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First successful backcrossing towards eggplant (*Solanum melongena*) of a New World species, the silverleaf nightshade (*S. elaeagnifolium*), and characterization of interspecific hybrids and backcrosses

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ABSTRACT

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a drought tolerant invasive weed native to the New World. Despite its interest for common eggplant (*S. melongena* L.) breeding, up to now no success has been obtained in introgression breeding of eggplant with American *Solanum* species. Using an interspecific hybrid between common eggplant and *S. elaeagnifolium* as maternal parent we were able to obtain several fruits with viable seed after pollination with *S. melongena* pollen. Twenty individuals of the first backcross (BC1) generation were crossed again to the *S. melongena* parent and second backcross (BC2) seed was obtained for 17 of them, suggesting that most of the genome of *S. elaeagnifolium* is likely to be represented in the set of BC2 families. Five plants of each of the two parents, interspecific hybrid and BC1 generation were characterized with morphological descriptors and for pollen viability. The interspecific hybrid was intermediate among parents, although in overall morphological characteristics more similar to the *S. elaeagnifolium* parent. However, pollen viability of the hybrid was very low (2.6%). The BC1 generation was intermediate in characteristics between the hybrid and the *S. melongena* parent, with pollen viability increasing to an average of 19.4%. The root system of the interspecific hybrid indicated that it is able to explore larger areas of the soil than the *S. melongena*

parent. The phenolics profile of the fruit of the two parents and hybrid revealed a higher diversity in phenolic constituents in *S. elaeagnifolium* compared to *S. melongena*, where the major phenolic compound was chlorogenic acid, while the interspecific hybrid was intermediate. By using flow cytometry it was found that *S. elaeagnifolium*, *S. melongena*, and their interspecific hybrid were diploid, although the genome size of *S. elaeagnifolium* was slightly smaller than that of *S. melongena*. Our results represent the first report of successful development of backcross generations of common eggplant with a New World *Solanum* species. This makes available a relatively unexplored, phylogenetically distant genepool for eggplant breeding. The backcross materials obtained can make a relevant contribution to developing new eggplant cultivars with new nutritional and environmental properties.

Keywords:
Backcrosses
Introgression breeding
Flow cytometry
Phenolics profile
Solanum elaeagnifolium
Solanum melongena

1. Introduction

Crop wild relatives can contribute to widening the genetic background of crops and adapting them to new challenges, such as climate change (Dempewolf et al., 2014). The economic impact of the utilization of crop wild relatives in crop breeding has been estimated at the global level in $164.5 \cdot 10^9$ US$ annually, while the current value of crop wild relatives for breeding in the most important crops could triple in a climate change scenario (Tyack and Dempewolf, 2015). This clearly shows how research in crop wild relatives and its utilization in breeding may have an important economic impact by developing new cultivars with improve characteristics. In this way, a new approach known as “introgressiomics” calling for the systematic development of plant materials

containing introgressions from wild species has recently been proposed (Prohens et al., 2017).

One of the vegetable crops in which significant efforts are being done in the last years for introgression breeding from related species for adaptation to climate change is the common eggplant (*Solanum melongena* L.) (Toppino et al., 2008; Liu et al., 2015; Kouassi et al., 2016; Plazas et al., 2016). The common or brinjal eggplant is an Old-World crop domesticated in Southeast Asia (Meyer et al., 2012), and is related to wild species of spiny solanums (Leptostemonum clade) occurring in Asia and Africa (Knapp et al., 2013; Vorontsova et al., 2013; Aubriot et al., 2016; Vorontsova and Knapp, 2016).

Interspecific hybrids and backcrosses of eggplant have been obtained with many related Old World species, and this has included the development of introgression materials with different species and one set of introgression lines with *S. incanum* (Rotino et al., 2014; Kouassi et al., 2016; Plazas et al., 2016; Gramazio et al., 2017; Gramazio et al., 2018). In addition, sexual and somatic hybridization have also been used to develop interspecific hybrids between eggplant and several New World species. In this way, *Solanum aculeatissimum* Jacq. (Zhou et al., 2018), *S. elaeagnifolium* Cav. (Kouassi et al., 2016), *S. sisymbriifolium* Lam. (Gleddie et al., 1986), *S. torvum* Sw. (Jarl et al., 1999; Collonnier et al., 2003), and *S. viarum* Dunal (Prabhu et al., 2009) are of great interest for breeding for its resistance or tolerance to biotic and abiotic stresses (Kashyap et al., 2003; Rotino et al., 2014; Kouassi et al., 2016; Zhou et al., 2018). In fact, some of these New World species, like *S. torvum*, are regularly used as eggplant rootstocks due to their resistance to multiple soil diseases and nematodes (Arao et al., 2008; King et al., 2010; Gisbert et al., 2012; Sabatino et al., 2018). However, interspecific hybrids between brinjal eggplant and New World *Solanum* species have to date been highly sterile (Lester and Kang, 1998; Prohens et al., 2012; Rotino et al., 2014; Liu et al., 2015; Çürük and Dayan, 2017; Afful et al., 2018). Ploidy modification techniques, like the development of tetraploids containing the full chromosome complements of both parental species allowed fertility restoration in hybrids of common eggplant with the Old World relative *S. aethiopicum* L. (Isshiki and Taura, 2003) but not in hybrids with New World *S. torvum* (Sihachakr et al., 1989). Thus, to our knowledge no backcrosses have been obtained for the introgression of genes or genomic fragments of interest from New World *Solanum* species into the genetic background of eggplant.

One of the New World species of greatest interest in the improvement of eggplant is the silverleaf nightshade (*S. elaeagnifolium*). This distant wild relative of eggplant is native to deserts and dry forests of North and South America and belongs to the sister group of all Old World spiny solanums, the *Elaeagnifolium* clade (Knapp et al., 2017). It is highly tolerant to drought (Christodoulakis et al., 2009) and has spread as an invasive noxious weed in arid and semi-arid regions of the world, where it causes considerable economic damage (Mekki, 2007). In addition, *S. elaeagnifolium* has been barely explored for other traits that may be of interest for eggplant breeding such as the content of nutritionally important bioactive phenolics (Kaushik et al., 2015). Despite its evident interest for eggplant breeding, obtaining interspecific hybrids between common eggplant and *S. elaeagnifolium* has not been described until recently (Kouassi et al., 2016). After multiple crosses between six different accessions of *S. melongena* and one of *S. elaeagnifolium* a few fruit set when using one *S. melongena* accession (MEL3) as female parent, and nine hybrid plants could be obtained after embryo rescue of immature fruits by Kouassi et al. (2016).

Within the Leptostemonum Clade, New World *Solanum* species of the *Elaeagnifolium* clade are those phylogenetically closest to the Old World species (Vorontsova and Knapp, 2016; Knapp et al., 2017). This led us to hypothesize that, compared to other New World species, using interspecific hybrids with *S. elaeagnifolium* would result in higher success in achieving introgression breeding in eggplant. In this way, a New World genepool could be accessible for breeding and for widening the genetic background of eggplant.

In this paper, using the hybrids obtained by Kouassi et al. (2016) we describe the characteristics of interspecific hybrids between *S. melongena* and *S. elaeagnifolium*, and we make a first report of the development and characteristics of backcross generations between eggplant and this New World species. We consider that these results open a way to the use of the characteristics of interest of *S. elaeagnifolium* and its closest relatives for eggplant improvement. Given the high tolerance to drought of *S. elaeagnifolium* (Christodoulakis et al., 2009) these materials may be of great interest for developing a new generation of eggplant varieties adapted to climate change.

2. Material and methods

2.1. Plant material and hybridizations

Parental materials consisted of one accession of *S. melongena* (MEL3) and one accession of *S. elaeagnifolium* (ELE2). *Solanum melongena* MEL3 is an accession from Ivory Coast used in an introgression breeding programme (Kouassi et al., 2016; Plazas et al., 2016) having semi-long fruits. *Solanum elaeagnifolium* ELE2 was collected as a weed in Greece and has small round fruits (Kouassi et al., 2016). Both parents have green fruits with dark green stripes (Figure 1) that ripen to yellow or orange-brown. Also, materials used included clonal replicates of a plant of the interspecific hybrid *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 obtained after embryo rescue (Kouassi et al., 2016).

In order to obtain backcross generations towards the *S. melongena* parent, the *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 interspecific hybrid, due to its low pollen viability, was always used as female parent in crosses for obtaining the first backcross (BC1) generation. Also, the plants obtained of the BC1 generation were used as female parents for developing the second backcross (BC2) generation. All plants used for hybridizations were grown in an insect-free greenhouse in 15 l pots filled with coconut fiber. Plants were watered and fertilized using a drip irrigation system. Hybridizations were performed early in the morning. Basically, flower buds one or two days before anthesis were opened and emasculated with a forceps and pollen from the male parent was gently deposited on the stigma of the female parent using a glass slide. Flowers were tagged and fruits were harvested when physiologically ripe, with the exception of a first fruit of the backcross between the interspecific hybrid *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 and *S. melongena* MEL3, which was harvested physiologically unripe for embryo rescue using the protocol indicated in Plazas et al. (2016). For fruits left to ripen, seeds were extracted from each individual fruits and left on filter paper for drying at room temperature. Subsequently they were placed in paper bags and stored at 4ºC in hermetic glass jars which contained silica gel for maintaining seed moisture low. Seed germination was performed using the protocol described in Ranil et al. (2015).

