



## Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*)



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### ABSTRACT

Introgression breeding can contribute to broadening the genetic background of eggplant (*Solanum melongena*). We used six eggplant varieties and 44 interspecific hybrids between these eggplant accessions and 10 accessions of wild relatives from the primary gene pool species *S. insanum* and secondary gene pool species *S. anguivi*, *S. dasphyllum*, *S. incanum*, *S. lichtensteinii*, and *S. tomentosum* to obtain first backcross generations to *S. melongena*. Pollen viability in cultivated and wild parents and interspecific hybrids with *S. insanum* was high, while for interspecific hybrids with secondary gene pool species it was variable. First backcross generations to *S. melongena* were obtained with interspecific hybrids of all the wild species, with the best results being obtained in crosses with hybrids between *S. melongena* and *S. insanum*. However, ample differences were observed among eggplant varieties in the success of the crosses. Additionally, the six eggplant varieties were crossed with secondary gene pool species *S. campylacanthum*, *S. lidii* and *S. vespertilio* and with tertiary gene pool species *S. bonariense*, *S. elaeagnifolium* and *S. sisymbriifolium* with the aim of obtaining new interspecific hybrids. Successful interspecific hybridization was achieved with the three new secondary gene pool species tested and, using embryo rescue, with the tertiary gene pool species *S. elaeagnifolium*. The new backcross generations and interspecific hybrids obtained will contribute to broadening the genetic background of the eggplant and to the genetic enhancement of this crop.

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### 1. Introduction

Despite the large morphological diversity of cultivated eggplant (*Solanum melongena* L.) (Portis et al., 2015; Kaushik et al., 2016) its genetic diversity is narrow, in particular in the modern cultivars (Muñoz-Falcón et al., 2009). The low genetic diversity of eggplant, compared to its wild relatives (Furini and Wunder, 2004; Vorontsova et al., 2013), is very likely a result of the genetic bottleneck during domestication from its ancestor, the weedy *S. insanum*,

in Southeast Asia and China (Meyer et al., 2012). Although introgression from *S. insanum* has probably been common during the evolution of the crop (Davidar et al., 2015), artificial selection coupled with limited genetic diversity of *S. insanum* has contributed to maintaining a narrow genetic background of eggplant.

Depending on the phylogenetic relationships with eggplant and potential for hybridization with eggplant, its crop wild relatives are assigned to the primary, secondary or tertiary gene pools (Syfert et al., 2016). *Solanum insanum* is the only species included in the primary gene pool of eggplant (Knapp et al., 2013). However, eggplant is also related to a large number of Old World species of *Solanum* section *Leptostemonum*, most of which are native to Africa, where the greatest diversity of wild relatives of eggplant can be found (Weese and Bohs, 2010; Vorontsova et al., 2013; Aubriot et al., 2016). In this respect, around 50 wild species of the Anguivi, Melon-gena and Climbing phylogenetic groups of the eggplant secondary gene pool have been identified as potentially most interesting as

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genetic resources for eggplant breeding (Syfert et al., 2016). Also, a number of other *Solanum* species, some of them from the New World, are part of the tertiary gene pool species of eggplant (Daunay and Hazra, 2012; Syfert et al., 2016). Among them, *S. torvum* and *S. sisymbriifolium* have been traditionally considered as very interesting for eggplant breeding due to their multiple resistances to the most important eggplant fungal and bacterial diseases, like those caused by *Fusarium oxysporum*, *Verticillium dahliae*, or *Ralstonia solanacearum*, as well as to nematodes (Bletsos et al., 2003; Gousset et al., 2005; Daunay and Hazra, 2012). Although sexual and somatic interspecific hybrids with *S. torvum* have been obtained, they are highly sterile and although reciprocal crosses have been attempted, no backcross generations have been produced (Sihachakr et al., 1989a, 1989b; Bletsos et al., 1998; Kumchai et al., 2013). Regarding *S. sisymbriifolium*, no viable sexual interspecific hybrids have been obtained and tetraploid somatic hybrids have been sterile (Gleddie et al., 1986; Bletsos et al., 1998). Other tertiary gene pool species that have recently raised interest for eggplant breeding are *S. bonariense*, which is a relative of *S. torvum* (Nurit-Silva et al., 2012) with high vigour and that has largely been unexplored but that may share some of the useful characteristics of *S. torvum* for eggplant breeding, and *S. elaeagnifolium* which is an invasive weed highly tolerant to drought (Christodoulakis et al., 2009). To our knowledge, no interspecific hybrids have been obtained between eggplant and either *S. bonariense* or *S. elaeagnifolium*. Although Plazas et al. (2016) attempted crossing *S. melongena* with *S. elaeagnifolium*, no fruit set was obtained.

Contrarily to many other crops, in which wild species have been used for introgression breeding (Hajjar and Hodgkin, 2007; Warschefsky et al., 2014), the use of wild species in eggplant breeding has been very limited (Daunay and Hazra, 2012; Rotino et al., 2014). Although interspecific hybrids, with different degrees of viability and fertility, between eggplant and wild relatives have been obtained with some 25 species (Rotino et al., 2014; Devi et al., 2015; Plazas et al., 2016) most of the studies have been for taxonomic purposes and preliminary breeding works and have not undertaken the development of backcross generations. Nonetheless, in some instances introgression materials have been obtained with eggplant relatives, including eggplant lines with introgression of resistance to *Fusarium oxysporum* from *S. aethiopicum* (Toppino et al., 2008), or to *Verticillium dahliae* from *S. linnaeanum* (Liu et al., 2015), or backcross generations with *S. aethiopicum* or *S. incanum* (Prohens et al., 2012, 2013). Also, alloplasmic male-sterile *S. melongena* lines containing the cytoplasm of wild relatives of eggplant have been obtained (Khan et al., 2015). However, no systematic attempts have been undertaken to obtain multiple sets of eggplant materials containing introgressions from different wild relatives of eggplant.

