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*in memoriam*  
**Dr. Alain Palloix**



# PROCEEDINGS

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# Screening of solanaceous wild relatives for graft affinity with eggplant (*Solanum melongena* L.)

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## Abstract

Eggplant (*Solanum melongena* L.), an important solanaceous crop in the world, is susceptible to soilborne bio-agressors, the main of which, in temperate areas, being *Verticillium* wilt (*Verticillium* sp.) and root knot nematodes (*Meloidogyne* spp.). Grafting on resistant rootstock is an alternative method to soil disinfection. In France, the generalization of grafting spread over the 2000's. After several years of utilization of resistant tomato rootstocks for eggplant production, and to a lesser extent, of *Solanum torvum* rootstocks, damaging wilt-like-symptoms are often observed by growers. Hence it is necessary to identify alternative rootstocks, able to sustain the soil pathogenic complex by a combination of vigor and genetic resistance. However, graft affinity between potential rootstocks and eggplant (scion) is the first step to investigate. Solanaceae family offers a wide choice of candidate rootstock species. The objective of this research was to identify solanaceous species having a good graft affinity with eggplant. Screening of a wide set of genetic resources was carried out at the Centre Technique Interprofessionnel des Fruits et Légumes -Ctifl- (Lanxade and Balandran center) in collaboration with INRA. Experiments were carried out during a five years period (2011-2015). Several *Solanum* species displayed a good graft affinity with eggplant and are candidates for further agronomic evaluation.

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## 1. Introduction

Eggplant (*Solanum melongena* L.) is an important solanaceous crop in the world and represents an important source of income for growers. The species is susceptible to several soilborne bio-agressors. *Verticillium* wilt (*Verticillium* sp.) and root knot nematodes (*Meloidogyne* spp.) are the major limiting factors for eggplant production in temperate conditions, worldwide. Up to a recent past, both diseases were controlled by soil fumigation with methyl bromide prior to planting. However, since this chemical is no more authorized for this use in European countries since 2005, no alternative chemicals have provided sufficient control of soilborne diseases. Grafting on resistant rootstock is an alternative method to soil disinfection. In France, the research on grafting for vegetables production started in the 1950's, and grafting onto *Verticillium* resistant tomato rootstocks started in the 1960's for commercial production (Messiaen and *al.*, 1967; Beyries, 1974). The generalization of grafting spread over the 2000's and nowadays 63% of French eggplant surfaces are grafted (Torres and Brand, 2015). After several years of utilization of resistant tomato rootstocks for eggplant production, and to a lesser extent, of *Solanum torvum* rootstocks, damaging wilt-like-symptoms are often observed by growers. A survey by Villeneuve and *al.* (2016) in two main French production areas indicated that *Verticillium*, as well as at least two additional fungi, were responsible of the wilt symptoms observed on grafted plants, thus revealing the development of new pathogenic

complexes in intensively cultivated soils. Hence it is necessary to identify alternative rootstocks, able to sustain the new soil pathogenic complexes by a combination of vigor and genetic resistance. Graft affinity between potential rootstocks and eggplant (scion) is the first step to investigate. *Solanum* genus, and more largely, Solanaceae family, offer a wide choice of candidate rootstock species. The objectives of this research were to identify solanaceous species having a good graft affinity with eggplant and yielding good agronomic results. Screening of a wide set of genetic resources was carried out at the Centre Technique Interprofessionnel des Fruits et Légumes -Ctifl- (Lanxade and Balandran center) in collaboration with INRA.

## 2. Materials and method

Graft affinity between eggplant and candidate rootstocks was investigated in a two steps process, (i) screening of rootstocks on young plants in semi-controlled conditions, and (ii) for the best rootstock-scion combinations, evaluation of agronomic results in greenhouse conditions.

### Plant materials

Seventy five accessions of several genera and species belonging to the *Solanaceae* family, as well as five interspecific hybrids between eggplant and related *Solanum* species, were included in this study (table 1). Seeds were obtained from INRA (Vegetables Genetic Resources Centre, CRB-Leg, GAFL, Montfavet, and Ploudaniel) and also from the French institute of Tabaco (Bergerac, France), the Universitat Politècnica de València (Spain) and the Radboud University (Nijmegen, The Netherlands). The interspecific hybrids (F1) were created at INRA GAFL. Germination was secured with a 500 ppm gibberellic acid (GA<sub>3</sub>) treatment applied to the seeds 24 hours before sowing in a substrate containing 67% of compost and 33% of quartz. Two leaf stage plantlets were transplanted in pots containing the same substrate (1.5l).