Plants used for characterization were transplanted in June 2017 to soil in a screenhouse. Plants were watered and fertilized by drip irrigation and trellised using vertical strings. Weeds were removed manually and phytosanitary treatments against spider mites and whiteflies were performed when necessary. Five plants of each of the parentals, their interspecific hybrid, and of the first backcross (BC1) of the interspecific hybrid towards the *S. melongena* parent were used for the morphological

169 characterization of above-ground parts. Three additional plants of *S. melongena* and of
170 the interspecific hybrid were used for the evaluation of the root system.
171
172 *2.2. Characterization*
173
174 Traits used for the characterization of the aerial part included 18 qualitative
175 (Table 1) and 16 quantitative (Table 2) descriptors mostly based on EGGNET and
176 IBPGR descriptors (IBPGR, 1990; van der Weerden and Barendse, 2007; Kaushik et
177 al., 2016). Descriptors used included traits of the habit, leaf, inflorescence, flower, and
178 fruit. Except for plant height and stem diameter, for which only one measurement was
179 taken per plant, at least five measurements were taken from each individual plant in
180 order to obtain individual plant averages for the conventional morphological descriptors
181 (i.e., five measurements per replicate). Pollen viability was evaluated according to Aref
182 (1992) with some modifications. From a cell suspension with a concentration of
183 500,000 cells/ml, 1 ml of dilution was distributed in a 6 mm diameter Petri dish and
184 stained with 0.001% FDA solution (fluorescein diacetate, 1 µl per ml of suspension) and
185 allowed to incubate for 5 minutes. Fluorescence in FDA was determined by scoring the
186 percentage of fluorescing pollen grains under an ultraviolet (UV) source provided by a
187 mercury lamp. The principle is based on the uptake of non-fluorescing FDA by the
188 vegetative cells of a viable pollen grain and subsequent hydrolysis by esterase to release
189 fluorescein, which fluoresces under UV (excitation filter = 485 nm and barrier filter =
190 520 rim). In contrast, nonviable cells are incapable of hydrolyzing FDA and, therefore,
191 do not fluoresce (Heslop-Harrison et al., 1984). Each determination of pollen fertility
192 consisted was performed by counting 300 to 500 pollen grains by examining 10
193 locations in a series of random areas across the Petri dish that contained the sample
194 under test.
195 For the characterization of the root traits, four traits were measured. Firstly, the
196 plants were carefully removed from the ground, with the help of a hoe, to reduce root
197 damage; once extracted they were cleaned with water to eliminate the earth or the
198 accumulated mud. Finally, the characters indicated in Table 3 were evaluated manually
199 with the help of a phenotyping scoreboard.
200
201 *2.3. Phenolics content*

Chlorogenic acid (CGA), the main phenolic compound in the eggplant flesh (Stommel and Whitaker, 2003; Whitaker and Stommel, 2003; Prohens et al., 2013), and other hydroxycinnamic acid conjugates were extracted and analyzed using the methodology indicated in Plazas et al. (2014) in order to assess overall phenolic content of the fruit. Extractions were performed with 0.1 g of lyophilized sample homogenized in 1.8 ml of methanol:water (80:20, v/v) plus 0.1% (w/v) of 2,3-tert-butyl-4-hydroxyanisole (BHT). After that the extract was vortexed vigorously, sonicated for 1 h and centrifuged at 2000 rpm for 3 min and the supernatant filtered through 0.2-µm polytetrafluoroethylene (PTFE) membrane filters.

Extracts were analyzed on a HPLC 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) operated by the OpenLAB CDS ChemStation Edition software package (Agilent Technologies). Aliquots of 10 µL were injected into a ZORBAX Eclipse Plus C18 (3.5 µm; 4.6 mm × 12.5 mm; Agilent Technologies) column protected by a ZORBAX Eclipse Plus C18 guard column (5 µm; 4.6 mm × 12.5 mm; Agilent Technologies). A binary gradient consisting of 0.1% formic acid (Solvent A) and methanol (Solvent B) was used. The mobile phase gradient described in Plazas et al. (2014) was used and absorbance was measured at 325 nm for quantification. CGA concentration in the extracted samples was calculated using calibration curves. The CGA peak area and the total peak area (TPA) of other phenolic acids were determined.

2.4. Determination of ploidy level

Cell nuclei from leaf tissues were isolated mechanically according to Dpooležel et al. (1989) with some modifications. Approximately 0.5 cm^2 of fresh young leaf tissue was chopped with a razor blade in a glass Petri dish containing 0.5 ml lysis buffer LB01 (pH 7.5) containing 15 mM Tris (hydroxymethyl) aminomethane, 2 mM Na_2EDTA and 0.5 mM spermine, and was left to incubate for 5 min. Subsequently, the suspensions containing nuclei and cell fragments were passed through a 30µm CellTrics filter (Sysmex). The nuclei in the filtrate were stained by CyStain UV Ploidy (Sysmex) by incubation for 5 min. The fluorescence intensity of the homogenate was measured using CyFlow ploidy-analyzer (Partec, Münster, Germany), measuring at least 4000 nuclei for each sample.

3. Results

3.1 Backcrossing results

Twenty-two fruits developed after performing over 800 crosses between the interspecific hybrid *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 as a female parent and the recurrent *S. melongena* MEL3 as male parent. The first fruit to set was collected before physiological maturity and it was found to contain developing seeds, from which 10 embryos were rescued (in heart and torpedo stages), which developed well and gave phenotypically normal BC1 plants. Because opening of this first fruit revealed an apparently normal development of the seeds, several subsequent fruits were allowed to develop to physiological maturity; these yielded seeds with a germination rate higher than 50%. We subsequently, therefore, abandoned embryo rescue, and the fruits containing seeds with the BC1 zygotes were allowed to ripen on the plant for extraction of mature seeds. Except for a single fruit that was parthenocarpic, all of the other 21 fruits of the interspecific hybrid after pollination with the recurrent parent *S. melongena* MEL3, presented seeds with a range between 4 and 40 seeds (mean ± SD = 12.32 ± 8.13 seeds/fruit). No fruits were obtained from non-pollinated flowers, although some seedless pseudofruits occasionally formed from mon-pollinated flowers.

We put all 40 seeds from the fruit containing the largest seed number to germinate; this resulted in 50% germination, giving us 20 BC1 plants. These BC1 plants were grown for a next cycle of backcrossing for obtaining the BC2 generation. Multiple crosses (over 3,000) were performed using the BC1 plants as female parent, resulting in at least one fruit obtained in 17 out of the 20 BC1 plants. A total of 92 fruits (between 1 and 9 per individual plant) were obtained and all of them had seeds, with a range between 1 and 150 seeds/fruit (mean ± SD = 62.86 ± 35.99 seeds/fruit). Several BC2 seeds from each BC1 plant were germinated to obtain between 5 and 12 plants per individual BC2 family.

3.2 Characterization of parents, hybrid, and BC1 generations

Important differences were observed between the *S. melongena* MEL3 and *S. elaeagnifolium* ELE2 parents in the morphology of the vegetative part of the plant,

leaves, inflorescences and flowers, and fruits. In fact, for 21 out of the 37 characters evaluated, there was no overlap in the ranges of variation (Tables 1 and 2).

Regarding plant habit, *S. melongena* has an upright growth habit and is much taller (more than two-fold) and has a thicker stem than *S. elaeagnifolium* (Table 1 and 2). The hybrids have an upright growth habit and are intermediate for plant height and stem diameter, although the values are closer to those of *S. elaeagnifolium* (Tables 1 and 2; Figures 1A-C). For the BC1 generation, the five plants characterized had an upright growth habit, but a great segregation was observed for plant height and stem diameter, with ranges of variation wider than those of the parents for these characters (Tables 1 and 2; Figure 1D). Amazingly, interspecific hybrids displayed prickles between nodes, while none of the parents did. Some BC1 plants had prickles between nodes, but their degree of prickliness was much lower than that of the F1 (Table 2).

Leaf morphology also displayed great differences between the parents (Tables 1 and 2; Figures 1E-F). None of the two parents had prickly leaves; however, *S. melongena* leaves had stronger lobing, were more erect, and much larger than those of *S. elaeagnifolium*. The leaves of the hybrid were intermediate for all the observed characters, and again quite variable in the individuals of the BC1, which displayed segregation for the leaf lobing (Tables 1). As occurred with the prickles between nodes, some prickles appeared in the leaves of the hybrids. However, all BC1 plants had non-prickly leaves.

Many differences were observed among parents in inflorescence and flower traits. *Solanum melongena* had flowers with light violet corolla, connivent anther cone, and straight style, while those of *S. elaeagnifolium* had a darker bluish violet corolla, spreading anther cone, and curved style (Table 1; Figures 1G, 2A and 2C). Flowers of *S. melongena* were considerably larger than those of *S. elaeagnifolium*, while pollen viability was very high in *S. melongena* (>90%) and moderate (around 50%) in *S. elaeagnifolium* (Table 2). On the other hand, the number of flowers per inflorescence was similar among both parents and none of the parents displayed anthocyanins in the pistil. Flowers of *S. melongena* were fasciated and displayed higher numbers of petals, sepals and anthers than those of *S. elaeagnifolium*, which were strictly pentamerous (Tables 1 and 2; Figures 1G). Hybrids had light violet corolla and a connivent cone of anthers (like *S. melongena*) and curved style (like *S. elaeagnifolium*) and segregated for the presence of anthocyanins in the pistil (Table 1; Figure 2B). Flower number per inflorescence was transgressive to both parents (Table 2). Flowers of hybrids were

