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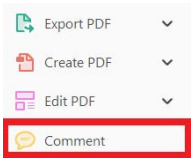

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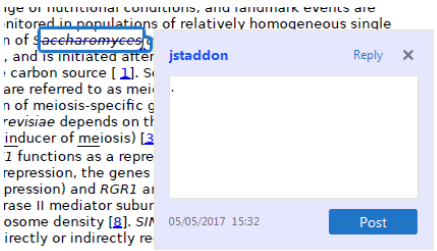

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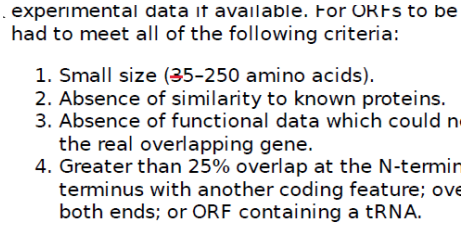

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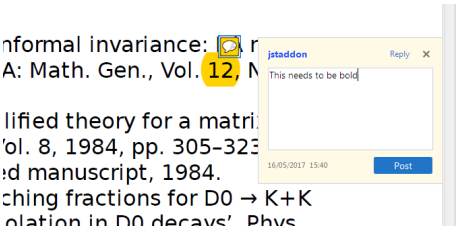

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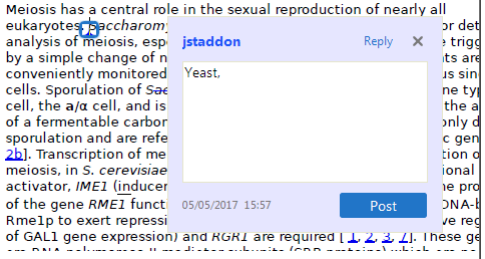

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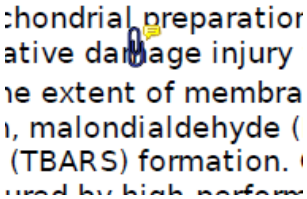

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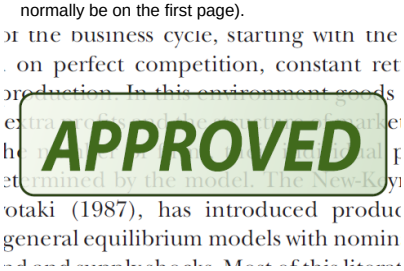

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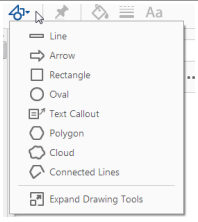

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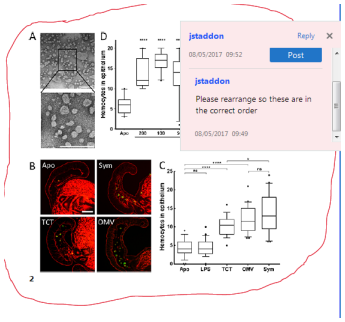

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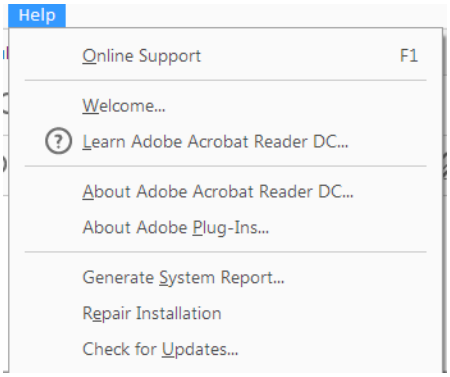

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Fitness cost but no selection for virulence in *Meloidogyne incognita* after two consecutive crops of eggplant grafted onto *Solanum torvum*