We have recently reported the development of interspecific hybrids with 10 different wild species (Plazas et al., 2016), which is a first step for developing materials of eggplant with introgressions from several wild species. Many of the hybrids obtained have been morphologically characterized (Kaushik et al., 2016), which has provided relevant information for breeders on the potential interest of the different wild species. Backcrossing of interspecific hybrids to the cultivated eggplant is the next natural step in an introgression breeding programme in this species (Toppino et al., 2008; Daunay and Hazra, 2012; Liu et al., 2015), but its success depends on the fertility of the hybrids, which may preclude or difficult the development of backcross generations, and the direction of the cross (Daunay and Hazra, 2012; Rotino et al., 2014). Also, developing new interspecific hybrids with other potentially interesting wild species from the secondary and tertiary gene pools may also contribute to the final objective of broadening the genetic base of eggplant with introgressions from wild species. In this respect, when obtaining interspecific hybrids, the use of *S. melongena* as female parent is

frequently preferred, as it has several advantages, like allowing the 100% recovery of the cytoplasm of the cultivated parent, easier manipulation of flowers, more regular fruit set, greater number of seeds per fruit and absence of dormancy in the seeds (Lester and Kang, 1998; Behera and Singh, 2002; Devi et al., 2015; Plazas et al., 2016).

In this work we evaluate the fertility of multiple interspecific hybrids of several eggplant varieties with accessions (i.e., distinct, uniquely identifiable sample of seeds representing a population, which is maintained in storage for conservation and use) corresponding to six different wild species, perform crosses between these interspecific hybrids and their respective *S. melongena* parents in order to develop backcross generations. In addition, we perform new interspecific hybridizations with different accessions of six other wild species of the secondary and tertiary gene pools in order to develop new interspecific hybrids. Our aim is to provide relevant information and new backcross generations and interspecific hybrids with the ultimate objective of broadening the genetic background of eggplant.

## 2. Material and methods

### 2.1. Plant material

#### 2.1.1. First backcross generations

The plant material used for obtaining first backcrosses to cultivated eggplant consisted of: a) six eggplant cultivars used as recurrent parents originating from Ivory Coast (MEL1 to MEL3) and Sri Lanka (MEL4 to MEL6); b) 10 accessions of wild eggplant relatives corresponding to three accessions of the only primary gene pool species *S. insanum* (INS1-INS3), and seven to the secondary gene pool species *S. anguivi* (2 accessions, ANG1-ANG2), *S. dasyphyllum* (1 accession, DAS1), *S. incanum* (1 accession, INC1), *S. lichtensteinii* (2 accessions, LIC1-LIC2), and *S. tomentosum* (2 accessions, TOM1-TOM2); and, c) 44 interspecific hybrids between the six eggplant accessions and the 10 wild species accessions, totaling 18 hybrids with *S. insanum*, 10 with *S. anguivi*, four with *S. dasyphyllum*, four with *S. incanum*, six *S. lichtensteinii*, and two with *S. tomentosum*. The codes of the hybrids used and their respective wild and cultivated parents are presented in Table 1. Further information on the origin of these cultivated and wild accessions can be found in Plazas et al. (2016). Five plants of each of the six *S. melongena* recurrent parents, 10 wild parental accessions and, as available, of the 44 interspecific hybrids were used. Only the *S. melongena* accessions and interspecific hybrids were used for making the crosses to obtain the backcrosses to *S. melongena*. All accessions were used for determining pollen viability.

#### 2.1.2. New interspecific hybrids

Twelve accessions from three other species of the secondary gene pool (*S. campylacanthum*, four accessions; *S. lidii*, one accession; *S. vespertilio*, two accessions) and three species of the tertiary gene pool (*S. bonariense*, one accession; *S. elaeagnifolium*, two accessions; *S. sisymbriifolium*, two accessions) (Table 2) were used for interspecific hybridization with the same six *S. melongena* accessions (MEL1-MEL6) used for obtaining the backcrosses. Five plants of each of the *S. melongena* parents and of the wild parental accessions were used for interspecific hybridization.

### 2.2. Cultivation conditions

Seeds of all materials were germinated according to the protocol of Ranil et al. (2015). Germinated seeds were transferred to seedling trays and kept in a climatic chamber with under a photoperiod and temperature regime of 16 h light (25 °C):8 h dark (18 °C). Seedlings were transplanted in April 2015 to a pollinator-free glasshouse at

**Table 1**

Materials used for obtaining first backcross combinations to *S. melongena*. They correspond to accessions of *S. melongena* (MEL1 to MEL6) and wild species of the primary and secondary gene pools, and the respective interspecific hybrids between *S. melongena* and wild species.