Species	Accession number	Seed source	Accessions used in confirmation trials
<i>Capsicum annuum</i>	RV6	Inra GAFL	
<i>C. baccatum</i>	PM 1034	Inra GAFL	
<i>Cestrum parqui</i>	874750007	Nijmegen	
<i>Cyphomandra betacea</i>	894750221	Nijmegen	2012-13
<i>Hyoscyamus niger</i>	88475005	Nijmegen	
<i>Iochroma australe</i>	904750118	Nijmegen	
<i>Lycianthes rantonetii</i>	814750064	Inra GAFL	
<i>Lycium barbarum</i>	MM 1378	Nijmegen	2013
<i>Nicandra physaloides</i>	884750065	Institut du tabac	
<i>Nicotiana tabacum</i> n°1	MS 270	Institut du tabac	
<i>N. tabacum</i> n°2	MS 33518	Inra GAFL	
<i>Physalis edulis</i>	MM 1321	Inra GAFL	2013
<i>P. peruviana</i>	MM 1358	Inra GAFL	2012-13
<i>Solanum acanthoideum</i>	MM 12296	Inra GAFL	
<i>S. aculeastrum</i>	MM 1425	Inra GAFL	2013-15
<i>S. aethiopicum</i> group Gilo	MM 369	Inra GAFL	2013-14-15
<i>S. aethiopicum</i> group <i>Aculeatum</i>	MM 232 bis	Inra GAFL	2013-14-15
<i>S. anguivi</i> agg.	MM 134	Inra GAFL	2015
<i>S. arundo</i>	MM 1689	Inra GAFL	2012-13
<i>S. atropurpureum</i>	MM 1369	COMAV	
<i>S. burchellii</i>		COMAV	
<i>S. canense</i>		COMAV	

<i>S. caripense</i>	MM 1526	Inra GAFL	2015
<i>S. catombelense</i>	MM 987	Inra GAFL	
<i>S. cerasiferum</i>	UPV 23386	Inra Ploudaniel	
<i>S. chacoense</i>	UPV 23372	Inra GAFL	
<i>S. citrullifolium</i>	MM 1218	Inra GAFL	2015
<i>S. coccineum</i>	MM 866	Inra GAFL	2014
<i>S. cyaneo-purpureum</i>		Inra GAFL	
<i>S. dasyphyllum</i>	MM 1174	Inra GAFL	
<i>S. dennekense</i>	MM 992	Inra GAFL	
<i>S. dinteri</i>	MM 994	Inra GAFL	2015
<i>S. elaeagnifolium</i>	MM 1137	Inra GAFL	
<i>S. erianthum (verbascifolium)</i>	MM 1137	Inra GAFL	2015
<i>S. glaucophyllum</i>	MM 1312	Inra Ploudaniel	
<i>S. hastifolium</i>	MM 1221	Inra GAFL	2012-13-15
<i>S. hougasii</i>	MM 1534	Inra GAFL	2013-15
<i>S. incanum</i> group A	MM 1326	Inra GAFL	2013-15
<i>S. incanum</i> group B	MM 1793	Inra GAFL	
<i>S. incanum</i> group C	MM 1349 bis	Inra GAFL	2013-15
<i>S. incanum</i> group C		Inra GAFL	
<i>S. incanum</i> group D		Inra GAFL	
<i>S. jatrophiifolium</i>	MM 716	Inra GAFL	2013
<i>S. kurzii (sanitwongsei)</i>	MM 1428	Inra GAFL	
<i>S. laciniatum</i>	MM 664	Inra GAFL	2013
<i>S. lidii</i>	MM 684	Inra GAFL	2013-15
<i>S. linnaeanum</i>	MM 1248	Inra GAFL	
<i>S. macrocarpon</i>	MM 1529	Inra GAFL	
<i>S. mammosum</i>	MM 1003	Inra GAFL	2012-2013
<i>S. marginatum</i>	MM 370	Inra GAFL	
<i>S. mauritianum</i>	MM 1005	Inra GAFL	
<i>S. melanospermum</i>	MM 195	Inra GAFL	2012-13-14
<i>S. muricatum</i>	MM 1136	Inra GAFL	
<i>S. palinacanthum</i>	MM 1715	Inra GAFL	2013
<i>S. pyracanthos</i>	MM 824	Inra GAFL	
<i>S. renschii</i>	MM 573	Inra GAFL	
<i>S. richardii</i>	MM 1350	Inra GAFL	2014
<i>S. rigescens</i>	MM 1821	Inra GAFL	2015
<i>S. rigescentoides</i>	MM 1821	Inra GAFL	
<i>S. rostratum</i>	MM 1762	Inra GAFL	
<i>S. rubetorum (rigescens auct. non Jacq)</i>	MM 1014	Inra GAFL	
<i>S. scabrum</i>	MM 1015	Inra Ploudaniel	
<i>S. schimperianum</i>	MM 1753	Inra GAFL	
<i>S. sisymbriifolium</i>	MM 1224	Inra GAFL	
<i>S. stoloniferum</i>	MM 1226	Inra GAFL	
<i>S. stramonifolium</i>	MM 1190	Vilmorin	2012-13-15
<i>S. supinum</i>	MM 1018	COMAV	2012-13
<i>S. tomentosum</i>	MM 831	Inra GAFL	2012-13-14
<i>S. torvum</i>	MM 12192	Inra GAFL	2012-13-14
<i>S. trachycarpum</i>	MM 284	Inra GAFL	
<i>S. trilobatum</i>		Inra GAFL	
<i>S. viarum (without spines)</i>		Inra GAFL	
<i>S. violaceum</i>	MM 416		
<i>S. virginianum</i>	MM 1022		
<i>Withania somnifera</i>			

