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Critical aspects of grafting as a possible strategy to manage soil-borne pathogens in Italy

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ABSTRACT

The management of soil-borne pathogens is nowadays complicated by the increasing restrictions in the usage of fumigants. Several factors need to be considered for a sustainable use of this practice such as the susceptibility of rootstocks against soil-borne pathogens which is age-dependent and the incomplete resistance of some of the rootstocks to one or more pathogens. There is an evidence of a pathogenic variation among some isolates of *Phytophthora* spp. on solanaceous crops and on rootstocks and the development of new diseases or the re-emergence of already known pathogens such as *Colletotrichum coccodes* after the phase out of methyl bromide. The critical aspects of grafting for soil-borne pathogens management in Italy are discussed.

Keywords: solanaceaous crops, *Phytophthora nicotianae*, *Phytophthora capsici*, *Colletotrichum coccodes*

1.Introduction

In Italy, 59 million vegetable plants, generally grown under greenhouse, are grafted (Morra and Billotto, 2010). In Piedmont (northern Italy) grafted plants are mostly used in tomato cropping system but is becoming more popular also in the case of bell pepper and, in some areas, on melon. Grafted tomatoes are used to reduce susceptibility against pest and root and wilt diseases and to increase yield (Louws et al., 2010; Rouphael et al., 2010). However, resistance may be broken down under high pathogen population pressure and new races of the pathogen may evolve on rootstocks. Recently, some emerging diseases on plants grafted on rootstocks have been recorded in Italy. Verticillium wilt incited by *Verticillium dahliae* was consistently observed on eggplant grafted on *Solanum torvum* (Garibaldi et al. 2005), while *Colletotrichum coccodes* attacks were observed on grafted tomato plants grown in several farms after repeated cropping cycles of tomato (Minuto et al. 2008). This last pathogen can infect several cultivated species, including pepper, potato, eggplant, lettuce, chrysanthemum and some species of Cucurbitaceae and Brassicaceae (Dillard, 1992; Last et al., 1966). Among *Phytophthora* group, both *P. nicotianae* and *P. capsici* can affect tomato (Erwin and Ribeiro, 1996). Rootstocks belonging to interspecific hybrids of *Solanum lycopersicum* x *S. hirsutum* (cvs. Beaufort, He Man) and to *S. lycopersicum* (cv. Energy) were infected by *Phytophthora nicotianae* (Minuto et al. 2007 b). Pathogenic variation among the isolates of *Phytophthora nicotianae* requires further studies to evaluate the possibility of existence of different physiological races within *P. nicotianae* (Garibaldi and Gullino, 2010), while *P. capsici* is a pathogen showing a high degree of variability and variation among isolates (Foster and Hausbeck, 2010). Increased symptoms of basal rots caused by *Rhizoctonia solani* (anastomosys group AG4) were repeatedly observed on tomato grafted onto *S. lycopersicum* x *S. hirsutum* in Veneto, Piedmont, Liguria, Lazio, Campania, Sardinia under greenhouse conditions after 5 days to 6 weeks from transplant at 20-27°C (Minuto et al., 2007 a). *Phytophthora capsici*, *P. nicotianae* and

C. coccodes showed a broad host range among solanaceous and cucurbitaceous and the success of crop rotation is limited.

Due to an increased request of grafted plants, experimental activities have been carried out to evaluate the effectiveness of grafting on resistant rootstock against emerging soilborne diseases of tomato. The rootstocks used in this study are considered resistant to many other soil-borne diseases, but their tolerance to *P. nicotianae, P. capsici* and *C. coccodes* is not known.

2.Materials and methods

2.1. Experimental layout.

The first set of trials was carried out in 2010 under growth chamber conditions at Agroinnova (Univesity of Torino) to test the susceptibility of several tomato rootstocks to *Phytophthora nicotianae* and *P. capsici* under artificial inoculation conditions. The second set of trials was carried out in 2010 in a plastic tunnel at Boves (Cuneo) (northern Italy) in a soil with a history of several tomato crops during the seasons prior to the beginning of this study (Table 1).

2.1.1. Growth chamber trials.

Eight commercially available tomato rootstocks were used in this study: 'He-Man', 'Arnold', 'Armstrong', 'Maxifort', 'Beaufort', 'Unifort', 'Natalya' and 'Spirit' while the tomato 'Cuore di bue' was used as susceptible control. The strains of *Phytophthora nicotianae* (PHT7) and *P. capsici* (PHT22) isolated from *S. lycopersicum* were propagated for the production of zoospores according to the method described by Li et al. (2009) (Table 1).