303 pentamerous like those of *S. elaeagnifolium* although smaller than either parent any of
304 them (Table 2; Figure 1G), and pollen viability of the hybrid was very low (<3%). A
305 wide range of diversity was observed for color and size of flowers in the BC1
306 generation, with wide segregation for these traits (Tables 1 and 2; Figure 1G).
307 Segregation was observed for corolla color, presence of anthocyanins in the pistil, and
308 style curvature, with most plants displaying anthocyanin pigmentation and curved
309 styles. The number of flowers per inflorescence was similar to that of the parents, and
310 like *S. elaeagnifolium* and the interspecific hybrid, flowers of BC1 plants were
311 exclusively pentamerous (Table 2). Corolla diameter was very variable, but average
312 corolla diameter was similar to that of the F1 hybrid (Table 2; Figure 1G). Finally,
313 pollen viability also exhibited a wide range of variation, with a considerable increase
314 (average of around 20%) over pollen fertility levels of the F1 (Table 2).
315 Few differences existed among parents in fruit firmness and color, except that
316 the predominant fruit color at physiological ripeness was yellow-orange for *S.*
317 *melongena* and orange for *S. elaeagnifolium*, and the fruit flesh was white and green,
318 respectively (Table 1). However, large differences were observed in fruit size and shape
319 (Table 2). Fruits of *S. melongena* were much larger and more elongated than those of *S.*
320 *elaeagnifolium*, with fruit length and width on average 15.7-fold and 6.4-fold larger in
321 *S. melongena* than in *S. elaeagnifolium* (Table 2). Also, fruits of *S. melongena* had a
322 thicker peduncle than those of *S. elaeagnifolium*. Regarding the calyx, its relative length
323 in relation to the berry length was shorter in *S. melongena* than in *S. elaeagnifolium*, and
324 it was non-prickly in *S. melongena* and prickly in *S. elaeagnifolium* (Table 2). The F1
325 fruits were less firm than those of either of the two parents, probably as a consequence
326 of being parthenocarpic, and in color were similar or intermediate (for those traits that
327 display differences among parents) to the two parents. Fruit size and shape was
328 intermediate to those of the parents, although much more similar to the *S.*
329 *elaeagnifolium* parent (Table 2). For fruit calyx characteristics, F1 hybrids had a relative
330 calyx length and prickliness similar to the ones observed in *S. elaeagnifolium*. The BC1
331 plants were also similar to the parents and F1 in color characteristics (Table 1).
332 Although variation was observed for fruit size and shape, fruits from BC1 plants were
333 generally intermediate between those of the F1 and the *S. melongena* parent, although
334 much more similar to the former than to the latter (Table 2). For fruit calyx
335 characteristics, the relative fruit calyx length of BC1 individuals was similar to that of *S.*

melongena, while calyx prickliness was variable, with a range from 0 to 10 prickles and an average value slightly lower than that of the F1 hybrid (Table 2).

The root system of developed mature plants of the *S. melongena* parent and of the F1 hybrid S. *melongena* × *S. elaeagnifolium* was characterized and considerable differences were observed (Table 3; Figure 3). Unfortunately the root system of *S. elaeagnifolium* could not be scored, as when the plants were uprooted out plants were already senescent and the root system damaged. The main differences observed were that, compared to *S. melongena*, the F1 had a somewhat longer and thinner main root, a reduced whorl diameter, a higher number of roots of diameter >2 and a lower density of lateral roots in the main root (Table 3). It is also evident from Figure 3 that the F1 hybrid has a root system that explores the soil to longer distances than *S. melongena*, which has a large part of the root system concentrated to a few centimeters around the stem.

3.3 Phenolics profile of parents and hybrids

The analysis of phenolic acids reveals clear differences in the profiles obtained for *S. melongena* and *S. elaeagnifolium* (Figure 4). For *S. melongena*, chlorogenic acid (CGA) was the main compound, representing over 85% of the chromatogram total peak area (TPA) (Figure 4A); also, an unidentified peak very close to the CGA peak and probably representing an isomer or a derivative of CGA (Whitaker and Stommel, 2003) makes a secondary peak. *Solanum elaeagnifolium* also has an important CGA peak, but it represents slightly less than 30% of the TPA, although another peak close to CGA, which probably is also a CGA isomer or derivative, accounts for almost 19% of the TPA. Another important peak corresponding to an unidentified phenolic compound that appears at a retention time of 20.2 minutes in *S. elaeagnifolium*. Also, several other minor peaks appear in the *S. elaeagnifolium* chromatogram that do not appear, or have very low percentage of TPA, in *S. melongena* (Figure 4B). For example, a peak at 15.9 min is detected in *S. elaeagnifolium* but not in *S. melongena*, and a small peak in *S. melongena* at 19.1 min is much higher in *S. elaeagnifolium*. The individuals of the F1 present a chromatogram in which all the major peaks present in the chromatograms of the parents are also present. In this case, the secondary CGA peak disappears, revealing a clear CGA peak representing almost 70% of the TPA (Figure 4C). The unidentified compound from *S. elaeagnifolium* with a peak at 20.2 minutes also appears in the F1

hybrid, although it only represents around 10% of the TPA. In the F1 chromatogram most of the minor peaks observed in the chromatograms of both parents also appear (Figure 3C). Here, the peak at 15.9 min is similar in area to the one found in the *S. elaeagnifolium* parent (Figures 3B and 3C).

3.4 Flow cytometry analysis of parents and hybrids

The analysis with flow cytometry revealed that both parents and the interspecific hybrid were diploid (Figure 5). No large differences were apparent for the genome size among parents and interspecific hybrid, although ELE2 seems to have a slightly smaller genome than *S. melongena*.

4. Discussion

The use of crop wild relatives in breeding has demonstrated in many crops that can make a significant economic impact (Tyack and Dempewolf, 2015). For example, in tomato, introgressions in commercial varieties from a wild relative have contributed to the increase of 2.4% in the soluble solids content of the fruit, which has had an economic impact of $250 \cdot 10^6$ US$ annually only in the US (Hunter and Heywood, 2011). This latter example reveals that although at the global level eggplant has less economic value than tomato (around 6.5-fold less) (FAO, 2018), wild relatives may make an important economic impact in eggplant breeding. However, up to now, to our knowledge, no commercial cultivars of eggplant with introgressions from crop wild relatives are available, and the potential of wild eggplant relatives for the development of commercial cultivars remains untapped. Here we report the successful backcrossing, up to the BC2 generation, of a species native to the New World (*S. elaeagnifolium*) with *S. melongena*, an Old World domesticate (Meyer et al., 2012). According to our knowledge, it is the first time that introgression materials of eggplant with a New World species have been obtained. This has important implications for eggplant breeding, as the introgression materials obtained (up to BC2 generation) indicate that a new distant untapped genepool has become available for eggplant breeding.

By using the interspecific hybrid *S. melongena* × *S. elaeagnifolium* as a female parent and *S. melongena* as a male parent, fruits containing viable seeds were obtained, although the degree of success was lower compared to backcrosses made with other

interspecific hybrids of eggplant with Old World species (Kouassi et al., 2016). This is probably due to a greater sterility of the hybrid, as indicated by low pollen fertility. The fact that no seeded fruits appeared in the non-pollinated flowers suggests that pollen sterility in *S. melongena* × *S. elaeagnifolium* hybrids is a more limiting factor than that of the ovules, as has been shown in other crops (Dwivedi et al., 2008; Prohens et al., 2017). The extraction of embryos made in the first fruit of the hybrid pollinated with *S. melongena* pollen revealed a normal appearance in all immature seeds, suggesting that there are no major problems of embryo degeneration and abortion. In this way, mature seeds containing the BC1 zygotes presented a percentage of germination higher than 50%.

Of the 20 BC1 plants we used to develop the BC2 generation, seeds and BC2 offspring were obtained from 17 of them. Assuming a normal segregation and recombination in the F1 hybrid gametes, it would mean that the percentage of *S. elaeagnifolium* genome represented in the BC1 17 plants would be $1-0.5^{17}$ (>0.99999). Even though it is likely that distortions in the segregation and lack of recombination in some areas of the genome may have occurred (Kreike and Stiekema, 1997; Gramazio et al., 2018), a large part of the genome of *S. elaeagnifolium* is likely represented in the BC1 plants and the BC2 offspring. On the other hand, although variations in the ploidy degree are common in *S. elaeagnifolium* (Moscone E., 1992; Acosta et al., 2005; Powell and Weedin, 2005; Scaldaferro et al., 2012; Knapp et al., 2017) the results of flow cytometry indicate that the accession used of *S. elaeagnifolium* is diploid and presents a genome size similar to that of *S. melongena*, which could have contributed to the success of the backcrosses.

The interspecific hybrids were intermediate in most of the parental characteristics, although generally closer to *S. elaeagnifolium*. This is a common phenomenon in interspecific hybrids in eggplant with wild relatives (Prohens et al., 2013: Kaushik et al., 2016). However, unlike many other interspecific hybrids of eggplant (Kaushik et al., 2016), hybrids between common eggplant and *S. elaeagnifolium* did not display heterosis for vigor, perhaps due to the great phylogenetic distance between the two species (Vorontsova et al., 2013). The hybrid displayed prickles in the leaf, although none of the parents had prickly leaves. This is a common phenomenon in interspecific eggplant hybrids when crossing with a non-prickly species, probably because the mutations that confer lack of leaf prickles are different in the two