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The eggplant *Solanum melongena* 'Cristal', either ungrafted or grafted onto the *Solanum torvum* 'Brutus' rootstock, was cultivated for two consecutive years in the same plots in a plastic greenhouse to assess the level of resistance to *Meloidogyne incognita* and crop yield. At the end of the second crop, the putative selection for virulence of the nematode subpopulations coming from infected ungrafted and grafted eggplant was assessed in the eggplant and in *S. torvum* in a pot experiment. Nematode population densities at transplantation in 2017 ranged from 2 to 378 100 cm^{-3} of soil and did not differ between ungrafted and grafted eggplant. At the end of each crop, higher galling index and number of nematodes in soil and in roots were registered in ungrafted than grafted eggplant. The grafted eggplant was categorized as resistant in 2017 and as highly resistant in 2018. Eggplant yield did not differ irrespective of grafting in 2017 after being cultivated for 135 days, but did differed after 251 days of cultivation in 2018. In the pot experiment, *S. torvum* was categorized as resistant to both *M. incognita* subpopulations. However, the *M. incognita* subpopulation obtained from roots of *S. torvum* produced 49.4% fewer egg masses and 56% fewer eggs per plant in the eggplant than the nematode subpopulation obtained from roots of the eggplant cv. Cristal. The results of this study reveal that the infective and reproductive fitness of the nematode decreased without having been selected for virulence.

Keywords: eggplant yield, grafting, resistance durability, root-knot nematode, rootstock, *Solanum melongena*

Introduction

Eggplant, *Solanum melongena*, is one of the most cultivated solanaceous crops with an estimated worldwide production of *c.* 52 million tonnes in 1.8 million ha (FAOSTAT, 2017). Root-knot nematodes (RKN), *Meloidogyne* spp., are one of the most damaging soilborne pathogens in solanaceous crops, especially under protected cultivation (Hallman & Meressa 2018). Maximum eggplant yield losses of 95% have been reported (Greco & Di Vito, 2009). The use of resistant plants is an effective and economically profitable management strategy to control RKN (Sorribas *et al.*, 2005) that is more environmentally friendly than the common soil nematicides (Nyczepir & Thomas, 2009). In nematology, plant resistance is defined as the ability of a plant to suppress infection development and/or reproduction of plant-parasitic nematodes (Roberts, 2002). Grafting onto resistant rootstocks has become a common method to control soilborne pathogens when no commercial resistant cultivars are available (Lee & Oda, 2002; Thies *et al.*, 2015). That is the case for eggplant, which is usually grafted onto resistant tomato or interspecific hybrids such as *S. lycopersicum* × *S. habrochaites* (Daunay, 2008). However, the expression of resistance can be limited by several factors such as constant soil temperatures above 30 °C (Araujo *et al.*, 1982), and the genetic background of the rootstock along with that of the nematode species (Cortada *et al.*, 2008). Moreover, the repeated cultivation of plant species carrying the same resistance gene can select virulent nematode populations capable of overcoming the plant defence mechanisms (Verdejo-Lucas *et al.*, 2009; Giné & Sorribas, 2017; Expósito *et al.*, 2019). Thus, other resistance sources have been assessed, including *S. melongena* lines, interspecific hybrid of *S. integrifolium* × *S. melongena*, and the wild related species *S. integrifolium*, *S. sisymbrifolium* and *S. torvum* (Daunay, 2008). Nonetheless, *S. torvum* is currently the only wild species commercially available for use as rootstock for eggplant worldwide (Uehara *et al.*, 2017; Öçal *et al.*, 2018). Several *S. torvum* accessions and cultivars have been previously described as resistant to *M. incognita* (Dhivya *et al.*, 2014), *M. luci* (Öçal *et al.*, 2018), and to some populations of *M. arenaria* and *M. javanica* (Tzortzakakis *et al.*, 2006; Uehara *et al.*, 2017; Öçal *et al.*, 2018), but

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susceptible to *M. hapla* (Öçal *et al.*, 2018). Some *S. torvum* rootstocks have also been recently described as valuable tools for managing *M. incognita* and *M. javanica* populations from Spain, irrespective of their (a) virulence status to the resistance *N* and *Mi-1.2* genes in pepper and tomato, respectively (García-Mendívil *et al.*, 2019a). Nevertheless, no information has been previously reported about the effect of continuous cultivation of *S. torvum* on selecting for virulence and on the nematode fitness. Therefore, experiments were conducted to estimate the effect of two-year cultivation in the same plot under plastic greenhouse conditions on *M. incognita* population densities, disease severity, eggplant yield, selection for virulence and nematode fitness.

