein similar to fructose-bisphosphate aldolase 3 from *Arabidopsis thaliana* (*AtFBA3*) and *SlFBA4* was mapped to the region of the detected QTL for C content in leaf, at the beginning of chr. 10. This might be a potential candidate gene since FBAs are key enzymes involved in glycolysis, gluconeogenesis, and the Calvin cycle (Cai et al., 2016). Furthermore, three candidate genes for QTLs associated with TPC and CGA were

detected. One candidate gene encoding 4-hydroxycinnamoyl-CoA ligase 2 (4CL2), located on chr. 3 at 80.8 Mb, was related to the stable QTL for CGA (*cga3.HN*, *cga3.LN*) and the QTL for TPC (*tpc3.LN*). Furthermore, another homologous 4CL gene (4CL5) and a phenylalanine ammonia-lyase (PAL)-encoding gene, located at 17.7 Mb and 134.8 Mb, respectively, on chr. 7, were putative candidates to be associated with the QTL for CGA identified under HN (*cga7.HN*). The search through the genome assembly failed to highlight candidate genes on the introgressed regions of chr. 5 and 12. However, genes encoding a PAL and cinnamate 4-hydroxylase (C4H) were also located at the beginning of chr. 5 in the '67/3' eggplant reference genome assembly (V3 version) (Barchi et al., 2019).

4. Discussion

4.1. Changes in soil with different N fertilization

Interactions occur among the different components of soil and inorganic fertilizers during cultivation that significantly affect soil properties and the nutrient absorption capacity of the plant. Soil analysis would be therefore an essential task when planning the most appropriate fertilization for a sustainable agriculture model, although this has usually not been taken into account (Soto et al., 2015).

A similar trend to that of our findings, towards decreasing available N in soil, was observed for banana cultivation under high N fertilization doses above a certain optimal level (Sun et al., 2020). The authors found the highest N rates significantly decreased soil enzymatic activity, which played a key role in assimilable N and P regeneration in soil. Long-term N fertilization supply can also affect soil micro-organisms and organic matter (SOM), which are the most important factors for soil fertility (Zeng et al., 2016). However, in our study, after a short-time cultivation period, no differences were found for SOM associated with the two fertilization solutions applied, which was in agreement with other studies (Nascente et al., 2013; Sun et al., 2020). In addition, according to the Spanish standard classifications (Yáñez Jiménez, 1989), the average SOM was low in our study. More studies regarding soil microbial activity would be necessary to confirm the effect of the N inputs. On the other hand, Sun et al. (2020) found that higher yields, achieved with higher N fertilization rates, led to a higher nutrient removal from soil by plants, thus reducing soil available nutrients. In this respect, our results showed higher yields under HN. Furthermore, plants accumulated significantly more N in leaves and fruits under HN than under LN. These results suggest increased N extraction from the soil by plants in the HN treatment, which may potentially be the main reason for the differences found for the N content in soil between the two N treatments.

The decrease in soil potassium, as well as the micronutrients iron and copper, observed after cultivation for both HN and LN were probably also associated with their removal from the soil by plants through root absorption and also by leaching. The significant increase of calcium and magnesium in soil after cultivation may be due to the high concentration of these cations in the irrigation intake water. The slight increase in soil pH with fertilization may have also influenced it, insofar as a sandy, alkaline soil favours phosphorus precipitation in the form of insoluble

phosphates by binding cations such as calcium and magnesium (Miller et al., 1970).

In brief, a combination of conditions, including the interaction of N fertilization rate with soil enzymatic activity, plant yield and nutrient uptake, the rapid leaching nature of nitrates in soil (Wang and Li, 2019), and sandy soil texture which is weak in nutrient and water retention (Tracy et al., 2013), might explain our results.