437 species (Lester, 1986; Varoquaux et al., 2000; Kouassi et al., 2016; Plazas et al., 2016;
438 Prohens et al., 2012).
439 In the hybrids the style was curved as in *S. elaeagnifolium*; in addition, some
440 plants of the F1 presented anthocyanins in the style while others did not. Differences
441 among F1 hybrids in style pigmentation could be due to environmental effects or
442 epigenetic modifications (Hahlbrock and Scheel, 1989; Dixon and Harrison, 1990;
443 Shichijo et al., 1993; Noda et al., 2004). Something similar, in addition to segregation,
444 could be taking place in the individuals of the BC1. The fact that the flowers of the
445 hybrid are smaller than those of both parents, but at the same time there are more
446 flowers per inflorescence, has already been observed in hybrids between eggplant and
447 other relatives (Daunay et al., 1993; Kaushik et al., 2016). The fruits of the hybrid have
448 intermediate characteristics between those of both parents, although more similar to
449 those of the wild species, again common in interspecific eggplant hybrids (Prohens et
450 al., 2013; Kaushik et al., 2016).
451 As in other studies (Prohens et al., 2012, 2013), a regression towards the
452 characteristics of the *S. melongena* parent was observed in the BC1 generation, although
453 an important segregation was observed for all the characters in which the parents
454 differed, except for the number of parts of the flower, which was consistently five, as in
455 the wild parent. Amazingly, the fertility of pollen increased considerably, with an
456 average of 19.4% and a minimum value of 7.4% in one of the five plants characterized,
457 which suggests a rapid recovery of fertility already in this first generation of
458 backcrossing, as described in other crops (Wall, 1970; Prohens et al., 2017).
459 Although the aerial part of interspecific hybrids is smaller than that of the *S.*
460 *melongena* parent, the main root of both materials has a similar length. In addition, the
461 fact that the relative density of lateral roots in the main root of the hybrid is smaller than
462 that of *S. melongena* suggests that the hybrid explores other areas of the soil (Chen et
463 al., 2014), whereas the *S. melongena* root system is mostly concentrated in the area
464 where the drip irrigation system supplies water and nutrients. This suggests that an
465 improved eggplant root system can be obtained through introgression from *S.*
466 *elaeagnifolium* which, apart from being drought tolerant (Christodoulakis et al., 2009),
467 has a rhizomatous root system (Knapp et al., 2017)
468 Wild species of eggplant generally present a more diverse phenolic profile that
469 that of *S. melongena*, in which chlorogenic acid is the main component (Stommel and
470 Whitaker, 2003; Whitaker and Stommel, 2003; Prohens et al., 2013). In our case, the

profile of phenolic compounds shows that *S. elaeagnifolium* and *S. melongena* are also considerably different. In addition, *S. elaeagnifolium* presents a greater total peak area in the chromatogram, while the hybrid presents an intermediate profile, although more similar to that of *S. elaeagnifolium*. This suggests that *S. elaeagnifolium* can contribute to improving the content of phenolic bioactive compounds of eggplant (Kaushik et al., 2015) without lowering the chlorogenic acid content. In this way, the use of another New World species (*S. viarum*) has been suggested as a potential source of variation to improve the caffeoylquinic acid content and its derivatives in eggplant (Wu et al., 2012).

In conclusion in this study we present for the first time, to our knowledge, the development of backcross generations of a hybrid between eggplant and a wild relative from the New World belonging to its tertiary germplasm pool (Kouassi et al., 2016). Our results suggest that these introgression materials will be of great interest for the genetic improvement of eggplant; they may have an tremendous potential to increase tolerance to abiotic stresses, such as to drought by improving the eggplant root system, as well as by enhancing its bioactive properties by increasing the contents in bioactive phenolics and modifying its profile (Kaushik et al., 2015). In addition, the introgression materials may also contribute to other traits that remain unexplored in *S. elaeagnifolium*, such as tolerance to pests and diseases. Also, because *S. elaeagnifolium* is not phylogenetically closely related to *S. melongena* (Vorontsova et al., 2013) the introgression materials obtained may represent an appropriate model to study epigenetic modifications occurring in the genome following distant hybridization and introgression breeding (Wang et al., 2005; Dong et al., 2006). Finally, we hope that this seminal study opens the way for the incorporation of the *Elaeagnifolium* clade New World genepool (Knapp et al., 2017) for eggplant breeding, ultimately contributing to the development of a new generation of plants adapted to climate change and with improved nutritional and diseases and pest resistance properties.

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References

Acosta, M., Bernardello, G., Guerra, M., Moscone, E., 2005. Karyotype analysis in several South American species of *Solanum* and *Lycianthes rantonnei* (Solanaceae). Taxon 54, 713–723.

Afful, N.T., Nyadanu, D., Akromah, R., Amoatey, H. M., Annor, C., Diawouh R. G., 2018. Evaluation of crossability studies between selected eggplant accessions with wild relatives *S. torvum*, *S. anguivi* and *S. aethiopicum* (Shum group). J. Plant Breed. Crop Sci. 10, 1–12.

Arao, T., Takeda, H., Nishihara, E., 2008. Reduction of cadmium translocation from roots to shoots in eggplant (*Solanum melongena*) by grafting onto *Solanum torvum* rootstock. Soil Sci. Plant Nutr. 54, 555–559.

Aref A., 1992. Determination of pollen viability in tomatoes. J. Amer. Soc. Hort. Sci. 117, 473–476.

Aubriot, X., Singh, P., Knapp, S., 2016. Tropical Asian species show that the Old World clade of 'spiny solanums' (*Solanum* subgenus *Leptostemonum pro parte*: Solanaceae) is not monophyletic. Bot. J. Linn. Soc. 181, 199–223.

539 Chen, X., Zhang, J., Chen, Y., Li, Q., Chen, F., Yuan, L., Mi, G., 2014. Changes in root
540 size and distribution in relation to nitrogen accumulation during maize breeding in
541 China. Plant Soil 374, 121–130.
542 Christodoulakis, N.S., Lampri, P.N., Fasseas, C., 2009. Structural and cytochemical
543 investigation of the leaf of silverleaf nightshade (*Solanum elaeagnifolium*), a
544 drought-resistant alien weed of the Greek flora. Aust. J. Bot. 57, 432.
545 Collonnier, C., Fock, I., Mariska, I., Servaes, A., Vedel, F., Siljak-Yakovlev, S.,
546 Souvannavong, V., Sihachakr, D., 2003. GISH confirmation of somatic hybrids
547 between *Solanum melongena* and *S. torvum*: assessment of resistance to both
548 fungal and bacterial wilts. Plant Physiol. Biochem. 41, 459–470.
549 Çürük, S., Dayan, A., 2017. Morphological characteristics of interspecific hybrids of
550 *Solanum melongena* and *Solanum torvum*. Mediterr. Agric. Sci. 30, 179–188.
551 Daunay, M.C., Chaput, M.H., Sihachakr, D., Allot, M., Vedel, F., Ducreux, G., 1993.
552 Production and characterization of fertile somatic hybrids of eggplant (*Solanum*
553 *melongena L.*) with *Solanum aethiopicum* L. Theor. Appl. Genet. 85, 841–850.
554 Dempewolf, H., Eastwood, R.J., Guarino, L., Khoury, C.K., Müller, J. V., Toll, J., 2014.
555 Adapting agriculture to climate change: A global initiative to collect, conserve, and
556 use crop wild relatives. Agroecol. Sustain. Food Syst. 38, 369–377.
557 Dixon, R.A., Harrison, M.J., 1990. Activation, structure, and orga- nization of genes
558 involved in microbial defense in plants. Adv. Genet. 28, 165–234.
559 Dong, Z.Y., Wang, Y.M., Zhang, Z.J., Shen, Y., Lin, X.Y., Ou, X.F., Han, F.P., Liu, B.,
560 2006. Extent and pattern of DNA methylation alteration in rice lines derived from
561 introgressive hybridization of rice and *Zizania latifolia* Griseb. Theor. Appl. Genet.
562 113, 196-205.
563 Dpooležel, J., Binarová, P., Lcretti, S., 1989. Analysis of nuclear DNA content in plant
564 cells by flow cytometry. Biol. Plant. 31, 113–120.
565 Dwivedi, S.L., Upadhyaya, H.D., Stalker, H.T., Blair, M.W., Bertioli, D.J., Nielen, S.,
566 Ortiz, R., 2008. Enhancing crop gene pools with beneficial traits using wild
567 relatives, in: Plant Breeding Reviews. John Wiley & Sons, Inc., Hoboken, NJ,
568 USA, pp. 179–230.
569 FAO, 2018. FAOSTAT Food and Agriculture Data. Available at
570 http://www.fao.org/faostat/ (Accessed October 6, 2018).
571 Gisbert, C., Prohens, J., Nuez, F., 2012. Performance of eggplant grafted onto
572 cultivated, wild, and hybrid materials of eggplant and tomato. Int. J. Plant. Sci. 5,

367–380.
Gleddie, S., Keller, W.A., Setterfield, G., 1986. Production and characterization of somatic hybrids between *Solanum melongena* L. and S. *sisymbriifolium* Lam. Theor. Appl. Genet. 71, 613–621.
Gramazio, P., Prohens, J., Plazas, M., Mangino, G., Herraiz, F.J., Vilanova, S., 2017. Development and genetic characterization of advanced backcross materials and an introgression line population of *Solanum incanum* in a *S. melongena* background. Front. Plant Sci. 8, 1477.
Gramazio, P., Prohens, J., Plazas, M., Mangino, G., Herraiz, F.J., García-Fortea, E., Vilanova, S., 2018. Genomic tools for the enhancement of vegetable crops: A case in eggplant. Not. Bot. Horti Agrobot. 46, 1–13.
Hahlbrock, K., Scheel, D., 1989. Hahlbrock, K., Scheel, D., 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annu. Rev. Plant Physiol. Mol. Biol. 40, 347–369.
Heslop-Harrison, J., Heslop-Harrison, Y., Shivanna, K.R., 1984. The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67, 367–375.
Hunter, D., Heywood, V.H. (Eds.), 2011. Crop Wild Relatives: A Manual of *In Situ* Conservation. Earthscan, London.
IBPGR, 1990. Descriptors for Eggplant. International Board for Plant Genetic Resources, Rome.
Isshiki, S., Taura, T., 2003. Fertility restoration of hybrids between *Solanum melongena* L. and *S. aethiopicum* L. Gilo Group by chromosome doubling and cytoplasmic effect on pollen fertility. Euphytica 134, 195–201.
Jarl, C.I., Rietveld, E.M., de Haas, J.M., 1999. Transfer of fungal tolerance through interspecific somatic hybridisation between *Solanum melongena* and *S. torvum*. Plant Cell Rep. 18, 791–796.
Kashyap, V., Vinod Kumar, S., Collonnier, C., Fusari, F., Haicour, R., Rotino, G.., Sihachakr, D., Rajam, M., 2003. Biotechnology of eggplant. Sci. Hort. 97, 1–25.
Kaushik, P., Andújar, I., Vilanova, S., Plazas, M., Gramazio, P., Herraiz, F., Brar, N., Prohens, J., 2015. Breeding vegetables with increased content in bioactive phenolic acids. Molecules. 20, 18464–18481.
Kaushik, P., Prohens, J., Vilanova, S., Gramazio, P., Plazas, M., 2016. Phenotyping of Eggplant wild relatives and interspecific hybrids with conventional and phenomics