Materials and methods

Experimental plots

The experiment was conducted in a 700 m^2 plastic greenhouse located at Viladecans (Barcelona, Spain). The soil texture was sandy loam with 83.8% sand, 6.7% loam and 9.5% clay, pH 8.7, 1.8% of organic matter (w/w) and 0.5 dS m^{-1} electrical conductivity. The soil was infested in 2014 with the avirulent *Mi1.2* gene isolate Agropolis from *M. incognita* coming from a single egg mass and multiplied in the susceptible tomato cv. Durinta (Expósito *et al.*, 2019). The plots used in this experiment were previously cultivated with the rotation lettuce-French bean-eggplant. The experiment consisted of two treatments: eggplant cv. Cristal (Semillas Fitó) grafted onto the *S. torvum* 'Brutus' (Semillas Fitó), and ungrafted eggplant cv. Cristal as standard for comparison. Each treatment was replicated 10 times in plots with a narrow variation in nematode densities between treatments at transplantation. Crops were grown from 16 June to 29 October 2017 (135 days) and from 20 March to 26 November 2018 (251 days) and plots maintained in black fallow between cropping seasons. Individual plots consisted of a row of 2.5 m with four plants spaced 0.6 m apart and plots within a row were spaced 1 m apart. Plants of each treatment were cultivated in the same plot each year to determine the effect on *M. incognita* population densities, the disease severity, the crop yield and the durability of the resistance. Soil of each plot was prepared individually to avoid cross contamination. Plants were irrigated as needed through a drip irrigation system and fertilized with a solution of NPK (15-5-30) at 31 kg ha^{-1}, iron chelate and micronutrients at 0.9 kg ha^{-1}. Weeds were removed manually before and during the cropping season. Fruit yield was determined weekly between 8 and 17 weeks after transplantation in 2017, and between 11 and 31 weeks in 2018. Soil temperatures and water content were recorded daily at 1 h intervals with digital probes 5TM (Decagon Devices, Inc.) placed at 15 cm depth.

Nematode population quantification

The initial nematode population densities (*Pi*) in soil were quantified at transplantation and the final population densities (*Pf*) at the end of the crop. Soil samples were taken from each experimental plot and consisted of eight cores, taken from the first 30 cm of soil with an auger of diameter 2.5 cm, that were mixed and passed through a 4 mm pore sieve to remove stones. The second-stage juveniles (J2) were extracted from 500 cm^3 of soil, and incubated at 27 ± 2 °C for one week, using Baermann trays (Whitehead & Hemming, 1965). J2 were collected using a 25 μm aperture screen, counted, and expressed as J2 per 100 cm^3 of soil. At the end of the crop, roots were carefully uprooted, washed, and the galling index (GI) evaluated on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plants and roots dead (Zeck, 1971). The number of eggs per plant was then assessed by extracting them from roots by blender maceration in a 5% commercial bleach solution (Hussey & Barker, 1973) and counting them. Reproduction index (RI) was calculated as the percentage of eggs produced in the rootstock compared to that in the eggplant cultivar. The response of the rootstock was categorized according to the RI as highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%) or susceptible (RI ≥ 50%) (Hadisoeganda & Sasser, 1982).

Selection for virulence and nematode fitness

At the end of the plastic greenhouse experiment in 2018, two nematode subpopulations were considered according to the plant species in which they were produced, i.e. eggplant or *S. torvum*. The eggs extracted from roots of the ungrafted and grafted eggplants were incubated in Baermann trays at 27 ± 2 °C to allow J2 emergence to determine the putative selection for virulence in a pot experiment. The J2 emerged in the first 24 h were discarded. Nematodes were collected daily for 10 days using a 25 μm sieve, and stored at 9 °C until inoculation. Seeds of *S. torvum* 'Brutus' were pretreated with a KNO_3 solution to improve germination (Ranil *et al.*, 2015), transferred to vermiculite filled trays and incubated in a growth chamber at 25 ± 2 °C and 16:8 h (light:-dark) photoperiod for 4 weeks. Afterwards, the seedlings were transplanted to 200 cm^3 pots containing sterile sand and maintained in a growth chamber at 25 ± 2 °C with a 16:8 h (light:-dark) photoperiod for 1 week, and inoculated with one J2 per cm^3 sand. Each treatment was replicated 10 times. Plants were maintained in the growth chamber for 55 days, watered as needed throughout the experiment and fertilized with a slow-release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with PT100 probes (Campbell Scientific Ltd).

