SUPPLEMENTARY FILE S1. Detailed description of the methods used for fruit chemical composition analyses.

Fruit composition of eggplant lines with introgressions from the wild relative *S. incanum*: interest for breeding and safety for consumption

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S1.1. Determination of anthocyanins and total chlorophylls in peel

Anthocyanin content was evaluated in two subsamples per replicate of 1 cm^2 freezed-dried peel from the part of the fruit with a darker colour. A solid-liquid extraction procedure was performed using 5 mL of methanol:HCl:water (50:1:49 v/v) during 24 h in the dark at 4 °C. Pigment content was calculated from absorbance values of the final extract at 530 nm as cyanidin-3-galactoside equivalents with molar extinction coefficient of 3.43×10^4 L cm^{-1} mol^{-1} [18], using the formula $Total\ anthocyanins\ \left(\frac{mol}{cm^2}\right) = \frac{Abs}{3.43\times10^4} \times \frac{5}{1000} \times \frac{1}{S}$, where *Abs* is the absorbance given by the spectrophotometer, and *S* is the surface of the peel portions in cm^2. The results were expressed as mg cm^{-2} of dry weight.

Total chlorophylls in peel were extracted using 0.2 g freeze-dried powdered peel with 20 mL acetone (80% v/v) for 1 hour under stirring at 200 rpm. After a filtration step, quantification of chlorophylls was done spectrophotometrically, measuring the solution absorbance at 645 and 663 nm, and following the formulas described in Herraiz et al. [19]. The results were expressed as mg g^{-1} of dry weight.

For all spectrophotometric measures a UviLine 9400 UV-VIS spectrophotometer (Schott Instruments, Mainz, Germany) was used.

S1.2. Determination of sugars and organic acids

Contents in sugars and organic acids were determined by High Performance Liquid Chromatography (HPLC) using a 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA). A subsample of 0.1 g of freeze-dried material was dissolved in 1.5 mL distilled water for the analyses and filtered through 0.22 mm PVDF MILLEX-GV filters (Merck Millipore, MA, USA). The same sample was used to perform the analysis of sugars and organic acids. Fructose (FRU), glucose (GLU) and sucrose (SUC) were separated using a Luna® Omega SUGAR column (3 µm; 151 150 × 4.6 mm; Phenomenex, Torrance, CA, USA), with a mobile phase of 75% acetonitrile: 25% HPLC-grade water at isocratic gradient, flow rate of 1 mL min^{-1} and total runtime of 15 min; and then detected by refractive index using a 350 RI detector (Varian, Palo Alto, CA, USA). Malic (MAL) and citric (CIT) acids were separated and detected by HPLC-UV at 210 nm using a Luna® Polar C18 column (3 µm; 150 mm × 4.6 mm; Phenomenex). The HPLC conditions were a mobile phase of 95% 1.8 mM H_2SO_4: 5% methanol with an isocratic gradient, a flow rate of 0.4 mL min^{-1} and a total runtime of 15 min. Quantifications were done using a purified external standard curve for each compound.

Contents in total sugars and total acids were calculated from concentrations of individual compounds as FRU+GLU+SUC and CIT+MAL, respectively. Contents of all compounds were expressed as mg g^{-1} of dry weight.

S1.3. Determination of chlorogenic acid

For chlorogenic acid analysis, 0.1 g freeze-dried powdered material was homogenized in 1.5 mL methanol:water (80:20, v/v) with 0.1% (w/v) of 2,3-tert-butyl-4-hydroxyanisole (BHT), sonicated for 1 h at room temperature, and filtered through 0.2-µm PTFE membrane filters (Merck Millipore). Chlorogenic acid content was determined by HPLC-UV at 325 nm using a 1220 Infinity LC System (Agilent Technologies), a BRISA "LC²" C18 column (3 µm particle size; 150 mm x 4.6 mm) (Teknokroma, Barcelona, Spain) and a mobile phase of 0.1% formic acid (A) and methanol (B) The mobile phase gradient was as follows: 0 min, 95% A:5% B at 0.5 mL/min; 0–5 min linear increase to 10% B at 0.5 mL/min; 5–10 min, linear increase to 20% B at 0.5 mL/min; 10–18 min, linear increases to 83% B and 0.5 mL/min; 18–23 min, linear increase to 100% B at 0.5 mL/min; 23–27 min, 100% B at 1.0 mL/min; 27–30 min, decrease to 5% B at 1.0 mL/min; 30–40 min, 95% A:5% B at 0.5 mL/min [20]. Quantification was done using a purified external standard curve of chlorogenic acid, and results were expressed as mg g^{-1} of dry weight.