607 descriptors provides insight for their potential utilization in breeding. Front. Plant
608 Sci. 7, 677.
609 King, S.R., Davis, A.R., Zhang, X., Crosby, K., 2010. Genetics, breeding and selection
610 of rootstocks for Solanaceae and Cucurbitaceae. Sci. Hort. 127, 106-111.
611 Knapp, S., Sagona, E., Carbonell, A.K.Z., Chiarini, F., 2017. A revision of the *Solanum*
612 *elaeagnifolium* clade (*Elaeagnifolium* clade; subgenus *Leptostemonum*,
613 Solanaceae). PhytoKeys 84, 1–104.
614 Knapp, S., Vorontsova, M.S., Prohens, J., 2013. Wild relatives of the eggplant (*Solanum*
615 *melongena* L.: Solanaceae): New understanding of species names in a complex
616 group. PLOS ONE 8, e57039.
617 Kouassi, B., Prohens, J., Gramazio, P., Kouassi, A.B., Vilanova, S., Galán-Ávila, A.,
618 Herraiz, F.J., Kouassi, A., Seguí-Simarro, J.M., Plazas, M., 2016. Development of
619 backcross generations and new interspecific hybrid combinations for introgression
620 breeding in eggplant (*Solanum melongena*). Sci. Hort. 213, 199–207.
621 Kreike, C.M., Stiekema, W.J., 1997. Reduced recombination and distorted segregation
622 in a *Solanum tuberosum* (2x) × *S* . *spegazzinii* (2x) hybrid. Genome 40, 180–187.
623 Lester, R.N., 1986. Taxonomy of scarlet eggplants, *Solanum aethiopicum L*. Acta Hort.
624 125–132.
625 Lester, R.N., Kang, J.H., 1998. Embryo and endosperm function and failure in *Solanum*
626 species and hybrids. Ann. Bot. 82, 445–453.
627 Liu, J., Zheng, Z., Zhou, X., Feng, C., Zhuang, Y., 2015. Improving the resistance of
628 eggplant (*Solanum melongena*) to *Verticillium* wilt using wild species *Solanum*
629 *linnaeanum*. Euphytica 201, 463–469.
630 Mekki, M., 2007. Biology, distribution and impacts of silverleaf nightshade (*Solanum*
631 *elaeagnifolium* Cav.). EPPO Bull. 37, 114–118.
632 Meyer, R.S., Karol, K.G., Little, D.P., Nee, M.H., Litt, A., 2012. Phylogeographic
633 relationships among Asian eggplants and new perspectives on eggplant
634 domestication. Mol. Phylogenet. Evol. 63, 685–701.
635 Moscone E., 1992. Estudios sobre cromosomas meióticos en Solanaceae de Argentina.
636 Darwiniana 39, 668–687.
637 Noda, N., Kanno, Y., Kato, N., Kazuma, K., Suzuki, M., 2004. Regulation of gene
638 expression involved in flavonol and anthocyanin biosynthesis during petal
639 development in lisianthus (*Eustoma grandiflorum*). Physiol. Plant. 122, 305–313.
640 Plazas, M., Prohens, J., Cuñat, A.N., Vilanova, S., Gramazio, P., Herraiz, F.J., Andújar,

641 I., 2014. Reducing capacity, chlorogenic acid content and biological activity in a
642 collection of scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*)
643 eggplants. Int. J. Mol. Sci. 15, 17221–17241.
644 Plazas, M., Vilanova, S., Gramazio, P., Rodriguez-Burruezo, A., Rajakapasha, R.,
645 Ramya, F., Niran, L., Fonseka, H., Kouassi, B., Kouassi, A., Kouassi, A., Prohens,
646 J., 2016. Interspecific hybridization between eggplant and wild relatives from
647 different genepools. J. Am. Soc. Hortic. Sci. 141, 34–44.
648 Powell, A., Weedin, J., 2005. Documented chromosome numbers 2005: 2. Counts from
649 western Texas, mostly trans-Pecos cacti. Sida 21, 1665–1668.
650 Prabhu, M., Natarajan, S., Veeraragavathatham, D., Pugalendhi, L., 2009. The
651 biochemical basis of shoot and fruit borer resistance in interspecific progenies of
652 brinjal (*Solanum melongena*). EurAsia J. BioSci. 3, 50–57.
653 Prohens, J., Gramazio, P., Plazas, M., Dempewolf, H., Kilian, B., Díez, M.J., Fita, A.,
654 Herraiz, F.J., Rodríguez-Burruezo, A., Soler, S., Knapp, S., Vilanova, S., 2017.
655 Introgressiomics: a new approach for using crop wild relatives in breeding for
656 adaptation to climate change. Euphytica 213, 158.
657 Prohens, J., Plazas, M., Raigón, M.D., Seguí-Simarro, J.M., Stommel, J.R., Vilanova,
658 S., 2012. Characterization of interspecific hybrids and first backcross generations
659 from crosses between two cultivated eggplants (*Solanum melongena* and *S.*
660 *aethiopicum* Kumba group) and implications for eggplant breeding. Euphytica 186,
661 517-538.
662 Prohens, J., Whitaker, B.D., Plazas, M., Vilanova, S., Hurtado, M., Blasco, M.,
663 Gramazio, P., Stommel, J.R., 2013. Genetic diversity in morphological characters
664 and phenolic acids content resulting from an interspecific cross between eggplant,
665 *Solanum melongena*, and its wild ancestor (*S. incanum*). Ann. Appl. Biol. 162,
666 242-257.
667 Ranil, R.H.G., Niran, H.M.L., Plazas, M., Fonseka, R.M., Fonseka, H.H., Vilanova, S.,
668 Andújar, I., Gramazio, P., Fita, A., Prohens, J., 2015. Improving seed germination
669 of the eggplant rootstock *Solanum torvum* by testing multiple factors using an
670 orthogonal array design. Sci. Hort. 193, 174–181.
671 Rotino, G.L., Sala, T., Toppino, L., 2014. Eggplant, in: Alien Gene Transfer in Crop
672 Plants, Volume 2. Springer, New York, NY, pp. 381–409.
673 Sabatino, L., Iapichino, G., D'Anna, F., Palazzolo, E., Mennella, G., Rotino, G.L.,
674 2018. Hybrids and allied species as potential rootstocks for eggplant: Effect of

675 grafting on vigour, yield and overall fruit quality traits. Sci. Hort. 228, 81-90.
676 Scaldaferro, M., Chiarini, F., Santiñaque, F., Bernardello, G., Moscone, E., 2012.
677 Geographical pattern and ploidy levels of the weed *Solanum elaeagnifolium*
678 (Solanaceae) from Argentina. Genet. Resour. Crop Evol. 59, 1833–1847.
679 Shichijo, C., Hamada, T., Hiraoka, M., Johnson, C.B., Hashimoto, T., 1993.
680 Enhancement of red-light-induced anthocyanin synthesis in sorghum first
681 internodes by moderate low temperature given in the pre-irradiation culture period.
682 Planta 191, 238–245.
683 Sihachakr, D., Haicour, R., Chaput, M.-H., Barrientos, E., Ducreux, G., Rossignol, L.,
684 1989. Somatic hybrid plants produced by electrofusion between *Solanum*
685 *melongena* L. and *Solanum torvum* Sw. Theor. Appl. Genet. 77, 1–6.
686 Stommel, J.R., Whitaker, B.D., 2003. Phenolic acid content and composition of
687 eggplant fruit in a germplasm core subset. J. Amer. Soc. Hort. Sci. 128, 704–710.
688 Toppino, L., Valè, G., Rotino, G.L., 2008. Inheritance of *Fusarium* wilt resistance
689 introgressed from *Solanum aethiopicum* Gilo and *Aculeatum* groups into cultivated
690 eggplant (*S. melongena*) and development of associated PCR-based markers. Mol.
691 Breed. 22, 237–250.
692 Tyack, N., Dempewolf, H., 2015. The economics of crop wild relatives under climate
693 change, in: Crop Wild Relatives and Climate Change. John Wiley & Sons, Inc.,
694 Hoboken, NJ, USA, pp. 281–291.
695 van der Weerden, G.M., Barendse, G.W.M., 2007. A web- searchable database
696 developed for the EGGNET project and applied to the Radboud University
697 Solanaceae database. Acta Hortic. 745, 503–506.
698 Varoquaux, F., Blanvillain, R., Delseny, M., Gallois, P., 2000. Less is better: new
699 approaches for seedless fruit production. Trends Biotechnol. 18, 233–242.
700 Vorontsova, M.S., Knapp, S., 2016. A revision of the "spiny *Solanums*" *Solanum*
701 subgenus *Leptostemonum* (Solanaceae), in Africa and Madagascar. Syst. Bot.
702 Monogr 99, 1–432.
703 Vorontsova, M.S., Stern, S., Bohs, L., Knapp, S., 2013. African spiny *Solanum*
704 (subgenus *Leptostemonum* , Solanaceae): a thorny phylogenetic tangle. Bot. J.
705 Linn. Soc. 173, 176–193.
706 Wall, J., 1970. Experimental introgression in the genus *Phaseolus.* I. Effect of mating
707 systems on interspecific gene flow. Evolution. 24, 356–366.
708 Wang, Y.M., Dong, Z.Y., Zhang, Z.J., Lin, X.Y., Shen, Y., Zhou, D., Liu, B., 2005.