At the end of the experiments, the roots were carefully washed, the GI evaluated, the number of egg masses and eggs per plant determined, the number of eggs per egg mass calculated as well as the RI, and the level of resistance categorized, following the procedures previously stated.

Statistical analysis

Statistical analyses were performed using R statistical software v. 3.5.1 (R Foundation for Statistical Computing). The data were not normally distributed according to the normal Shapiro–Wilk *W*-test. The nonparametric analyses Mann–Whitney *U*-test was then used for paired comparisons between plant species per cropping season, between plant species per each *M. incognita* subpopulation and between *M. incognita* subpopulations per each plant species.

Results

Effect of *S. torvum* on *M. incognita* reproduction, disease severity and eggplant yield

The minimum and maximum soil temperatures during the cropping season in 2017 ranged from 21.1 to 29.9 °C,

and from 14.6 to 31.3 °C in 2018. The nematode population densities in soil at transplantation in 2017 ranged from 2 to 378 J2 per 100 cm^3 of soil and did not differ ($P < 0.05$) between treatments. At the end of the crop, a higher ($P < 0.05$) GI, and number of J2 in soil and eggs per plant were registered in the ungrafted than the grafted eggplant, but fruit yield did not differ (Table 1). In 2018, the nematode population densities in soil at transplantation were between 94% and 98% less than those registered at the end of the crop in 2017 but differed ($P < 0.05$) between treatments. At the end of the crop, a higher ($P < 0.05$) GI, number of J2 in soil and eggs per plant were also registered in ungrafted than grafted eggplant. Grafted eggplant yielded 2.1 more kg of fruit per plant ($P < 0.05$) than ungrafted. The *S. torvum* rootstock performed as resistant (1% ≤ RI < 10%) to *M. incognita* in 2017, and highly resistant (RI < 1%) in 2018 (Table 1).

Virulence selection

The *S. torvum* 'Brutus' was resistant (1% ≤ RI < 10%) to both *M. incognita* subpopulations obtained from roots of the ungrafted eggplant cv. Cristal or grafted onto the *S. torvum* 'Brutus' after being cultivated for two consecutive years in the same plots in a plastic greenhouse. Both *M. incognita* subpopulations caused between 53% and 69% lower ($P < 0.05$) GI and produced 97% less egg masses and eggs per plant, and between 21% and 31% fewer eggs per egg mass in *S. torvum* than in eggplant (Table 2). The *M. incognita* subpopulation from roots of *S. torvum* 'Brutus' produced 49.4% less ($P < 0.05$) egg masses and 56% less eggs per plant in the eggplant cv. Cristal than the nematode subpopulation from roots of eggplant cv. Cristal, but these parameters did not differ between subpopulations when inoculated in *S. torvum* (Table 2).

Discussion

This study demonstrates for the first time that two consecutive crops of *S. torvum* in the same plots do not select for virulence in *M. incognita* but have an infective and reproductive fitness cost for the nematode in the susceptible eggplant. The resistance of *S. torvum* seems to

Table 1 *Meloidogyne incognita* population densities in soil at transplantation (*Pi*) and at the end of the crop (*Pf*), galling index (GI), number of eggs per plant, reproduction index (RI) and eggplant, *Solanum melongena* 'Cristal' yield ungrafted or grafted onto the *Solanum torvum* 'Brutus' rootstock cultivated from June to October 2017 (135 days) and March to November 2018 (251 days) in the same plots in a plastic greenhouse