Seeds of individual cultivars were sown in 60-plug trays filled with steamed mix soil (Tecno 2: Tiesse 3, 1:1 vol/vol), and maintained in a glasshouse at 28°C with 12 h fluorescent light per day. Fourteen and 21 day-old seedlings were transplanted into plastic pots (14cm×14cm×10cm) filled with organic soil. Plants were watered previous to inoculation in order to keep the soil wet and facilitate zoospore movement. One millilitre zoospore suspension (5×10^4 ml^{-1}) was pipetted around the base of the plant. Inoculated and uninoculated seedlings were watered daily and maintained in a growth chamber with 12 h fluorescent light per day, at temperatures of 25±1°C. One pot with 4 seedlings was considered as one replicate and three replicates were used for each treatment, arranged in a completely randomized block design. Each trial was repeated three times.

2.1.2.Trials in plastic tunnel.

Tomato plants (cv. Ingrid and Tomahawk), 45 to 55 days old, not grafted and grafted on several rootstocks (Table 1), were transplanted in a soil with a history of 5 tomato cycles during the seasons prior to the beginning of this study. Grafted plants were transplanted 15–20 cm from the bed edge and 40–50 cm apart, and at 40-cm spacing along each row into soil covered with black plastic mulch by following a randomized block design, with three replicates and 5 plants/replicate.

2.2. Data collection and analysis.

The reaction of tomato rootstocks to the *P. nicotianae* and *P. capsici* isolates was evaluated 14 days after inoculation. Disease severity was measured according to an arbitrary scale from 0-100: 0, no symptoms; 25, some blight on stem; 50, blight and some necrosis; 75, blight, necrosis and wilting; 100, dead plant. *C. coccodes* infection in the tunnel trial was checked by evaluating the percentage of damaged plants and of infected roots at the end of the experiment, six months after transplanting.

At the end of each trial, the causal fungus was isolated from roots on selective medium for *Phytophthora* (Masago et al., 1977) and on potato dextrose agar in order to confirm the presence of *C. coccodes*. All data collected were statistically analysed, according to Tukey's test.

3. Results

Phytophthora infection resulted in rapid wilt and collapse on younger plants (14 days after sowing) 3 days after the artificial inoculation with zoospores, while on older plants (21 days from sowing), the symptoms appeared 4-5 days after inoculation. PHT7 strain of *P. nicotianae* appeared more virulent on the tested tomato rootstocks than PHT22, identified as *P. capsici* (Table 2). The main differences between the two *Phytophthorae* species were observed when the plants were inoculated 21 days after sowing: 'Beaufort', 'Unifort', 'Natalya' and 'Spirit' were resistant when inoculated with *P. capsici* isolate and susceptible when the *P. nicotianae* was used. 'He-Man' was partially resistant to *P. caspici* isolate and highly susceptible to *P. nicotianae* strain used. Young or old plants of the rootstock 'Arnold' exhibited a consistent and broad spectrum of disease resistance against both the *Phytophthora* isolates.

At the end of the tunnel trial, *C. coccodes* interested from 86.7 to 89.2% of roots in the cv. Tomahawk and cv. Ingrid grown in control plots. The most susceptible rootstocks were 'Unifort', 'Maxifort', 'Big Power' and 'Optifort' that showed a disease severity ranging from 36.7 to 55% of infected roots. The best results in terms of disease resistance were observed on tomato grafted on 'Armstrong' and 'Arnold', 'SuperPro', and 'Brigeor' (Table 3).