709 Extensive de novo genomic variation in rice induced by introgression from wild
710 rice (*Zizania latifolia* Griseb.). Genetics 170, 1945–1956.
711 Whitaker, B.D., Stommel, J.R., 2003. Distribution of hydroxycinnamic acid conjugates
712 in fruit of commercial eggplant (*Solanum melongena* l.) cultivars. J. Agric. Food
713 Chem. 51, 3448–3454.
714 Wu, S.B., Meyer, R.S., Whitaker, B.D., Litt, A., Kennelly, E.J., 2012. Antioxidant
715 glucosylated caffeoylquinic acid derivatives in the invasive tropical soda apple,
716 *Solanum viarum*. J. Nat. Prod. 75, 2246–2250.
717 Zhou, X., Bao, S., Liu, J., Yang, Y., Zhuang, Y., 2018. Production and characterization
718 of an amphidiploid derived from interspecific hybridization between *Solanum*
719 *melongena* L. and *Solanum aculeatissimum* Jacq. Sci. Hort. 230, 102–106.
720

721 **Table 1**

722 Descriptor states for the qualitative traits evaluated in *S. melongena* MEL3 (P1), *S. elaeagnifolium* ELE2 (P2), the interspecific hybrid *S.*
723 *melongena* MEL3 × *S. elaeagnifolium* ELE2 (F1), and first backcross (BC1) towards *S. melongena* (BC1) generations. For each generation, five
724 plants (n=5) were evaluated. Where segregation was observed within generation the numbers of plant of each class are indicated.

Trait	P1	P2	F1	BC1
Vegetative part				
Plant growth habit	Upright	Intermediate	Upright	Upright
Prickle color	-	-	Green	Green
Leaf				
Leaf blade lobing	Strong	Weak	Intermediate	1 Intermediate : 3 Strong : 1 Weak
Leaf surface	Flat	Flat	Flat	Flat
Inflorescence and flower				
Corolla color	Light violet	Bluish violet	Light violet	2 Pale violet : 3 Light violet
Style curvature	Straight	Curved	Curved	4 Curved : 1 Straight
Presence of anthocyanins in pistil	No	No	3 Yes : 2 No	4 Yes : 1 No
Fruit				
Fruit apex shape	Rounded	Rounded	Rounded	Rounded
Firmness in the wide part	Very firm	Very firm	Firm	Very firm
Size of the stylar scar	Small	Small	Small	Small

Fruit predominant color (at commercial ripeness)	Green	Green	Green	Green
Fruit predominant color (at physiological ripeness)	Yellow-Orange	Orange	Orange	Yellow-Orange
Fruit predominant color intensity (at commercial ripeness)	Clear	Clear	Dark	Clear
Fruit additional color (at commercial ripeness)	Dark green	Dark green	Dark green	Dark green
Fruit additional color distribution	Striped	Striped	Striped	Striped
Fruit flesh color (cut fruit at commercial ripeness)	White	Green	Intermediate	Intermediate
Fruit calyx color	Green	Green	Green	Green
Fruit color intensity under calyx	Medium	Medium	Medium	Medium

725

726

Table 2

Mean value, standard error and range of the morphological quantitative traits evaluated in *S. melongena* MEL3 (P1), *S. elaeagnifolium* ELE2 (P2), the interspecific hybrid *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 (F1), and first backcross (BC1) towards *S. melongena* (BC1) generations. For each generation, five plants (n=5) were evaluated.

	P1		P2		F1		BC1	
Trait	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Vegetative part								
Plant height (cm)	205 ± 4	190-215	84 ± 10	50-108	109 ± 12	80-140	117 ± 21	87-200
Stem diameter (cm)	4.5 ± 0.3	4.0-5.5	3.3 ± 0.3	3.0-4.0	2.9 ± 0.1	2.5-3.0	2.7 ± 0.4	1.5-4.0
Prickles between nodes (n)	0.0 ± 0.0	0-0	0.0 ± 0.0	0-0	2.8 ± 0.4	2-4	0.3 ± 0.2	0-1
Leaf								
Leaf prickles[a]	0 ± 0	0-0	0 ± 0	0-0	1 ± 0	1-1	0 ± 0	0-0
Length of the largest prickle (cm)	-	-	-	-	0.1 ± 0.0	0.1-0.1	-	-
Leaf pedicel length (cm)	6.4 ± 0.2	6.0-7.0	1.6 ± 0.3	1.0-2.5	3.1 ± 0.2	2.5-3.5	3.2 ± 0.6	2.0-5.5
Leaf apex angle[b]	3 ± 0	3-3	5 ± 0	5-5	3 ± 0	3-3	5 ± 0	5-5
Leaf blade length (cm)	15.8 ± 0.4	15.0-17.0	8.5 ± 0.2	8.0-9.0	11.9 ± 1.2	8.5-15.0	13.2 ± 1.0	11.0-17.0
Leaf blade width (cm)	9.4 ± 0.2	9.0-10.0	2 ± 0	1.8-2.2	6.1 ± 0.4	5.5-7.0	6.9 ± 0.6	6.0-9.0
Inflorescence and flower								
Flowers/inflorescence (cm)	4.4 ± 0.2	4-5	4.8 ± 0.2	4-5	7.6 ± 0.4	6-8	4.8 ± 0.6	3-6
Petals/flower	5.4 ± 0.3	5.2-5.5	5 ± 0	5-5	5 ± 0	5-5	5 ± 0	5-5

Sepals/flower	5.2 ± 0.1	5.0-5.3	5 ± 0	5-5	5 ± 0	5-5	5 ± 0	5-5
Stamens/flower	5.5 ± 0.4	5.2-5.6	5 ± 0	5-5	5 ± 0	5-5	5 ± 0	5-5
Corolla diameter (cm)	4.9 ± 0.1	4.5-5.0	3.7 ± 0.1	3.5-4.0	2.4 ± 0.1	2.0-2.5	2.5 ± 0.5	1.5-4.5
Pollen viability (%)	91.5 ± 2.4	84.2-97.3	50.7 ± 4.3	41.7-61.4	2.6 ± 0.2	2.3-2.8	19.4 ± 8.7	7.4-53.3
Fruit								
Fruit length (cm)	11.8 ± 0.6	11.0-14.0	0.75 ± 0.16	0.5-1.0	1.25 ± 0.13	1.0-1.5	2.75 ± 0.52	1.5-4.0
Fruit width (cm)	5.4 ± 0.5	4.0-7.0	0.85 ± 0.09	0.7-1.0	1.25 ± 0.13	1.0-1.5	2.0 ± 0.0	2.0-2.0
Fruit pedicel length (cm)	6.0 ± 1.0	5.0-7.0	2.3 ± 0.2	2.0-2.5	1.8 ± 0.1	1.5-2.0	2.0 ± 0.0	2.0-2.0
Fruit pedicel thickness (cm)	1.0 ± 0.0	1.0-1.0	0.35 ± 0.03	0.3-0.4	0.3 ± 0.0	0.3-0.3	0.4 ± 0.1	0.3-0.5
Relative fruit calyx length[c]	1 ± 0	1-1	3 ± 0	3-3	3 ± 0	3-3	1 ± 0	1-1
Fruit calyx prickles (n)	0 ± 0	0-0	5 ± 0	5-5	5 ± 0	5-5	3.75 ± 2.14	0-10

731 [a]Measured in a scale (0=Absent; 1= 1 to 2; 3= 3 to 5; 5= 6 to 10; 7: 11 to 20; 9= More than 20).

732 [b]Measured in a scale (1= Less than 15^{o}; 3=aprox. 45^{o}; 5= aprox. 75^{o}; 7= aprox. 110^{o}; 9= aprox. 160^{o}).

733 [c]Measured in a scale (0=Less than 10%; 3=aprox. 20%; 5=aprox. 50%; 7=aprox. 70%; 9=More than 75%).

734 **Table 3**

735 Mean value, standard error and range of the root morphological traits evaluated in *S.*
736 *melongena* MEL3 (P1), and interspecific hybrid *S. melongena* MEL3 × *S.*
737 *elaeagnifolium* ELE2 (F1). For each generation, three plants (n=3) were evaluated.

	P1		F1	
Trait	Mean	Range	Mean	Range
Whorl angle (°)	136.0 ± 3.0	130-140	151.3 ± 5.9	140-160
Main root length (cm)	42.7 ± 0.9	28-68	54.0 ± 2.0	33-66
Main root diameter (mm)	8.7 ± 0.2	8-9	5.0 ± 0.0	5-5
Relative density of laterals in the main root	Intermediate		Low	