Accession	<i>S. melongena</i>					
	MEL1	MEL2	MEL3	MEL4	MEL5	MEL6
Primary gene pool						
<i>S. insanum</i>						
INS1	MEL1 × INS1	MEL2 × INS1	MEL3 × INS1	MEL4 × INS1	INS1 × MEL5	MEL6 × INS1
INS2	MEL1 × INS2	MEL2 × INS2	MEL3 × INS2	MEL4 × INS2	MEL5 × INS2	MEL6 × INS2
INS3	INS3 × MEL1	INS3 × MEL2	INS3 × MEL3	INS3 × MEL4	MEL5 × INS3	INS3 × MEL6
Secondary gene pool						
<i>S. anguivi</i>						
ANG1	–	MEL2 × ANG1	MEL3 × ANG1	MEL4 × ANG1	MEL5 × ANG1	–
ANG2	MEL1 × ANG2	MEL2 × ANG2	ANG2 × MEL3	ANG2 × MEL4	MEL5 × ANG2	ANG2 × MEL6
<i>S. dasyphyllum</i>						
DAS1	MEL1 × DAS1	MEL2 × DAS1	MEL3 × DAS1	–	MEL5 × DAS1	–
<i>S. incanum</i>						
INC1	INC1 × MEL1	–	MEL3 × INC1	–	MEL5 × INC1	MEL6 × INC1
<i>S. lichtensteini</i>						
LIC1	MEL1 × LIC1	–	–	–	MEL5 × LIC1	MEL6 × LIC1
LIC2	MEL1 × LIC2	–	MEL3 × LIC2	MEL4 × LIC2	–	–
<i>S. tomentosum</i>						
TOM1	–	MEL2 × TOM1	TOM1 × MEL3	–	–	–

**Table 2**

Twelve wild species of the secondary and tertiary gene pools used for obtaining new interspecific hybrids with cultivated eggplant (*S. melongena*), including the germplasm accession code and the FAO germplasm bank of origin of each accession.

Wild species	Accessions	Germplasm accession code <sup>a</sup>	FAO germplasm bank code	Country of origin
Secondary gene pool				
<i>S. campylacanthum</i>	CAM5	MM680	FRA030	Tanzania
	CAM6	MM700	FRA030	Tanzania
	CAM7	MM1414	FRA030	Kenya
	CAM8	MM1426	FRA030	Kenya
<i>S. lidii</i>	LID1	4788	ESP026	Spain
<i>S. vespertilio</i>	VES1	4601A	ESP026	Spain
	VES2	BGV-3213	ESP003	Spain
Tertiary gene pool				
<i>S. bonariense</i>	BON1	BON1	ESP026	Spain
<i>S. eleagnifolium</i>	ELE1	MM1627	FRA030	Senegal
	ELE2	ELE2	ESP026	Greece
<i>S. sisymbriifolium</i>	SIS1	SOLN-78	ESP026	Unknown
	SIS2	1180	ESP026	Unknown

<sup>a</sup> Accessions with MM codes originate from the INRA (Avignon, France) germplasm bank, while the rest of the accessions belong to the COMAV (Valencia, Spain) germplasm collection.

the campus of the Universitat Politècnica de València Spain (greenhouse GPS coordinates: 39°29'01" N, 0°20'27" W). Plants were grown in 25 L pots filled with commercial growing substrate and irrigated and fertilized using a drip irrigation system. Plants were pruned and trained with vertical strings. Phytosanitary treatments against spider mites were performed when necessary.

### 2.3. Pollen viability

Pollen viability of the 44 interspecific hybrids and their parents was evaluated in a mixture of pollen extracted from several mature fully-opened flowers using an enzymatic method. Enzymatic-based pollen viability was assessed using the fluorescein diacetate (FDA) fluorochromatic reaction modified from Heslop-Harrison et al. (1984). Pollen samples were incubated in the dark with a solution of FDA (10 mg L<sup>-1</sup>) in water for 10 min, and then observed at 200x under a Zeiss Axiovert 40 CFL (Carl Zeiss AG, Oberkochen, Germany) inverted microscope equipped for fluorescence microscopy, using a 494 nm excitation filter and a 510 nm emission filter to excite/visualize FDA emission. Viable pollen grains were considered as those displaying intense fluorescence,

while those that displayed no or weak fluorescence were considered as non-viable.

In an attempt to correlate the FDA-based viability estimation with a faster, cheaper, easy-to-measure and therefore more convenient pollen morphological analysis, we performed a parallel morphological study. Using the same inverted microscope, pollen grains were observed under phase contrast at 200 x. Pollen grains with normal shape and size, and with a filled and turgent appearance were considered as viable, while those appearing dwarf, wrinkled or empty were considered as non-viable. For each parental or interspecific hybrid, between 400 and 800 pollen grains were counted from at least three microscope fields for both FDA-based viability estimation and for morphological assessment.

### 2.4. Backcrossing and interspecific hybridization

In order to obtain first backcross generations, reciprocal crosses were performed between *S. melongena* accessions and interspecific hybrids, although priority was given to hybridizations in which the interspecific hybrids were used as female parents, due to their lower pollen fertility. *Solanum melongena* was used to cross with new wild species as female parent, their flowers were emasculated.

**Table 3**  
Average values (based on accession means) for morphologically normal pollen and FDA-based pollen viability of cultivated eggplant (*S. melongena*), wild species from the primary and secondary gene pools, and of interspecific hybrids between *S. melongena* and wild species. Between 400 and 800 pollen grains were counted from at least three microscope fields for each accession or hybrid.