4.2. Effect of N treatment on the IIs and AN-S-26 and potential materials for breeding

The significant average reduction in plant growth (plant height and aerial biomass), chlorophyll content (SPAD), and N content in leaves and fruits observed under the LN treatment in this study is in agreement with Mauceri et al. (2019), which evaluated chlorophyll content index and N content in fruits and leaves in several eggplant accessions with different origins. To our knowledge, no other studies evaluating plant growth, yield parameters, fruit morphology, and antioxidant compounds in eggplant as a response to different N fertilization doses has been reported. However, our results were also congruent with studies in other species (Liu and Wiatrak, 2011; Han et al., 2014; Agegnehu et al., 2016; Truffault et al., 2019; Si et al., 2020). In this way, N is a major component of chlorophyll, as well as proteins and nucleic acids, thus it is the main macronutrient on which plant growth and development depend. The reduction of chlorophyll content in leaves, the photosynthesis rate, leaf area, plant height, or biomass allocation by promoting the root system are part of how plants adapt to N deficiency (Yan et al., 2019; Stagnari et al., 2021). Sugars can be transported from leaves to roots where they act as signalling molecules for biomass allocation and changes in root architecture in response to N and P deficiencies (Hermans et al., 2006). In addition, sugars can also act as repressors of photosynthetic activity in leaves under N shortage (Paul and Driscoll, 1997). Transport of sugars as signalling compounds, the reduction of photosynthetic rate and sink strength may explain the lower average content of C in leaves under LN compared to HN, while no significant differences were found for C content in fruit. Furthermore, in both leaves and fruits, C:N ratio was higher under LN, which has been reported as the driving factor for sugars to act as photosynthesis repressors (Paul and Driscoll, 1997). Plants under high N fertilization doses tend to grow vegetatively rather than reproductively (Elia and Conversa, 2012), thus the contrary seemed to be happening in our collection under the LN treatment.

The results regarding yield parameters (early and final yield, and total fruit number) are in agreement with Hernández et al. (2020), which found that N fertilization affected tomato yield in terms of fruit load instead of fruit weight. This suggests that minimizing N inputs promotes a more efficient N uptake and utilization in our collection, after a first period of recession and adaptation. In this sense, much higher NUE values were obtained under the LN treatment compared to HN. However, early yield is a trait of utmost relevance for farmers as it implies a much earlier market release of the product. Identifying the best lines for this trait, as well as studying an optimal N fertilization level between the HN and LN doses for eggplant sustainable production in our

conditions would be effective strategies for eggplant breeding for NUE in early stages of the harvest period.

The absence of genotype per N treatment ($G \times N$) interactions for most traits implies that the lines would show a similar trend in the phenotype in response to lowering N inputs. The distribution of the lines according to the two principal components of the PCA also reflected this similar trend, except for the IL SMI_12.6, which could be an interesting material for adaptation to low N inputs, as it performed better in terms of morpho-agronomic traits under the LN compared to the HN treatment. Besides SMI_12.6, the ILs SMI_5.1 and SMI_9.5 could also be potential materials for breeding, as they performed similarly under the two N treatments.

Correlations among traits can assist the breeder in predicting the phenotype for some traits by determining the phenotype of only a few. The positive correlations found among the traits related to plant growth, N and C in leaves, and those related to yield and NUE, are in agreement with other studies (Dinh et al., 2017; Getahun et al., 2020; Villanueva et al., 2021). Interestingly, these correlations were not N-treatment dependent. The same happened among traits related to fruit morphology, also in agreement with several studies (Portis et al., 2015; Mangino et al., 2021).

Furthermore, minimizing N inputs slightly reduced the average dry matter of fruits, which was in agreement with other studies reporting an increased dry matter content in fruits of diverse crops with increasing N fertilization (Sharifi et al., 2007; Ronga et al., 2019). The negative correlations found for fruit dry matter with fruit length and fruit pedicel length may suggest that under N shortage, bigger fruits tend to have a higher water proportion, which might be related to a reduced photoassimilate production as well as low dry matter allocation to fruit under LN.