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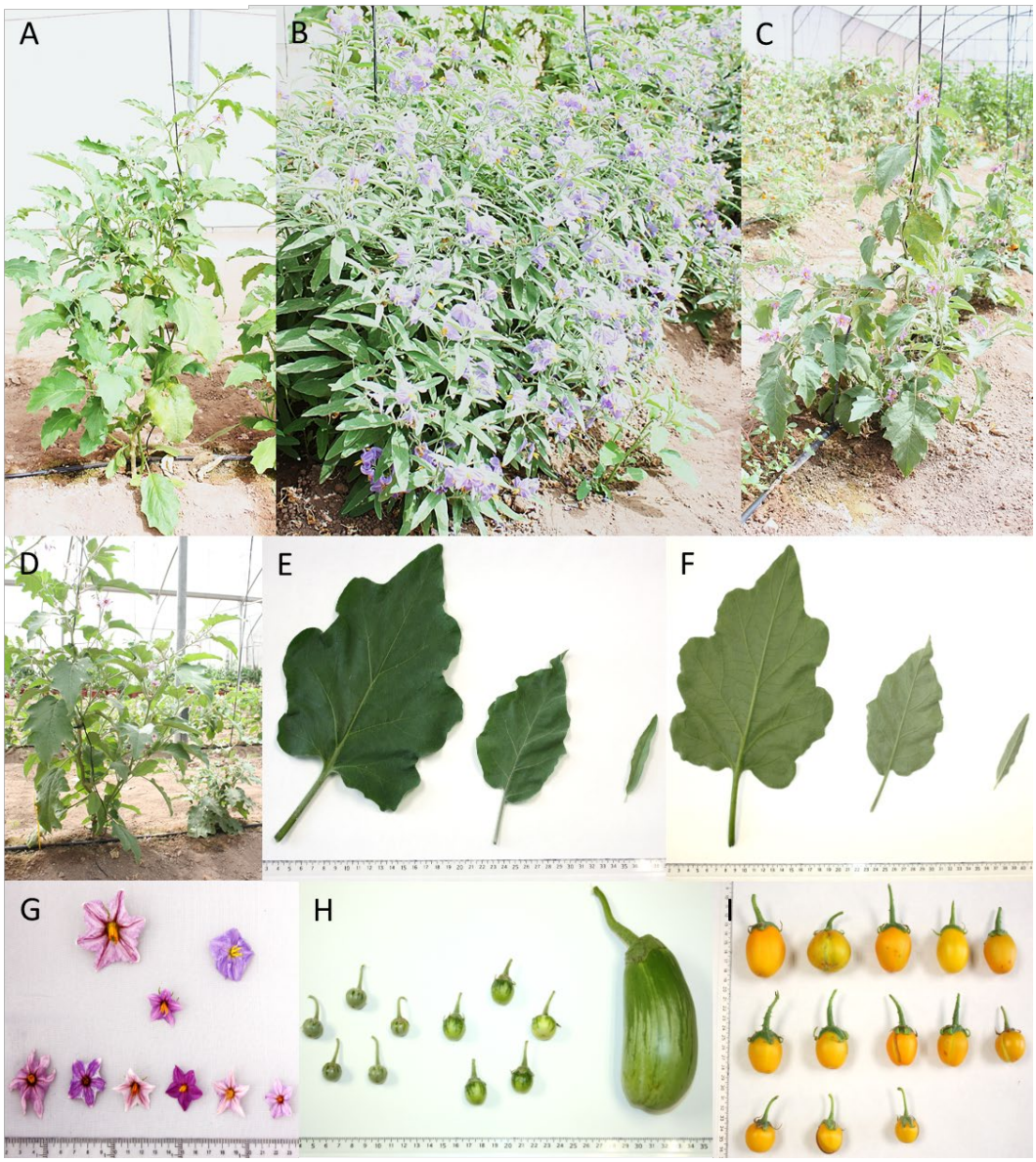

Figure 1

Morphology of plant materials evaluated: *S. melongena* MEL3 plant (A); *S. elaeagnifolium* ELE2 plant (B); *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 interspecific hybrid (F1) plant (C); two plants of the first backcross (BC1) of the F1 interspecific hybrid towards *S. melongena* displaying extreme difference in plant size (D); adaxial part of the leaf of *S. melongena* (left), F1 interspecific hybrid (center) and *S. elaeagnifolium* (right) (E); abaxial part of the leaf of *S. melongena* (left), F1 interspecific hybrid (center) and *S. elaeagnifolium* (right) (F); Flowers of *S. melongena* (left), F1 interspecific hybrid (center), *S. elaeagnifolium* (right) and BC1 individuals (below) (G); Fruits of *S. elaeagnifolium* (left), F1 interspecific hybrid (center) and *S. melongena* (right) (H); Segregation for fruit size and shape in physiologically mature fruits of the first backcross (BC1) of the F1 interspecific hybrid towards *S. melongena* (I). Scale in cm.

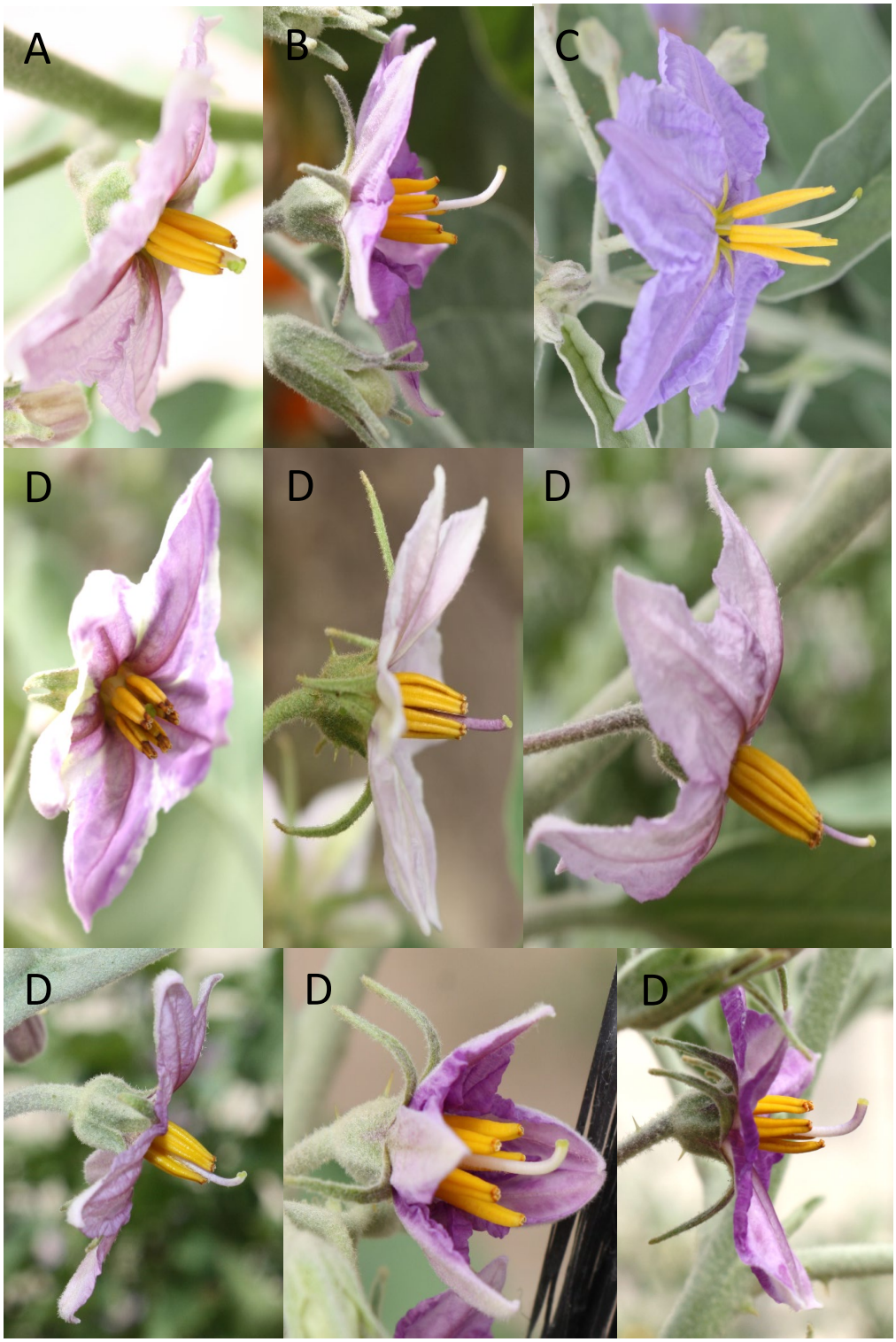

Figure 2

Variation for flower morphology in: *S. melongena* MEL3 (A); *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 interspecific hybrid (B); *S. elaeagnifolium* ELE2 (C); Flowers of different plants of the first backcross (BC1) of the F1 interspecific hybrid towards *S. melongena* (D). Large variation is observed in BC1 plants for flower size, corolla color, style length, curvature and color, anthers length, and opening of the anthers cone.

