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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.

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. Materiels 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_3) 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
Capsicum annuum	RV6	Inra GAFL	
C. baccatum	PM 1034	Inra GAFL	
Cestrum parqui	874750007	Nijmegen	
Cyphomandra betacea	894750221	Nijmegen	2012-13
Hyoscyamus niger	88475005	Nijmegen	
Iochroma australe	904750118	Nijmegen	
Lycianthes rantonetii	814750064	Nijmegen	
Lycium barbarum	MM 1378	Inra GAFL	
Nicandra physaloides	884750065	Nijmegen	2013
Nicotiana tabacum n°1	MS 270	Institut du tabac	
N. tabacum n°2	MS 33518	Institut du tabac	
Physalis edulis	MM 1321	Inra GAFL	
P. peruviana	MM 1358	Inra GAFL	
Solanum acanthoideum	MM 12296	Inra GAFL	2013
S. aculeastrum	MM 1425	Inra GAFL	2012-13
S. aculeatissimum	MM 369	Inra GAFL	
S. aethiopicum group Gilo	MM 232 bis	Inra GAFL	2013-15
S. aethiopicum group *Aculeatum*	MM 134	Inra GAFL	2013-14-15
S. anguivi agg.	MM 1689	Inra GAFL	2013-14-15
S. arundo	MM 1369	Inra GAFL	2015
S. atropurpureum		COMAV	2012-13
S. burchellii		COMAV	
S. canense			

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

	MM 1024 STT3 UPV 23392 MM 1025 MM 1602 MM 497 MM 511 MM 1262		
F1 (S.*aethiopicum* Gilo X *S. melongena*)	MM 232 x	Inra GAFL	2013-14-15
F1 (*S. linnaeanum* X *S. melongena*)	LF3	Inra GAFL	2013-14-15
F1 (*S. melongena* X *S. incanum* gr C)	MM 195 x	Inra GAFL	2014-15
F1 (*S. melongena* X *S.incanum* 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^2) 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	**Grafting success between 90 and 99,9%**
Cyphomandra betacea	Hyoscyamus niger
S. aethiopicum group aculeatum	S. aethiopicum Gilo X
S. coccineum	S. anguivi agg.
S. dasyphyllum	S. arundo
S. dennekense	S. atropurpureum
S. erianthum	S. dinteri
S. glaucophyllum	S. kurzii
S. incanum group A	S. marginatum
S. incanum group B	S. rigescens
S. incanum group C (MM 664)	S. viarum (without spines)
S. lidii	S. virginianum
S. pyracanthos	
S. rigescentoides	
S. aethiopicum Gilo X S. melongena	
S. melongena X S. incanum gr C	
S. melongena X S.incanum gr D	

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.

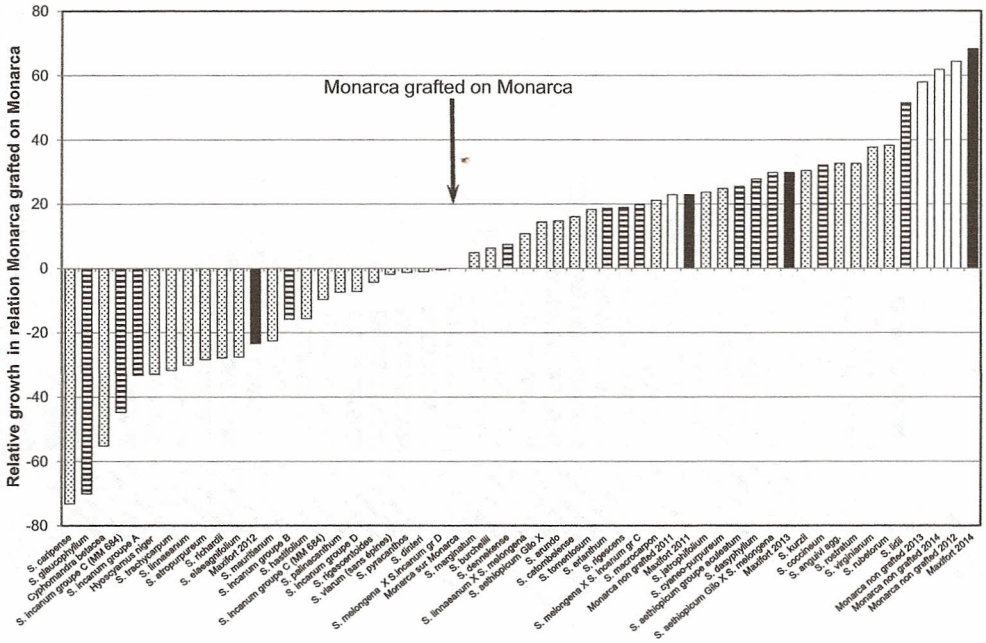

Figure 1:
Scion growth for the rootstocks displaying up to 80% graft success, expressed on the basis of the control Monarca auto-grafted, taken as a growth reference of zero,
rootstocks with 100% rate of grafting success; rootstocks with 80-99% rate of grafting success;
Monarca F1, not grafted (control); 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. mauritianum, 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).