	J2 per 100 cm^3 soil					
Plant	*Pi*	*Pf*	GI	Eggs per plant ($\times 10^2$)	RI (%)	Yield (kg per plant)
First crop						
Ungrafted	51 ± 36	29054 ± 8626	4.6 ± 0.3	12323 ± 2408		0.8 ± 0.1
Grafted	28 ± 12	2061 ± 818*	1.0 ± 0.1*	228 ± 76*	2.0 ± 1.00	0.8 ± 0.1
Second crop						
Ungrafted	686 ± 302	821 ± 179	7.5 ± 0.2	36422 ± 5895		1.9 ± 0.2
Grafted	127 ± 54*	124 ± 44*	0.9 ± 0.1*	883 ± 138*	0.1 ± 0.01	4.0 ± 0.4*

Data on nematode population densities in soil are the mean ± standard error of 10 replicates. Data on GI, eggs per plant, RI and yield are the mean ± standard error of 40 replicates.
Data followed by *in the same column and year indicate significant differences ($P < 0.05$) between germplasms according to the nonparametric Mann–Whitney *U*-test.
GI: galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck, 1971).
RI (reproduction index) = 100 × [(number of eggs/plant in the rootstock)/(number of eggs/plant on the eggplant cv. Cristal)].

Table 2 Galling index (GI), number of egg masses per plant, number of eggs per plant, reproduction index (RI) and number of eggs per egg mass of *Meloidogyne incognita* subpopulations obtained from roots of ungrafted eggplant, *Solanum melongena* 'Cristal', and grafted onto the *Solanum torvum* 'Brutus' rootstocks in Cristal and Brutus 55 days after cultivation in 200 cm^3 pots inoculated with 1 J2 cm^{-3}

	GI		Egg masses per plant		Eggs per plant		RI (%)		Eggs per egg mass	
Plant	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
Cristal	3.8 ± 0.2	2.9 ± 0.2†	79 ± 9	40 ± 3†	39746 ± 4392	17526 ± 2084†			533 ± 47	440 ± 27
Brutus	1.8 ± 0.3*	0.9 ± 0.2*,†	2 ± 1*	1 ± 0*	884 ± 230*	532 ± 138*	2.2 ± 0.6	3 ± 0.8	364 ± 55*	347 ± 103*

The nematode inoculum was obtained after cultivating the ungrafted and grafted eggplant cv. Cristal onto the rootstock cv. Brutus over two consecutive cropping seasons in the same plots in a plastic greenhouse.
Data are mean ± standard error of 10 replicates. Values followed by * in the same column show significant differences ($P < 0.05$) between germplasms according to the nonparametric test Mann–Whitney *U*-test. Values of each parameter followed by † show significant differences ($P < 0.05$) between nematode subpopulations per each plant according to the nonparametric test Mann–Whitney *U*-test.
GI: galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck, 1971).
RI (reproduction index) = 100 × [(number of eggs/plant in the rootstock)/(number of eggs/plant in the eggplant cv. Cristal)].

be more stable than other resistance genes in fruiting solanaceous crops such as tomato and pepper. In tomato, the selection for virulence to the *Mi-1.2* gene can be acquired progressively crop by crop of resistant tomato cultivars (Giné & Sorribas, 2017) or rootstocks (Verdejo-Lucas *et al.*, 2009), or suddenly just after one tomato crop grafted onto the resistant rootstock cv. Aligator (Expósito *et al.*, 2019). Regarding pepper, the selection for virulence to the *Me3* gene has been reported after two consecutive pepper crops grafted onto the rootstock cv. Atlante (Ros-Ibáñez *et al.*, 2014). In relation to the *N* gene, virulence has been reported in the USA but without any information on the selection process (Thies, 2011). Yang *et al.* (2014) consider that the entire disease resistance pathway is amplified in *S. torvum* compared with tomato and potato, enhancing plant defence mechanisms and resistance durability.