Species/hybrid	Accessions/hybrids	Morphologically normal pollen (%) <sup>a</sup>	FDA-based viability (%) <sup>a</sup>
Cultivated eggplant <i>S. melongena</i>	6	95.0 c	68.9 cd
Primary gene pool <i>S. insanum</i>	3	96.9 c	67.7 cd
Secondary gene pool <i>S. anguivi</i>	2	93.1 c	87.6 d
<i>S. dasyphyllum</i>	1	88.8 c	54.7 c
<i>S. incanum</i>	1	92.3 c	67.3 cd
<i>S. lichtensteinii</i>	2	92.8 c	59.6 c
<i>S. tomentosum</i>	1	88.4 c	66.1 cd
Hybrids with primary gene pool species <i>S. melongena</i> × <i>S. insanum</i>	18	94.9 c	62.2 c
Hybrids with secondary gene pool species <i>S. melongena</i> × <i>S. anguivi</i>	10	24.6 ab	18.1 ab
<i>S. melongena</i> × <i>S. dasyphyllum</i>	4	25.5 ab	14.9 ab
<i>S. melongena</i> × <i>S. incanum</i>	4	77.4 c	61.0 c
<i>S. melongena</i> × <i>S. lichtensteinii</i>	6	31.5 b	25.7 b
<i>S. melongena</i> × <i>S. tomentosum</i>	2	9.6 a	5.5 b
Probability of F <sup>b</sup>		<0.0001	<0.0001

<sup>a</sup> Means within columns separated by different letters are significantly different at  $P < 0.05$ , according to the Student-Newman-Keuls test using arcsine transformed data (arcsine of the squared root of percentage/100).

<sup>b</sup> Based on arcsine transformed data (arcsine of the squared root of percentage/100).

lated before flower opening, then pollen deposited on the stigma by gently rubbing a glass slide covered with pollen of the male parent. Pollinated flowers were tagged and a record was kept for calculating the percentage of fruit set.

### 2.5. Seed extraction and embryo rescue

Fruits that had set and developed corresponding to both first backcrosses and new interspecific hybrids with secondary gene pool species were left on the plant until they reached full physiological maturity. Seeds from these fruits were extracted manually at the lab and the seed was placed on filter paper and left to dry at room temperature. Seeds obtained for each fruit were subsequently counted. For fruits corresponding to interspecific hybridizations of *S. melongena* with tertiary gene pool species, the immature fruits were harvested between 20 and 30 days after pollination and brought to the laboratory where immature seeds, when present, were extracted under sterile conditions and embryos cultivated in vitro using the protocol indicated in Plazas et al. (2016).

### 2.6. Data analysis

For pollen viability percentage values, the average of each of the cultivated and wild accessions and of interspecific hybrids were calculated from the replicates for each of the accessions or hybrids. The percentage data were transformed using the arcsine transformation, which consists in obtaining the arcsine of the squared root of percentage/100 (Little and Hills, 1978), and subjected to ANOVA factorial analysis using the species or interspecific hybrid parental species combination as factor. Significant differences among species or interspecific hybrid parental species combinations were detected using the Student-Newman-Keuls (SNK) multiple range test. The mean percentage value for each accession or hybrid for morphologically normal pollen and pollen viability estimated with FDA was used to calculate the linear coefficient of correlation between these two pollen characters. For comparison of fruit set percentage in the crosses between: a) *S. melongena* parentals and interspecific hybrids for obtaining first backcross generations, or b) *S. melongena* parentals and wild species

for obtaining new interspecific hybrids, we used the Marascuilo's post hoc multiple comparison procedure, which allows the comparison among several proportions or percentages (Marascuilo and McSweeney, 1977), at  $P < 0.05$ . The standard error of the mean (SE) was also calculated for the fruit set percentage of each of the *S. melongena* accessions and from the number of seeds per seeded fruit.

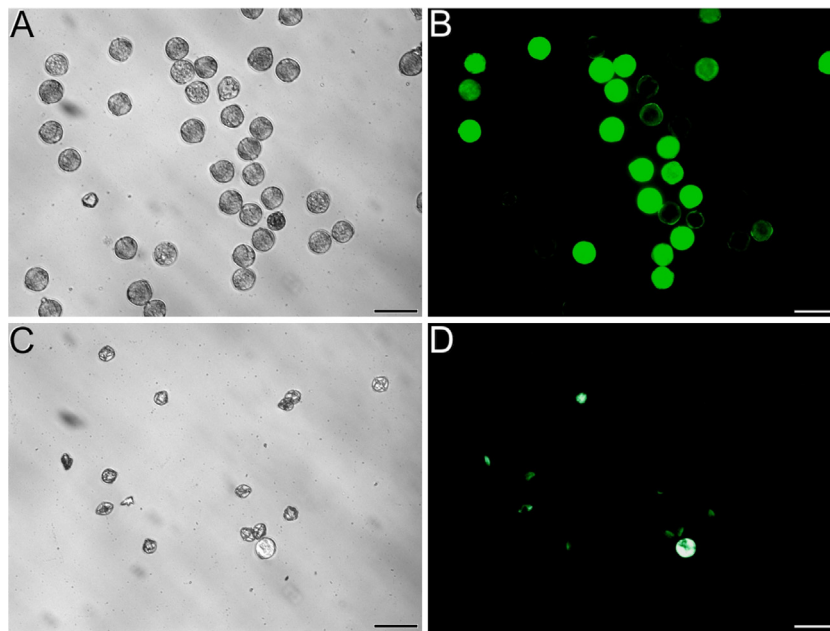
## 3. Results

### 3.1. Pollen viability

Pollen grains of the cultivated *S. melongena* and the wild species of the primary and secondary gene pools and the hybrids between *S. melongena* and the only primary gene pool species *S. insanum* were mostly of normal shape and size, with average values of morphologically normal grains above 88% and non-significant ( $P > 0.05$ ) differences among them (Table 3; Fig. 1). When considering the hybrids with secondary gene pool species, the hybrids between *S. melongena* and *S. incanum* displayed pollen grains which were mostly filled, turgent and normally shaped, with an average value of morphologically normal pollen of 77.4%, being non-significantly different from its parents; however, the rest of hybrids displayed a significantly lower frequency of morphologically normal pollen (Table 3; Fig. 1), with average values ranging between 9.6% for the hybrids of *S. melongena* with *S. tomentosum* to 31.5% for the hybrids between *S. melongena* and *S. lichtensteinii* (Table 3).