	MM 1024		
	STT3		
	UPV 23392		
	MM 1025		
	MM 1602		
	MM 497		
	MM 511		
	MM 1262		
F1 ( <i>S.aethiopicum</i> Gilo X <i>S. melongena</i> )	MM 232 x	Inra GAFL	2013-14-15
F1 ( <i>S. linnaeanum</i> X <i>S. melongena</i> )	LF3	Inra GAFL	2013-14-15
F1 ( <i>S. melongena</i> X <i>S. incanum</i> gr C)	MM 195 x	Inra GAFL	2014-15
F1 ( <i>S. melongena</i> X <i>S. incanum</i> gr D)	LF3	Inra GAFL	2013-15
	LF3 x MM		
	664		
	LF3 x MM		
	1248		

Table 1: Plant material used as experimental rootstocks

### Early screening for graft affinity

The experiments were carried out from 2011 to 2014 in greenhouse at Lanxade center of Ctifl located in southwestern France (lat.: 44.86, long.: 0.40). Controls were (i) the non-grafted 'Monarca F1' eggplant variety (Rijk Zwaan, Aramon, France), (ii) the self-grafted 'Monarca F1', and (iii) the commercial tomato rootstock 'Maxifort' (Monsanto, France) which is an interspecific hybrid *S. lycopersicum* X *S. habrochaites*.

Plants of the 'Monarca F1' scion at the 2-4 true leaves stages (20-50 days old) were grafted onto rootstock plants having 3-4 true leaves (40-50 days old) using the cleft grafting method. To be sure that scions and rootstocks were to have similar stem diameter at grafting time, sowing was made three times for the variety, at one week interval. After grafting, plantlets were kept for 5 days within a closed plastic shelter in a greenhouse with a day/night thermoperiode maintained between 25° and 18°C. Later on, grafted plantlets were progressively acclimatized by perforating the plastic. After acclimatization, grafted plants were placed in greenhouse under natural lighting for 150 days. Grafting combinations were randomized in a complete block design, with three replications of 5 plants per treatment.

Success of graft union was recorded as well as, 100 days after grafting, plant height and fresh weight of aerial part. Each graft union was longitudinally cut in order to observe the presence of browning (data not shown).

### Agronomic trials

The experiments were carried out in 2012 to 2015 in a greenhouse at Balandran center of Ctifl in southeastern region of France (lat.: 43°75', long.: 4°45'N). Seeds were sown at INRA and grafting (tongue approach) was realized by a professional nursery. Experimental design and controls were the same as for the early screening assays.