Regarding antioxidant compounds, N treatment did not significantly affect total phenolics (TPC) or individual chlorogenic acid content (CGA) in our eggplant ILs, although studies have reported an increased expression of the phenylpropanoid pathway in response to abiotic stresses, including N shortage (Galieni et al., 2015; Hernández et al., 2019). The high and positive intercorrelation found between TPC and CGA is in agreement with numerous studies reporting CGA as the major phenolic compound, by far, in the fruit flesh of *S. melongena* and *S. incanum*, usually accounting for more than 70% of TPC (Luthria et al., 2010; Prohens et al., 2013). This could facilitate eggplant breeding for the improvement of antioxidant capacity for a more sustainable agriculture, as we could expect a similar response of the phenotype under two contrasting N inputs.

4.3. Putative QTLs identified

Most of the putative QTLs identified in the present study were specific of the N treatment, which supported the strong effect of N levels in the changes of a considerable number of the traits analyzed. Nevertheless, identifying QTLs for each N treatment separately could represent an advantage for selection and breeding for specific conditions (Diouf et al., 2018).

The collocation of a several number of QTLs on chr. 2, compared with the significant inter-trait correlations found among plant growth, yield, and fruit size parameters could indicate the presence of genetic linkage or a pleiotropic locus

(Causse et al., 2002; Portis et al., 2014). Our results agree with the QTL cluster on chr. 2 for early and total yield, as well as fruit size traits, identified in an eggplant intraspecific F_2 population obtained by crossing the breeding lines “305E40” \times “67/3” (Portis et al., 2014). In addition, QTLs for fruit length and fruit weight were also detected on chr. 2 in an eggplant interspecific F_2 population with *Solanum linnaeanum* (Doganlar et al., 2002; Frary et al., 2014). Interestingly, the QTL for stem diameter (*pdiam2.LN*), fruit weight (*fw2.LN*), and fruit pedicel length (*fped4.LN*, *fped4.HN* and *fped12.HN*) were coincident with QTLs identified in a previous two-environment evaluation of a set of the eggplant lines with introgressions from *S. incanum* (Mangino et al., 2020). For all of them, the introgressed fragment had the same negative effect on the average trait value, except for the QTL for stem diameter on chr. 2, which had the opposite effect. This could be explained by the significantly larger introgressed fragment carried by the line SMI_2.9 in our study than the fragment in Mangino et al. (2020). Thus, the QTLs in each of the studies may have different locations on the chromosome or there may be interactions with other regions of the fragment that modify the QTL effect. Fruit weight is a trait of utmost interest for farmers, and its genetic basis has been extensively investigated for decades in Solanaceae, especially in tomato and pepper, and major QTLs have been mapped on chr. 2 in several studies (Paran and Van Der Knaap, 2007; Illa-Berenguer et al., 2015; Cambiaso et al., 2019). This supports the conservation of gene function among the Solanaceae, as well as the importance of synteny studies for genetic inferences in less studied species (Doganlar et al., 2002; Rinaldi et al., 2016).

The identified QTLs with a positive effect of the introgression (*fped9.LN*, *fl10.HN*, *fdm2.HN*, *fdm2.LN*, *fdm4.HN*, and *fdm4.LN*) were novel QTLs in eggplant, except for a QTL for fruit dry matter, which was also detected on chr. 2 on the eggplant “305E40” \times “67/3” intraspecific F_2 population (Toppino et al., 2016). In addition, QTLs for dry matter were also identified on syntenic fragments of chr. 2 and 4 in tomato (Causse et al., 2002; Lecomte et al., 2004; Bertin et al., 2009; Prudent et al., 2009).