The assessment of pollen viability with the FDA enzymatic reaction generally resulted in estimates of viability lower (~20%) than percentages of morphologically normal pollen (Table 3; Fig. 1). However, a high degree of correlation was observed between morphologically normal pollen and pollen viability estimated with FDA was observed, with a correlation coefficient among mean values for accessions and hybrids of  $r = 0.904$  ( $P < 0.0001$ ). When considering the *S. melongena* parents and wild species, the range of variation was much higher than with the morphological assessment, and significant differences ( $P < 0.05$ ) were observed between the highest value, which was found in *S. anguivi* (87.6%), and the lowest values of *S. dasyphyllum* (54.7%) and *S. lichtensteinii* (59.6%) (Table 3). The hybrids between *S. melongena* and the pri-





**Fig. 1.** Pollen grains of *S. melongena* (A, B) and of a hybrid between *S. melongena* and secondary gene pool *S. tomentosum* (C, D) stained with FDA and observed under phase contrast (A, C) and fluorescent light (B, D). Bars: 50  $\mu$ m.

mary gene pool species *S. insanum* and the secondary gene pool species *S. incanum* displayed average values (62.2% and 61.0%, respectively) that were not significantly different to those of any of its parents. Regarding the interspecific hybrids of *S. melongena* with other wild species of the secondary gene pool, they displayed significantly lower viability than its cultivated and wild parents, with the extreme values being the hybrids with *S. tomentosum* and *S. lichtensteinii*, which presented significant differences among them, with average values of 5.5% and 25.7%, respectively (Table 3).

### 3.2. Development of backcross generations

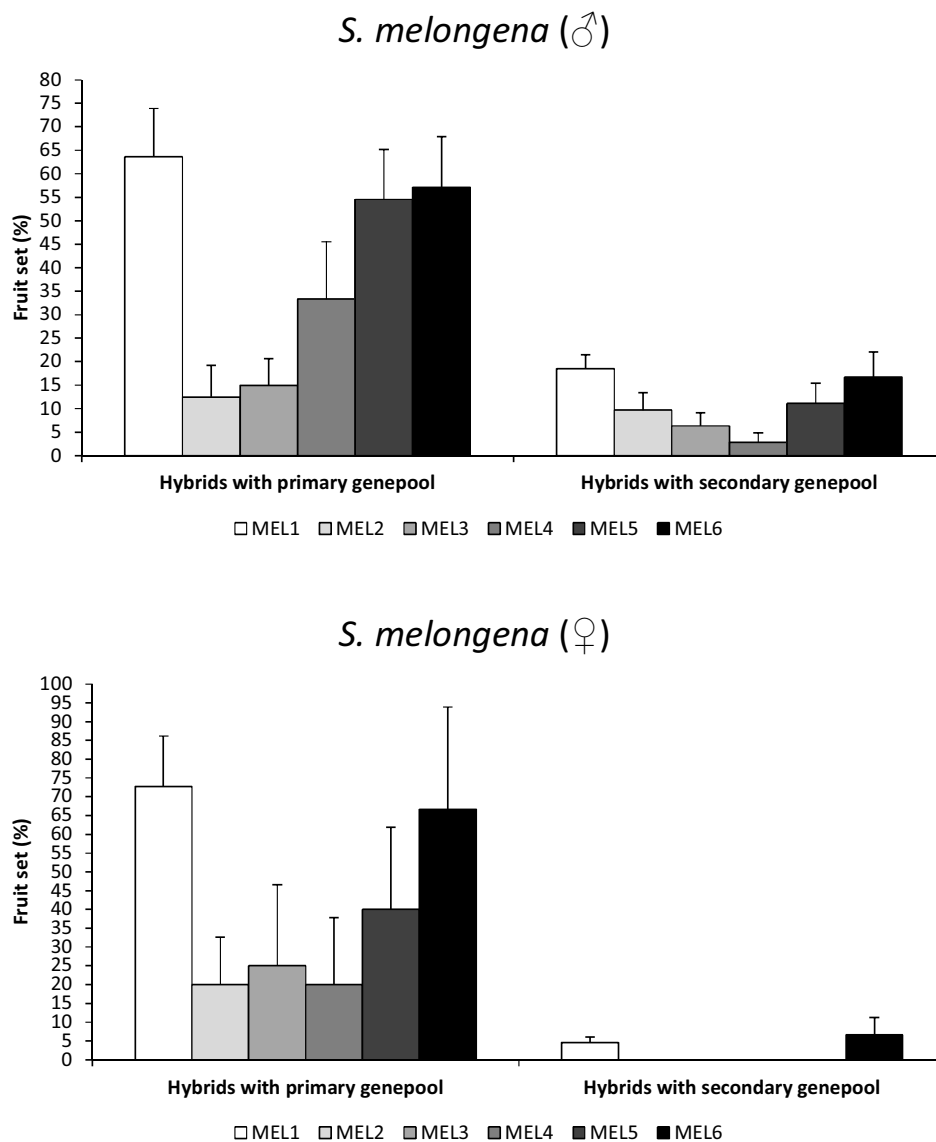
A total of 1052 crosses between the six cultivated *S. melongena* accessions and the 44 interspecific hybrids between *S. melongena* and wild relatives were performed, of which 615 were made using *S. melongena* as male parent and 437 as female parent (Table 4). Crosses of *S. melongena* with interspecific hybrids with the primary gene pool species *S. insanum* had a high degree of success, with a 38.4% and 43.2% of fruit set on the crosses made when using *S. melongena* as male or female parent, respectively (Table 4). Also, all fruits of the backcross with the hybrid with *S. insanum* were seeded, with a mean over 500 seeds/fruit in both directions, significantly higher than in the rest of backcrosses with secondary gene pool species (Table 4). The results of crossings of *S. melongena* with hybrids with secondary gene pool species were variable depending on the interspecific hybrid parental wild species and the direction of the cross. When using *S. melongena* as a male parent, fruit set in hybrids with *S. incanum* was significantly higher than in the rest of hybrids with secondary gene pool species (Table 4). Fruits of backcrosses were generally seeded, with the exception of a certain percentage of fruits in the crosses with hybrids *S. melongena*  $\times$  *S. dasyphyllum* (25.0%) and *S. melongena*  $\times$  *S. tomentosum* (42.9%), which were parthenocarpic. The number of seeds per fruit was very variable, with significantly higher values, of over 200 seeds/fruit, in the backcrosses between *S. melongena* and hybrids with *S. incanum* or *S. lichtensteinii*, than in the interspecific hybrids with the three other species (*S. anguivi*, *S. dasyphyllum*,