4. Conclusions

Grafting tomato is a technique increasingly adopted for several purposes: it increases the yield (Geboloğlu et al., 2011), and confers resistance to several pest and diseases, such as Fusarium wilt, bacterial wilt caused by *Ralstonia solanacearum* and root-knot nematodes (King et al. 2008; Lopez-Perez et al., 2006; Rivard and Louws 2008; Yamazaki et al. 2000 a; b). On the contrary, pathogens generally considered minor can became major on the rootstocks in the absence of soil fumigation (Garibaldi, A., Gullino, M.L., 2010). The Piedmont tomato growing system is conductive to both *P. capsici* and *P. nicotianae* infection. Our results show that some of the commercially available tomato rootstocks, such as 'Arnold' and 'Armstong' confer high levels of resistance to different isolates of Phytophthora crown and root rot and *C. coccodes*. Therefore, the results described in this study confirm that susceptibility of the rootstocks was age-dependent. Interestingly, from a practical point of view, rootstocks, such ‘Arnold’, also confer resistance to *P. nicotianae* strains isolated from *S.lycopersicum* × *S. hirsutum* ‘Beaufort’ (Gilardi et al., 2011). All the reported information confirms the potential risk due to the presence of *C. coccodes*, *P. capsici* and *P. nicotianae* in greenhouse tomatoes by considering also the practical difficulties of organizing effective crop rotation. The selection of resistant rootstocks should consider this aspect. Evaluating promising new rootstock candidates for their resistance or tolerance to Phytophthora crown and rot root is an important task of the tomato breeders in Italy. Other researches are needed, particularly in areas where grafting on resistant rootstocks is considered as an effective practice to avoid soil fumigation and to make it possible to continuously grow susceptible tomato cultivars such as ‘Cuore di bue’. In this regard, experimental programmes aimed at verifying the mid-term effects of repeated transplanting of commercial tomato rootstocks in infested greenhouse soil, will soon be concluded.

Acknowledgements

Work supported by a grant from LIFE project ‘Sustainable use of chemical fumigants for the control of soil-borne pathogens’ (LIFE08 ENV/IT/00432). The authors kindly thanks Dr. Pinxiang Lu and Dr. Michele Baudino for their technical support.

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Table 1. Experimental layout of the trials.

Trial description	Growth camber trials	Field trials
Target pathogen	*Phytophthora nicotianae* PHT7 *Phytophthora capsici* PHT22	*Colletotrichum coccodes*
Artificial inoculation	Zoospores at $5x10^4$	Natural infested soil
Host	Rootstocks at 14 and 21 day after sowing	Tomato cv. Tomawak and cv. Ingrid grafted on several rootstocks
End of the trials	30 days after transplanting	6 months after transplanting

Table 2. Disease reaction of different tomato rootstocks screened for resistance to *Phytophthora* crown rot.

Rootstock	Seed company	*P. nicotianae* strain PHT7		*P. capsici* strain PHT22	
		14[x]	21	14	21
He-Man	Syngenta Seeds	HS [y]	HS	PR	PR
Maxifort	De Ruiter	HS	PR	S	PR
Beaufort	De Ruiter	HS	S	HS	R
Unifort	De Ruiter	HS	S	HS	R
Arnold	Syngenta Seeds	PR	R	R	R
Armstrong	Syngenta Seeds	S	PR	S	R
Natalya	Esasem	HS	S	HS	PR
Spirit	Nunhems	HS	S	HS	PR
-[z]	Furia sementi	HS	PR	S	PR

[x] Age of the plants artificially inoculated (days from sowing).
[y] Reaction: Resistant (R, disease index from 0 to10), partly resistant (PR, DI: 11-30), susceptible (S, DI: 31-60) and highly susceptible (HS, DI: 61-100).
[z] Susceptible control tomato ‘Cuore di bue’ (Furia sementi).

Table 3. Disease severity of different tomato rootstocks screened for resistance to *C. coccodes* in a naturally infested soil.

Rootstock	Seed company	Disease severity (0-100) on tomato cv. Tomawak [x]		cv. Ingrid	
-[x]	-	86.7	f	89.2	e
Arnold	Syngenta	29.6	abc	21.3	ab
Armstrong	Syngenta	15.4	a	10.0	a
500294	Syngenta	20.4	ab	24.2	b
Unifort	De Ruiter	39.2	cde	46.9	cd
SuperProF1	Vilmorin	30.4	abc	22.9	b
Maxifort	De Ruiter	55.0	e	52.9	d
BigPower	RijkZwaan	36.7	bcd	52.1	d
Optifort	De Ruiter	52.9	de	42.9	cd
Emperador	RijkZwaan	21.7	ab	37.3	c
Brigeor	Gautier	31.3	abc	19.6	ab

[y] Within columns, values followed by a common letter do not differ significantly (Tukey test, $P < 0.05$)

[x] Not grafted control cvs. Tomawak and Ingrid