Mortality throughout the cultivation period, plant height at the end of trial (150 days after transplanting) -data not shown- were recorded. Early and total yield (kg/m<sup>2</sup>) were measured for each individual plant. Early yield was calculated over the first three weeks of harvest.

### 3. Results and discussion

#### Early screening for graft affinity

The best percentages of successful grafting are displayed in table 2. One hundred to 90% success rate was observed for 26 graft combinations; ■ 90-70% for 22 combinations; ■ 70-50% for 7; and ■ less than 50% for 19.

Overall, the species phylogenetically distant from *S. melongena* expressed a bad graft affinity, such as *Nicotinia* spp, *Physalis* spp, *Capsicum* spp., and *S. canense*. Our results for *S. sisymbriifolium*, a species valued for its resistance to *V. dahliae* (Bletsos *et al.*, 1998), don't confirm the good grafting affinity observed by Bletsos *et al.* (2003). We recorded only 13% of success grafting rate whereas these authors observed a rate over 70%. Our results are consistent with those of Rahman *and al.* (2002) who concluded that *S. sisymbriifolium* is not a promising rootstock for eggplant.

Conversely, some rootstocks, in particular the interspecific hybrids, exhibit a high rate of graft success, a good quality graft union and allow a good development of the scion. It should be noted that the grafting technique used in these early trials is not adapted to the commercial control rootstock 'Maxifort', since we obtained a random rate of grafting success (figure 1).

#### 100% of grafting success

Cyphomandra betacea  
*S. aethiopicum* group aculeatum  
*S. coccineum*  
*S. dasyphyllum*  
*S. dennekense*  
*S. erianthum*  
*S. glaucophyllum*  
*S. incanum* group A  
*S. incanum* group B  
*S. incanum* group C (MM 664)  
*S. lidii*  
*S. pyracanthos*  
*S. rigescentoides*  
*S. aethiopicum* Gilo X *S. melongena*  
*S. melongena* X *S. incanum* gr C  
*S. melongena* X *S. incanum* gr D

#### Grafting success between 90 and 99,9%

*Hyoscyamus niger*  
*S. aethiopicum* Gilo X  
*S. anguivi* agg.  
*S. arundo*  
*S. atropurpureum*  
*S. dinteri*  
*S. kurzii*  
*S. marginatum*  
*S. rigescens*  
*S. viarum* (without spines)  
*S. virginianum*

Table 2:  
The best accessions for grafting success in compatibility trials

The successful graft combinations induce however a wide growth range of the Monarca scion, from about 80% dwarfing to about 80% vigor boosting, when compared to the control Monarca auto-grafted (figure 1). The rootstocks inducing the highest scion vigor are *S. lidii*, *S. rubetorum*, *S. virginianum* and *S. rostratum*. The rootstocks depressing growth don't present a great interest.

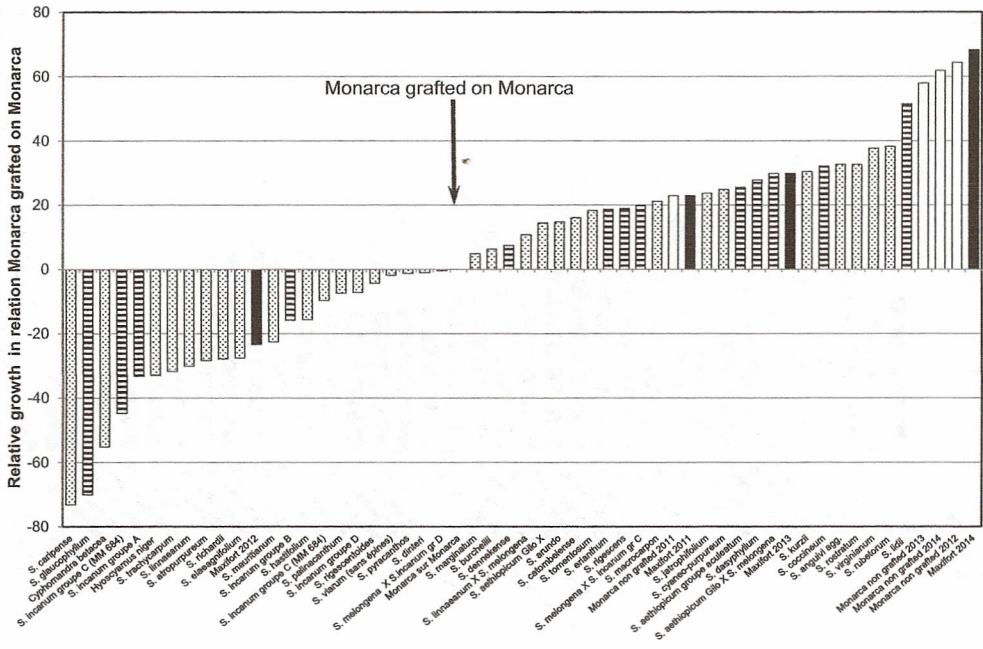


Figure 1:

Scion growth for the rootstocks displaying up to 80% graft success, expressed on the basis of the control Monarca auto-grafted, taken as a growth reference of zero,   
 ■ rootstocks with 100% rate of grafting success; ▨ rootstocks with 80-99% rate of grafting success;   
 □ Monarca F1, not grafted (control); ■ rootstock Maxifort (commercial control).

Different *Solanum* species are spiny, but as the spines are soft at an early plant development stage, they are not problematic.

**Agronomic trials**

We recorded some mortality shortly after plantation for *Cyphomandra betacea* and *Nicandra physaloides* and later (after the third harvest) for *S. atropurpureum* (75% of plants) and *S. coccineum* (53%). Mortality was less for *S. mauritanium*, *S. trachycarpum* and *S. viarum*.

Some *Solanum* species produce suckers, very strongly for *S. acanthoideum*, *S. trachycarpum* and *S. linnaeanum* and at a lower level for *S. aculeastrum*, *S. atropurpureum*, *S. pyracanthos* and the interspecific hybrid *S. linnaeanum x S. melongena*. When the suckers have dense and sharp spines, like *S. pyracanthos*, this may be a problem in cultivation.

Early production is an important aspect for growers. *Nicandra physaloides* and *S. rostratum* are part of the earliest rootstocks, with also good total commercial yield, unlike *S. trachycarpum* and *S. atropurpureum* which present also a good early production but a low total yield. By contrast, some botanical species used as rootstock induce a delayed production like *S. hastifolium*, *S. erianthum* and *S. coccineum* (figure 2).



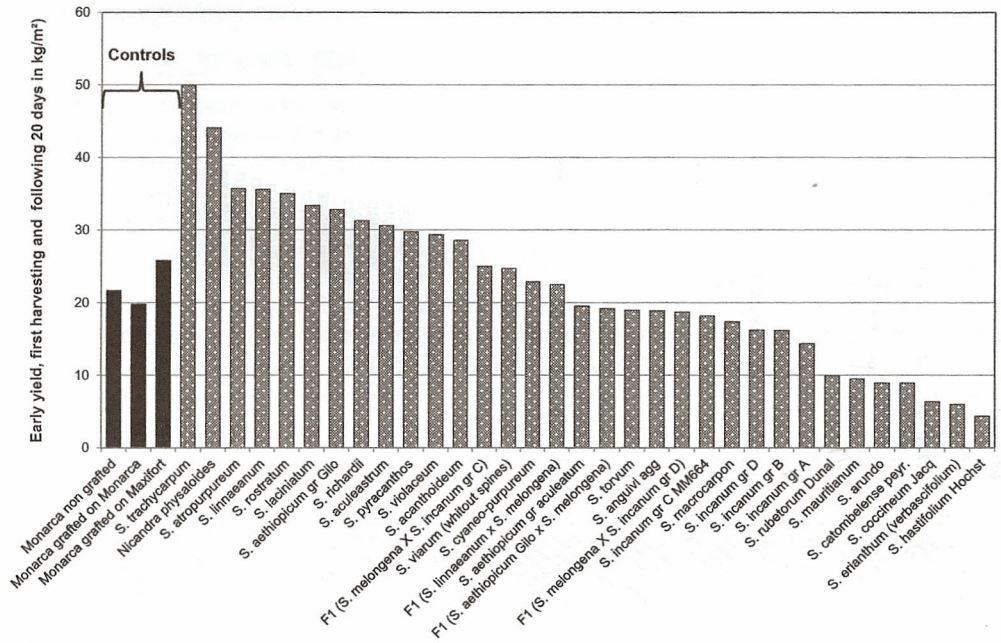


Figure 2:  
Effect of rootstock on early production (in kg/m<sup>2</sup>) for the three first weeks of production