and *S. tomentosum*), which had less than 20 seeds per fruit. When using *S. melongena* as a female parent, the fruit set percentage of backcrosses was much lower, and no fruit set was obtained when using hybrids with *S. lichtensteinii* or *S. tomentosum* as male parents, and the percentage was very low when using hybrids with *S. anguivi* (1.4%) or *S. dasyphyllum* (4.3%). Furthermore in the former case, all fruits obtained were parthenocarpic, while in the latter most of them were parthenocarpic (Table 4). Also, in the backcrosses with the hybrid with *S. incanum* the fruit percentage was much lower than when using it as a female parent and the number of seeds was also lower, with less than 100 seeds/fruit (Table 4). No significant differences were observed among the different hybrids of the same parental combination in the success of crossing when using them as male parent in the crossings with *S. melongena*.

Important differences were observed among *S. melongena* parents in the success of the crosses. When using *S. melongena* as a male parent it was observed that some accessions, like MEL1, MEL5 and MEL6 displayed a greater degree of success in the crosses with interspecific hybrids of *S. melongena* with both primary and secondary gene pool species (Fig. 2). When using *S. melongena* as a female parent, again important differences were observed among accessions, with MEL1, MEL5 and MEL6 having a greater degree of success when used as female parents than the rest of accessions in the crosses with hybrids with primary gene pool species. When crossing with secondary gene pool species, the two only accessions for which fruit set was obtained was with MEL1 and MEL6, with very low fruit set values (Fig. 2).

### 3.3. Development of new hybrids

A total of 263 crosses were made between *S. melongena* (as female parent) and the three secondary gene pool wild species *S. campylacanthum*, *S. lidii* and *S. vespertilio* (Table 5). The percentage of fruit set in the interspecific crosses with *S. melongena* varied between 2.6% for crosses with *S. lidii* to 24.5% with *S. campylacanthum*, which significant differences in fruit set among these two



**Fig. 2.** Fruit set percentage ( $\pm$ SE) of the first backcrosses between six *S. melongena* accessions (MEL1 to MEL6) and hybrids of *S. melongena* with wild species of the primary and secondary gene pools when using *S. melongena* as a male (above) or female (below) parent.

latter species (Table 5). All fruits obtained after crossing *S. melongena* with *S. lidii* and *S. vespertilio* were seeded and in the case of crosses with *S. vespertilio* had a large number of seeds (over 500 seeds per fruit). In the crosses with *S. campylacanthum*, despite the larger fruit set almost two thirds of the fruits were parthenocarpic and the number of seeds was much lower than in the hybrids with *S. vespertilio* and similar to the only fruit obtained with *S. lidii*. When considering crosses with tertiary gene pool species *S. bonariense*, *S. eleagnifolium* and *S. sisymbriifolium*, a total of 173 crosses were made. Considerable differences were found in fruit set, with significantly higher values in *S. eleagnifolium* (26.8%) than in *S. sisymbriifolium* (4.0%) and *S. bonariense* (0.0%). However, all fruits obtained after crossing with tertiary gene pool species were parthenocarpic or presented embryos aborted at an early stage, with the exception of a single fruit in which 11 embryos could be rescued and cultivated in vitro to produce viable hybrid plants (Table 5). As a result of the interspecific hybridizations with secondary and tertiary gene pool species performed 12 new interspecific hybrid combinations were obtained, of which 11 were with secondary gene pool accessions and 1 with the tertiary gene pool species *S. eleagnifolium*.

#### 4. Discussion

Broadening the genetic base of crops may help in coping with the challenges posed by the upcoming climate change (Dempewolf et al., 2014; Warschefsky et al., 2014). This is particularly true in crops with a narrow genetic base, like eggplant (Mace et al., 1999; Furini and Wunder, 2004; Muñoz-Falcón et al., 2009; Weese and Bohs, 2010; Vorontsova et al., 2013). Broadening the genetic background of eggplant can help not only to produce new varieties with traits introgressed from wild species, but also to improving the potential for obtaining hybrids heterotic for yield by crossing genetically distant parent lines (Rodríguez-Burruezo et al., 2008).