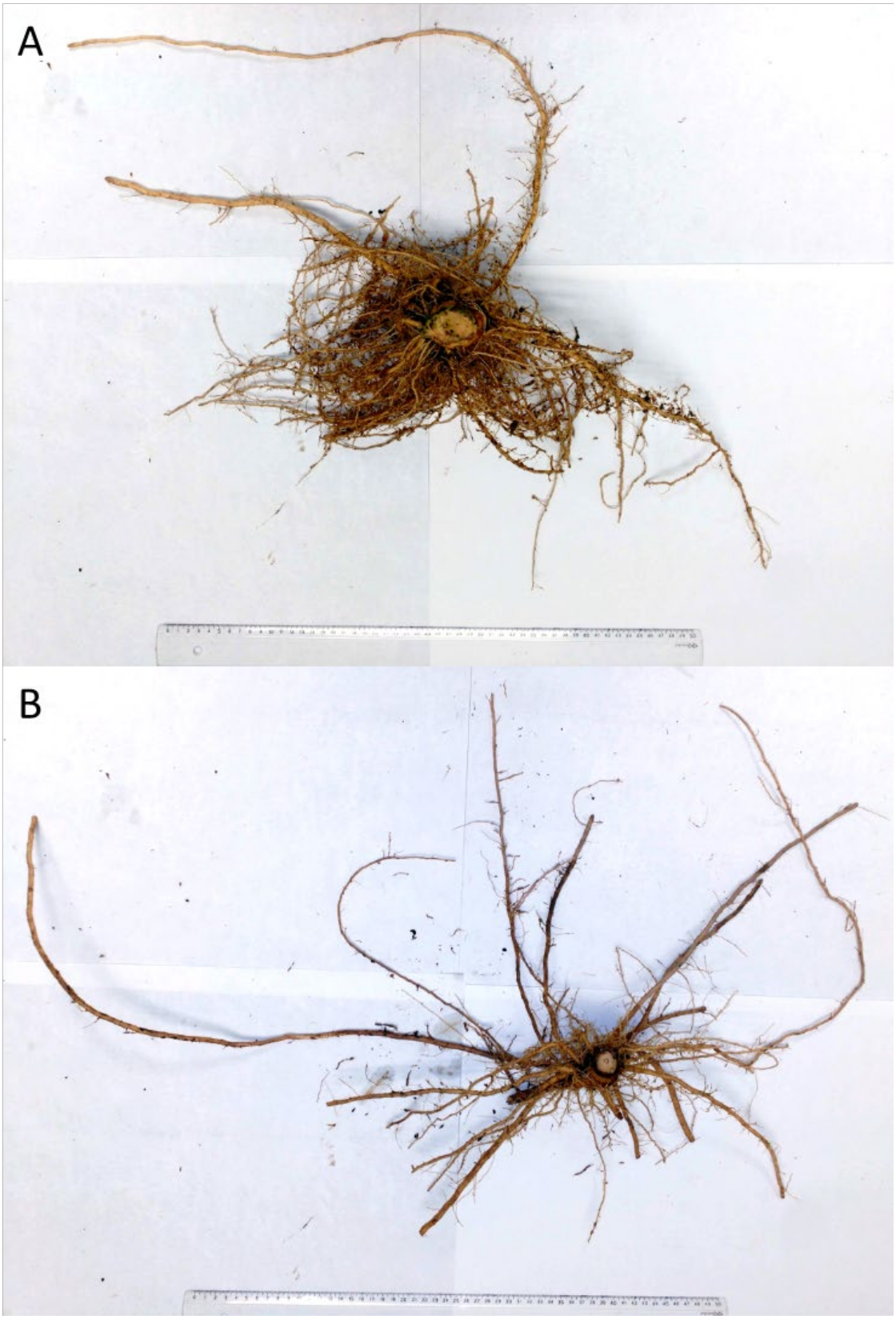

Figure 3

Root morphology in: *S. melongena* MEL3 (A) and F1 interspecific hybrid *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 (B).

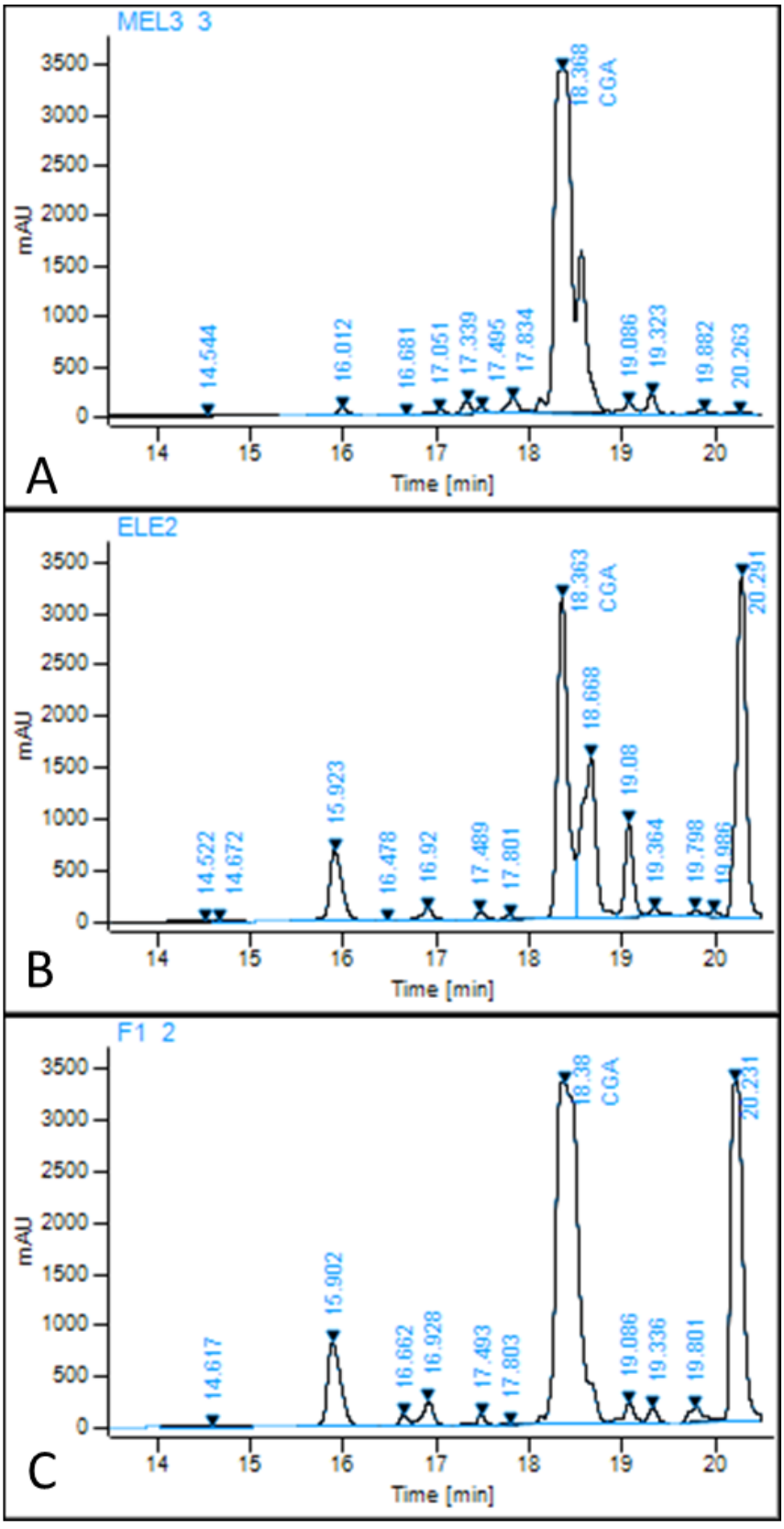

Figure 4

Phenolics profile of fruits of *S. melongena* MEL3 (A), *S. elaeagnifolium* ELE2 (B), and *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 F1 hybrid (C).

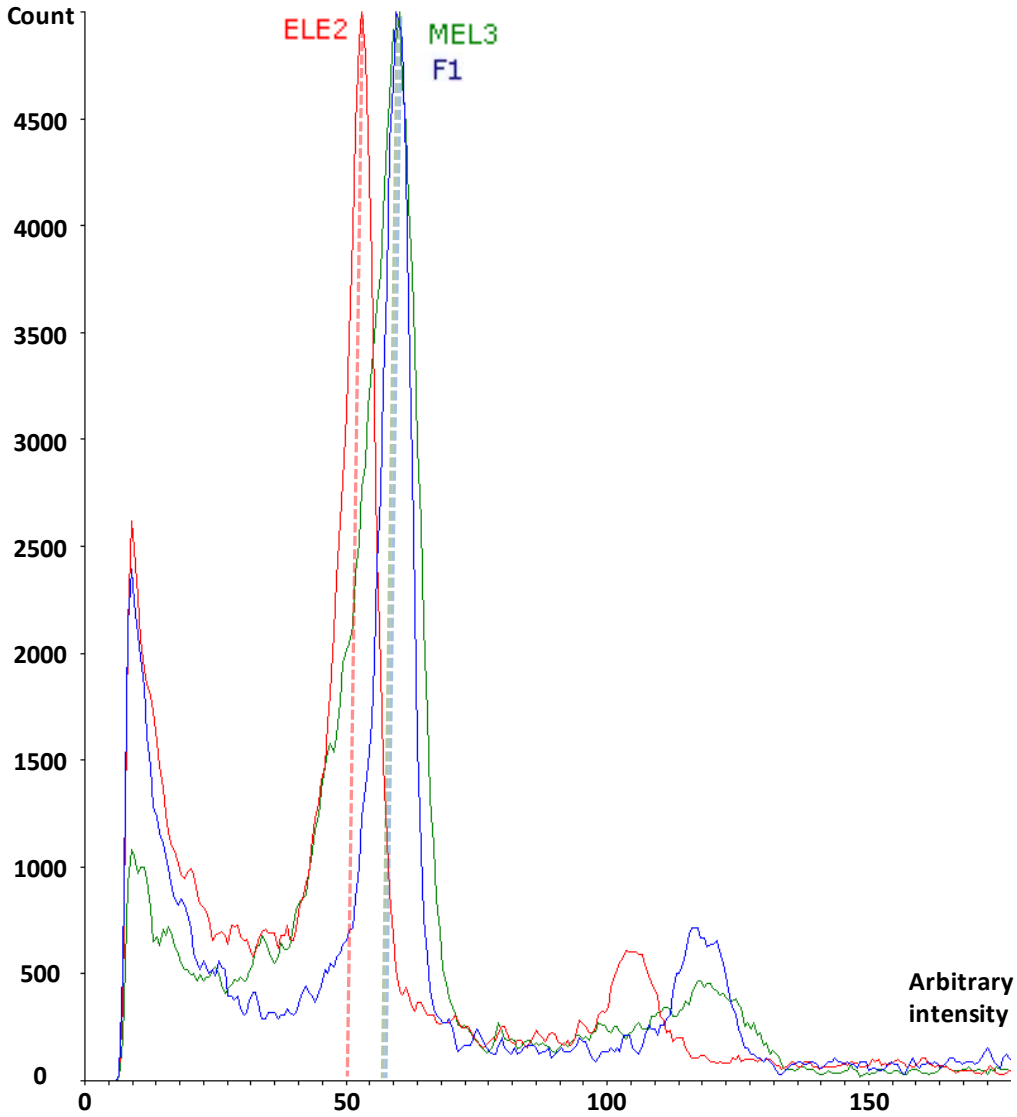

Figure 5

Flow cytometry histogram of the relative nuclear DNA contents of: *S. melongena* MEL3 (green), *S. elaeagnifolium* ELE2 (red) and F1 interspecific hybrid *S. melongena* MEL3 × *S. elaeagnifolium* ELE2 (blue). The *x*-axis represents the proportional fluorescence intensity level to the nuclear DNA quantity; the position of the main peak reflects the ploidy level. The *y*-axis indicates the number of nuclei analyzed.