For the total commercial yield, the interspecific hybrid (*S. melongena* X *S. incanum* group C) gives much better results than the commercial rootstock controls 'Maxifort' and *S. torvum* STT3 (figure 3). Yield of the two interspecific hybrids F<sub>1</sub> (*S. aethiopicum* Gilo X *S. melongena*) and F<sub>1</sub> (*S. linnaeanum* x *S. melongena*), as well as *S. aethiopicum* gr Gilo, *S. anguivi*, *S. incanum* group A and D, *S. macrocarpon*, *S. pyracanthos*, *S. rostratum*, and *S. violaceum* is similar to the yield of the two controls (figure 3). On the contrary, several rootstocks provide low to very low yields like *S. trachycarpum*, *S. mauritanum* and *S. hastifolium*. Globally the interspecific hybrids have comparable or better results than their botanical parent.

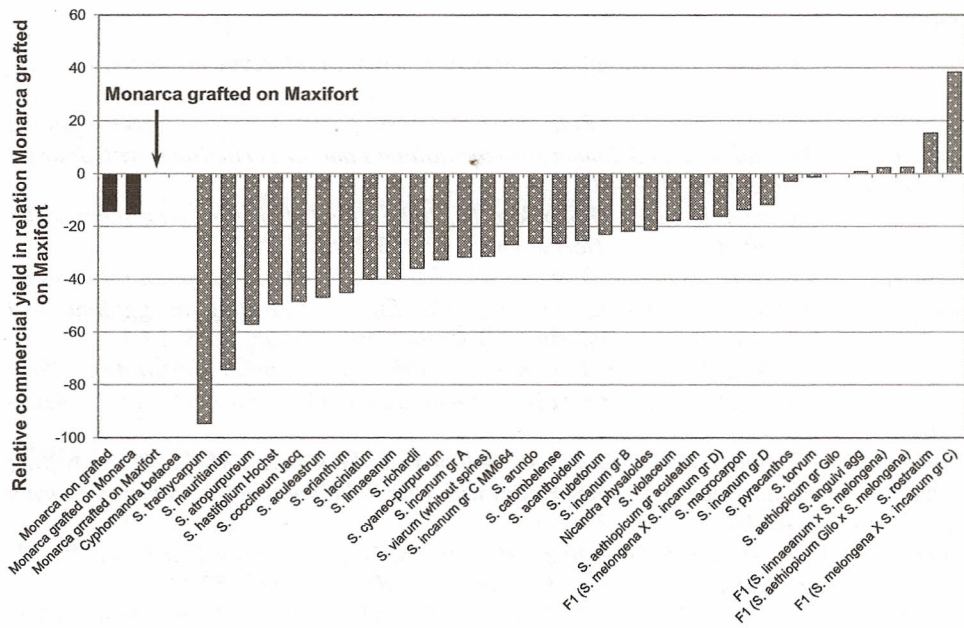


Figure 3:  
Relative total commercial yields 60 days after planting for the different botanical species tested as rootstock grafted onto the variety Monarca. The yield of the control Monarca grafted on Maxifort is taken as the reference of zero.

The data obtained during this prospective research on diverse parameters measuring graft affinity between eggplant (scion) and numerous Solanaceous species, from graft success to commercial yield, reveals the potentialities as rootstocks of several *Solanum* species, in particular *S. aethiopicum* gr. *aculeatum*, *S. aethiopicum* group Gilo, *S. anguivi*, *S. incanum* (group A, B, C, D) and *S. macrocarpon*, as well as interspecific hybrids ( $F_1$  *S. aethiopicum* group Gilo x *S. melongena*,  $F_1$  *S. melongena* x *S. incanum* group C and D and  $F_1$  *S. linnaeanum* x *S. melongena*) present also interest. Similar promising results, although obtained with less rootstocks germplasm, were obtained by Gisbert *and al.* (2011).

Further research is still needed before developing commercial new rootstocks. Indeed the agronomic performances of the best rootstocks identified has to be retested in different production conditions. Further, it is necessary to estimate their root vigor as well as their level of resistance to the major elements of the soil pathogenic complex, in particular *Verticillium dahliae*, *Colletotrichum coccodes* and *Meloidogyne* species. Furthermore, the alkaloid content of the eggplant fruits produced on these rootstocks has also to be looked at carefully.

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