Although interspecific hybridization of eggplant with related species has been reported with different degrees of success (Bletsos et al., 1998; Lester and Kang, 1998; Behera and Singh, 2002; Daunay and Hazra, 2012; Kumchai et al., 2013; Rotino et al., 2014; Devi et al., 2015; Plazas et al., 2016), successful introgression requires backcrossing of the hybrids to the cultivated parent. This step many times represents the most important challenge for introgression breeding (Rieseberg and Carney, 1998), due to sterility and/or lack of fruit set in some hybrid combinations. In previous works, back-

**Table 4**  
Number of crosses, fruit set, seedless fruits, seeds per seeded fruit (mean ± SE) in first backcrosses of interspecific hybrids between cultivated eggplant (*Solanum melongena*) and wild relatives from the primary and secondary gene pools, according to the direction of the cross.

Interspecific hybrids used as parents	<i>S. melongena</i> used as male parent				<i>S. melongena</i> used as female parent				
	Hybrids (n)	Crosses (n)	Fruit set (%) <sup>a</sup>	Seedless fruits (%)	Seeds/seeded fruit (n) <sup>a</sup>	Crosses (n)	Fruit set (%) <sup>a</sup>	Seedless fruits (%)	Seeds/seeded fruit (n)
Hybrids with primary gene pool species									
<i>S. melongena</i> × <i>S. insanum</i>	18	146	38.4 b	0.0	701.8 ± 36.6	37	43.2 b	0.0	551.5 ± 49.5
Hybrids with secondary gene pool species									
<i>S. melongena</i> × <i>S. anguivi</i>	10	200	11.5 a	0.0	16.0 ± 3.9	142	1.4 a	100.0	–
<i>S. melongena</i> × <i>S. dasycarpum</i>	4	103	7.8 a	25.0	17.3 ± 4.8	185	4.3 a	62.5	25.5 ± 14.7
<i>S. melongena</i> × <i>S. insanum</i>	4	36	41.7 b	0.0	205.0 ± 12.2	13	15.3 b	0.0	83.5 ± 18.5
<i>S. melongena</i> × <i>S. lichtensteinii</i>	6	85	11.8 a	0.0	221.8 ± 20.5	24	0.0 a	–	–
<i>S. melongena</i> × <i>S. tomentosum</i>	2	45	15.6 a	42.9	7.7 ± 0.4	36	0.0 a	–	–

<sup>a</sup> Means within columns separated by different letters are significantly different at  $P < 0.05$ , according to the Marascuilo's post-hoc multiple comparison procedure for proportions.

crosses have been obtained between eggplant and several wild species (Toppino et al., 2008; Mennella et al., 2010; Prohens et al., 2012, 2013; Liu et al., 2015); however, our work represents the first systematic attempt to obtain backcrosses between several varieties of cultivated eggplant a number of wild species accessions from the primary and secondary gene pools.

The high pollen viability observed in the parental cultivated and wild accessions indicates that the conditions in which the plants developed were appropriate for a normal pollen formation process (Giorno et al., 2013), which has allowed us to make an adequate assessment of the potential fertility of interspecific hybrids. The high pollen viability, similar to their parents, of the hybrids between *S. melongena* and *S. insanum* is a confirmation that both species are closely related (Weese and Bohs, 2010; Meyer et al., 2012; Knapp et al., 2013). Viability in the hybrids with secondary gene pool species has been very variable. Viability has been higher in hybrids with *S. insanum* and *S. lichtensteinii*, which are wild species phylogenetically close to eggplant (Weese and Bohs, 2010; Knapp et al., 2013; Vorontsova et al., 2013; Aubriot et al., 2016), than in the hybrids with the other phylogenetically more distant species. In any case, viable pollen, has been found in all the interspecific hybrids, indicating that potential exists for obtaining backcrosses with all the interspecific hybrids evaluated. The comparatively higher values estimated observed by us using morphology with respect to the FDA-based estimation was expected, as some normal-shaped pollen grains may not be able to germinate and fertilize the egg cells (Dafni and Firmage, 2000). However, we also showed that both methods are proportional. Thus, we propose that the study of pollen morphology may be useful for rapid estimations of viability in pollen of eggplant cultivated and wild accessions when high precision is not mandatory, or for situations where a fluorescence microscope is not available.

In general, the success of the backcrossing of the interspecific hybrids to *S. melongena* has matched the results obtained for interspecific hybridization between *S. melongena* and the respective wild species (Plazas et al., 2016). In this respect, backcrosses of the interspecific hybrids of *S. melongena* with *S. insanum* to the cultivated *S. melongena* parent have been highly efficient, with a high fruit set percentage and many seeds per fruit when the cross is made in both directions. These results are in agreement with the high success, comparable to that of intraspecific crosses in *S. melongena*, of interspecific hybridization between *S. melongena* and *S. insanum* (Davidar et al., 2015; Plazas et al., 2016). This is additional evidence supporting the claim that *S. insanum* is the wild ancestor of *S. melongena* (Meyer et al., 2012; Knapp et al., 2013). The most successful backcrosses with secondary gene pool species have been obtained with *S. insanum*, which was also the secondary gene pool species with higher pollen viability. *Solanum insanum* is also very close to *S. melongena* (Furini and Wunder, 2004; Knapp et al., 2013; Vorontsova et al., 2013). Also, the backcrosses of the interspecific hybrid with *S. lichtensteinii*, the other wild species used belonging to the Eggplant clade (Knapp et al., 2013; Syfert et al., 2016), have been relatively successful. The backcrosses with the hybrids with the three other species, which belong to the Anguivi phylogenetic group (Syfert et al., 2016) have been less successful, and the lower rate of success has been obtained with the hybrids with *S. tomentosum*, which presented low fertility, as estimated by pollen viability. In any case, backcrosses with interspecific hybrids of all species could be obtained, which will facilitate introgression breeding with all the species, especially taking into account fertility generally recovers in backcross generations, compared to the interspecific hybrids (Rieseberg and Carney, 1998). Our results also indicate that using the interspecific hybrids as female parents may be a better strategy for obtaining backcross generations, particularly when the pollen viability of the interspecific hybrid is low. Considerable differences have been found among the *S. melongena* genotypes in