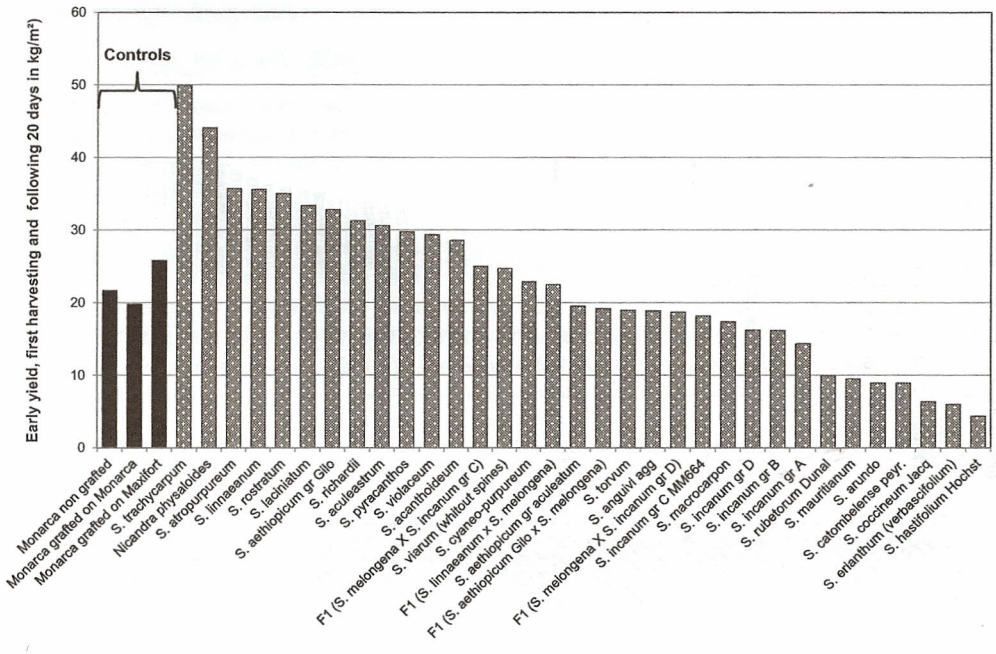

Figure 2:
Effect of rootstock on early production (in kg/m²) for the three first weeks of production

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_1 (S. *aethiopicum* Gilo X *S. melongena*) and F_1 (*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. mauritianum* and *S. hastifolium*. Globally the interspecific hybrids have comparable or better results than their botanical parent.

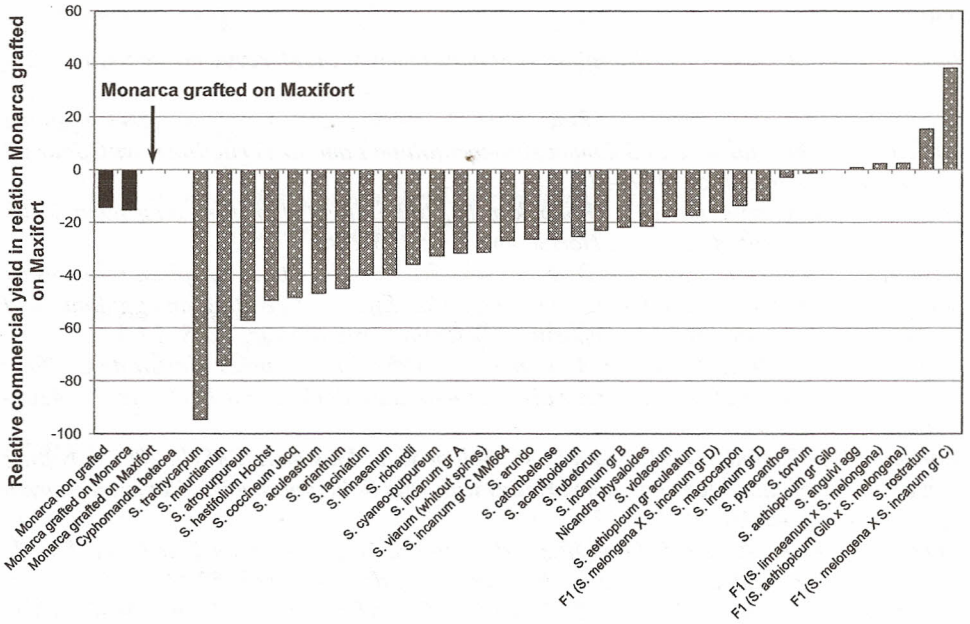

Figure 3:
Relative total commercial yields 60 days after planting for the different botanical species tested as rootstock grafted onto the variety Monarca. The yield of the control Monarca grafted on Maxifort is taken as the reference of zero.

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 developping 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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