The acquisition of virulence to a resistance gene can have a fitness cost for the nematode in susceptible cultivars of the same plant species (Castagnone-Sereno *et al.*, 2007; Djian-Caporalino *et al.*, 2011; Expósito *et al.*, 2019) after a minimum number of exposures to this resistance gene. For example, three resistant tomato crops were needed to affect the infectivity, reproduction and fecundity of a partially virulent *M. incognita* subpopulation in susceptible tomato compared to the avirulent subpopulation (Expósito *et al.*, 2019). Surprisingly, the results of the present study revealed that the infective and reproductive fitness of the nematode decreased without having been selected for virulence after two years of repeated cultivation. The causes for this loss of fitness as well as the stability of this characteristic should be investigated. In a nematode field population, a certain proportion of infective J2 can counteract the *S. torvum* resistance in a proportion that is maintained in the offspring, irrespective of the plant resistance status in which they were originated, as observed in the experiment here. Nonetheless, the proportion of the offspring originated in *S. torvum* has a fitness cost manifested in susceptible eggplant. This finding can have important consequences for managing *M. incognita* by agronomic methods because the nematode reproduction in susceptible eggplant decreased by about 56%. Therefore, the use of different resistant sources in rotation with susceptible ones will decrease the risk of selecting virulent nematode populations. Solanaceae and Cucurbitaceae are the two most common botanical families used in rotation under protected cultivation (Moncada *et al.*, 2013). In addition to the commercially available resistant tomato, pepper and eggplant cultivars and/or rootstocks, some other resistance sources in the Cucurbitaceae family such as *Cucumis metuliferus* and *Citrullus amarus* could be used as rootstocks in rotation schemes. Moreover, the resistance of *C. metuliferus*, *C. amarus* and *S. torvum* is also expressed against virulent populations to the *Mi-1.2* and *N* resistance genes (Expósito *et al.*, 2018; García-Mendívil *et al.*, 2019b). Plant resistance is an effective and economically profitable control method (Sorribas *et al.*, 2005) that can be durable if it is used in a proper manner (Djian-Caporalino *et al.*, 2011). Among the proposed strategies to increase resistance durability, alternating only two resistance genes reduces virulence selection but does not prevent it (Expósito *et al.*, 2019). Including more resistance sources, some of them having a fitness cost for the nematode such as that in *S. torvum*, could prevent the selection for virulence to specific resistance genes and could also reduce the infective and reproductive capability of the nematode in susceptible germplasm.

In relation to crop yield, significant differences between ungrafted and grafted eggplant were only detected after long cropping periods. An eggplant yield increase of 27% was recorded when cultivated over 9 months, but no differences were found for cropping periods shorter than 6.5 months (Çürük *et al.*, 2009; Gisbert *et al.*, 2011; Moncada *et al.*, 2013; Sabatino *et al.*, 2013; Bogoescu & Doltu, 2015). In the present study, grafted eggplant yielded a 110% more than the ungrafted when cropped over 8.3 months, but did not differ when cultivated over 4.5 months.

In summary, grafted eggplant onto *S. torvum* can yield significantly more in nematode-infested soil depending on *Pi* and/or crop duration and is a valuable tool for managing the three tropical *Meloidogyne* spp. irrespective of its (a)virulence status to other resistance genes in fruiting solanaceous crops; it reduces the infective and reproductive fitness of the nematode in susceptible eggplant after two consecutive crops without selecting for nematode virulence. However, special attention should be given in relation to variants of the nematode able to overcome resistance in *S. torvum*. Recently, the genotype A2-J of *M. arenaria* from Japan has been reported as virulent to *S. torvum*, but not the A2-O. Interestingly, the distribution area of the genotype A2-J overlaps with the cultivation area of eggplant (Uehara *et al.*, 2017). Additional long-term studies will be necessary to determine the resistance durability.

Acknowledgements

The authors thank MINECO and FEDER for funding projects AGL2013-49040-C2-1-R and AGL2017-89785-R, and CONACYT for providing PhD funding to H. A. García-Mendívil. Thank are also given to Laura Mateo from Semillas Fitó for providing seeds of *S. torvum* cv. Brutus, and to Sergi García, Alejandro Expósito and Miquel Masip for technical assistance. The authors have no conflict of interest to declare.

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