S1.4. Determination of total phenolics

Total phenolic content was estimated spectrophotometrically according to the Folin-Ciocalteu procedure optimized to carry out the redox reaction in a 96-well plate. For the phenolics extraction procedure, a subsample of 0.125 g freeze-dried powdered material was homogenized in 5 mL of acetone:water:glacial acetic acid (70:29.5:0.5, v/v/v) for 24 h under continuous stirring at room temperature. Then, 1.5 mL of the supernatant obtained after 5 min of centrifugation at 3500 rpm were kept at -20 °C until analysis. For spectrophotometric measures, 65 µL of the supernatant were mixed with 0.5 mL diluted (10%, v/v) Folin–Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany). After 5 min at room temperature, 0.5 mL of sodium carbonate (60 g/L) were added to the mixture and incubated during 90 min in the dark at room temperature. Subsequently, absorbance readings were taken at 750 nm in an iMark microplate spectrophotometer (Bio-Rad, Herts, UK) [20]. Quantification was done using a purified external standard curve of chlorogenic acid, and total phenolic content was expressed as mg of chlorogenic acid equivalents g^{-1} of dry weight.

S1.5. Determination of total antioxidant activity

Total antioxidant activity was evaluated using 0.15 g freeze-dried powdered material following the colourimetric assay of DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity [21], with slight modifications. After 1 h of incubation with 5 mL of acidified methanol (99:1, v/v) in the dark under continuous stirring and at room temperature, 0.1 mL of the diluted extract with methanol (1:10 v/v) was added to 3.9 mL DPPH• solution (0.025 g L^{-1}) and incubated for 1 h in the dark at room temperature. Total antioxidant activity was determined from absorbance values of the final solution at 515 nm using a UviLine 9400 UV-VIS spectrophotometer (Schott Instruments) and the antioxidant Trolox (Scharlab S.L., Barcelona, Spain) as standard. The results were expressed as µmol Trolox equivalents (TE) g^{-1} of dry weight.

S1.6. Determination of glycoalkaloids

Glycoalkaloids were extracted and quantified according to Mennella et al. [4], with slight modifications. A subsample of 0.1 g freeze-dried homogenized material was incubated with 95% ethanol during 30 min at 70 °C, and then filtered through 0.2-µm PTFE membrane filters (Merck Millipore). Subsequently, solamargine (SM) and solasonine (SS) were quantified by reversed phase HPLC-UV at 205 nm using a 1220 Infinity LC System (Agilent Technologies), a Luna® Omega Polar C18 column (3 µm; 150 mm × 4.6 mm; Phenomenex) at 50 °C and a mobile phase gradient of 2.5% (v/v) of an aqueous Triethylammonium phosphate solution (TEAP) (A) and acetonitrile (B) as follows: 0 – 20 min linear increase from 20% to 70% B; 20 – 25 min, linear decrease from 70% to 20% B. Quantifications were done using a purified external standard curve for each compound, and results were expressed as mg g^{-1} of dry weight. Total glycoalkaloids were calculated as the sum of SM + SS and expressed as mg g^{-1} of dry weight.

S1.7. Determination of crude protein

Crude protein content in fruit was estimated as 6.25 × total N, which was measured from 0.4 g of oven-dried powdered samples, following the Kjeldahl method [22]. First, a digestion process was performed adding 4 g

of catalysts K_2SO_4:$CuSO_4$:Se (10:1:0.1, w/w/w) and 10 mL of concentrated H_2SO_4:concentrated H_3PO_4 (95:5, v/v) to the subsample, and incubating the mix for 30 min at 420 °C. After cooling down, 50 mL of water was added. Secondly, a distillation process was performed using a Kjeltec 2100 Distillation Unit (Foss Tecator, Högamäs, Sweden). Subsequently, an acid-base titration with H_2SO_4 (0.05-0.06N) was performed. The estimated nitrogen content was calculated as: $Ntotal\ (\%) = \frac{(V-B)\times N(H_2SO_4)\times 14}{weight} \cdot 100$, where V is the volume of H_2SO_4 used in the titration, B is the volume of H_2SO_4 used for the blank and 14 is de atomic mass of nitrogen. The results were expressed as mg g^{-1} of dry weight.

S1.8. Determination of minerals

Mineralized samples were obtained from 2 g of oven-dried powder material, which were calcined for 2 h in a muffle at 450 °C [24]. After cooling down, ashes were hydrated and 2 mL of concentrated HCl were added. The solution was heated until first fumes appeared and then filtered with Whatman paper (Sigma-Aldrich, Saint Louis, MI, USA) and make it up to 100 mL volume with water. Content of minerals (Fe, Cu, Zn, Na, Mg, Ca, K, P) was then determined following the MAPA procedures [23]: by atomic absorption in a Thermo Elemental Solaar S Series spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) for Ca, Mg, Fe and Zn determinations, using a flame photometer Jenway PFP7 with stoichiometric flame (Jenway, Staffordshire, UK) for Na and K, and by the colourimetric phosphomolybdovanadate method for P determination. Units of minerals were expressed as mg g^{-1} of dry weight.

References

The references are embedded in the 'References' section of the article manuscript.