**Table 5**  
Number of crosses, fruit set, seedless fruits, seeds per fruit (mean  $\pm$  SE) in crosses between cultivated eggplant (*Solanum melongena*; as female parent) and wild relatives from the secondary and tertiary genepools (as male parents).

Wild species used as parentals	<i>S. melongena</i> (female parent)				
	Accessions (n)	Crosses (n)	Fruit set (%) <sup>a</sup>	Seedless fruits (%)	Seeds/seeded fruit (n)
<b>Secondary genepool</b>					
<i>S. campylacanthum</i>	4	184	24.5 b	62.2	133.3 $\pm$ 40.7
<i>S. lidii</i>	1	39	2.6 a	0.0	103 <sup>b</sup>
<i>S. vespertilio</i>	2	40	10.0 ab	0.0	562.5 $\pm$ 104.2
<b>Tertiary genepool</b>					
<i>S. bonariense</i>	1	65	0.0 a	–	–
<i>S. elaeagnifolium</i>	2	56	26.8 b	93.3	11 <sup>b,c</sup>
<i>S. sisymbriifolium</i>	2	52	4.0 a	100.0	–

<sup>a</sup> Means within column separated by different letters are significantly different at  $P < 0.05$ , according to the Marascuilo's post-hoc multiple comparison procedure for proportions.

<sup>b</sup> No SE is given, as only one fruit was obtained.

<sup>c</sup> Embryos rescued from immature seeds.

the success of backcrossing. According to the results obtained, we consider that best *S. melongena* accessions among those tested for successful backcrossing are MEL1 and MEL6, followed by MEL5. The rest of accessions in general had a poorer performance.

New interspecific hybrids with secondary genepool species *S. campylacanthum*, *S. lidii* and *S. vespertilio*, which may be used for subsequent backcrossing, have been obtained. Hybrids between eggplant and this three wild species have been reported previously (Lester and Kang, 1998; Daunay and Hazra, 2012; Rotino et al., 2014). *Solanum campylacanthum* is phylogenetically one of the closest relatives of eggplant (Mace et al., 1999; Knapp et al., 2013; Vorontsova et al., 2013; Syfert et al., 2016), has a wide range of distribution habitats and is extremely variable, which suggests that traits of interest for eggplant breeding for adaptation to climate change may be found in this species. *Solanum lidii* and *S. vespertilio* are endemic species from the Canary Islands (Spain) and are characterized by heterandrous and zygomorphic corolla, which in the case of *S. vespertilio* are also tetramerous (instead of the typical pentamerous corollas of *Solanum*) (Prohens et al., 2007). Apart from their potential interest for eggplant breeding, interspecific hybrids and backcross generations may also be an experimental material of great value for understanding the genetic basis of the odd flower morphology of these two species.

Interspecific hybridization with tertiary genepool species was unsuccessful with *S. bonariense* and *S. sisymbriifolium*. Although to our knowledge this is the first attempt for interspecific hybridization with *S. bonariense*, several previous attempts have been performed at crossing *S. melongena* and *S. sisymbriifolium* (Bletsos et al., 1998; Plazas et al., 2016) due to the interest of the latter as source of resistance to several diseases (Bletsos et al., 1998; Daunay and Hazra, 2012), but have always been unsuccessful at obtaining sexual interspecific hybrids. Amazingly, we have been able to obtain interspecific hybrids between *S. melongena* and *S. elaeagnifolium* through embryo rescue. To our knowledge, this is the first report of hybrids between these two species and represents a first step for introgressing the extreme tolerance to drought of *S. elaeagnifolium* (Christodoulakis et al., 2009) into the genetic background of *S. melongena*. However, the feasibility of using *S. elaeagnifolium* as a source of variation for eggplant breeding will depend on the fertility of the interspecific hybrids and the development of backcross generations.

## 5. Conclusions

Backcrosses to *S. melongena* have been obtained with the hybrids of eggplant with six wild relatives from the primary and secondary genepools, confirming that eggplant is amenable to introgression breeding from wild relatives. Fruit set percentage and number

of seeds per fruit have been highest in backcrosses with the only primary genepool species, *S. insanum*. Although pollen viability in eggplant is better estimated with FDA-based methods, the measurement of morphological parameters may be used as an alternative, easier although less precise method. Despite low pollen viability in some hybrids with some secondary genepool species, backcross generations have been obtained when using the hybrids as female parents. In addition, new interspecific hybrids have been obtained with three secondary genepool species, and with the tertiary genepool species *S. elaeagnifolium*, which is highly tolerant to drought. The information obtained and plant material developed will contribute to the development of a new generation of eggplant cultivars with a broader genetic background.

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