The search for candidate genes underlying the identified QTLs in our study was challenging because the introgressed fragments covered almost 100% of the chromosomes harboring the QTL, accounting for a massive number of genes. Obtaining sub-ILs, with shorter and overlapping introgression fragments, would be a future strategy for fine-mapping the QTLs. A relatively short introgressed fragment was obtained at the beginning of chr. 10 (0–2 Mb). Within this fragment, a QTL for carbon content in the leaf was identified under the LN treatment, which could be associated with N shortage response. No other QTLs was identified in eggplant for C metabolism, but a recent study using an IL population of *Solanum pennellii* into a cultivated tomato background reported the identification of a vast number of QTLs related to photosynthesis and primary metabolism, which were scattered over all chromosomes (Silva et al., 2018). The set of QTLs from this study included some associated with photosynthesis, respiration rates, and starch turnover in a region of chr. 5 syntenic to the beginning of chr. 10 (Silva et al., 2018). In addition, the potential candidate gene identified within the region of this QTL (SMEL_010g336770.1.01) encodes for a protein similar to a fructose-bisphosphate aldolase (FBA), which are key enzymes involved in glycolysis, gluconeogenesis, and the Calvin cycle. This gene family was also reported to change the expression levels as a response to various abiotic stresses (Lu et al., 2012; Cai et al., 2016). However, fine-

mapping of the QTL would be necessary for supporting this statement.

Finally, QTLs for total phenolics (TPC) and chlorogenic acid content (CGA) in fruit colocalized on chr. 3 and 12, which probably indicate the presence of a common QTL was associated with both traits on each chromosome, owing to the highly positive correlation between these under the two N treatments. For the other two identified QTLs for CGA, the introgressed fragment had a negative effect on the mean trait value, which was unexpected because a higher content in these antioxidant compounds was reported on *S. incanum* accessions compared to cultivated varieties (Prohens et al., 2013; Kaur et al., 2014). A possible explanation might be the occurrence of epistatic effects between loci of the wild introgressed fragment and the cultivated genetic background (von Korff et al., 2010). The major structural genes encoding the key enzymes involved in the CGA synthesis pathway are well known and have previously been located in a *S. melongena* × *S. incanum* linkage map (Gramazio et al., 2014). In addition, thanks to synteny to other important *Solanaceae* and the availability of various eggplant reference genome assemblies (Hirakawa et al., 2014; Barchi et al., 2019; Wei et al., 2020; Li et al., 2021), several orthologs of those core genes of the CGA synthesis pathway as well as transcription factors involved in its regulation have been positioned on the eggplant physical map. Several candidate genes encoding key enzymes of the CGA synthesis pathway were spotted within the regions of the identified QTLs for TPC and CGA, which provided new tools for elucidating the genetic basis of the accumulation of these compounds in eggplant. Although no candidate genes were identified for the QTLs on chr. 5 and 12, another QTL for increased CGA was recently detected at the beginning of chr. 5 in an advanced backcross population of eggplant with the wild relative *Solanum elaeagnifolium* (Villanueva et al., 2021). A better knowledge of the regulation of CGA synthesis pathway and its genetic determinants will be necessary to unravel all these QTL effects.

5. Conclusions

We reported herein the first morpho-agronomic and composition characterization of the response to N fertilization shortage on a set of eggplant introgression lines (ILs) and the recipient parent AN-S-26, from the first interspecific IL collection obtained in eggplant carrying fragments of the wild relative *S. incanum*. In addition, the effect of the two different N fertigation doses on soil composition was evaluated.

According to the interpretation of soil conditions after fertilization, the HN treatment might represent an excess of N in soil. However, the results of plant characterization indicated a lower performance under the N restrictive treatment for important agronomic traits. Therefore, an optimal fertilization between the two N fertilization levels used in the present study should be investigated to maximize plant yields without causing damage to the environment. In general, minimizing N inputs significantly affected traits related to plant growth, yield, and N and C distribution in plant and fruit dry matter, while it did not significantly affect fruit morphology and size, or the content in phenolic compounds in the fruit flesh. Among the ILs evaluated, some were identified as potential materials for breeding eggplant for adaptation to low N inputs. Finally, thanks to the available genotyping of the

ILs, several QTLs were identified associated with most of the traits analyzed under each of the N treatments, including seven QTLs stable between the two N treatments, which provided a useful tool for marker-assisted breeding in eggplant.

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Supplementary materials

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