

SHORT NOTE

Management of chili pepper root rot and wilt (caused by *Phytophthora nicotianae*) by grafting onto resistant rootstock

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Summary. Root rot and plant wilting caused by *Phytophthora nicotianae* is a severe disease of chili pepper (*Capsicum annuum* L.) in open fields and under greenhouse production in Tunisia. Chili pepper grafting for disease management is attracting increased interest in recent years. Using the tube grafting technique, different compatible scion/rootstock combinations were obtained with the wild-type pepper SCM334 and the local chili pepper cultivars 'Beldi' and 'Baker'. SCM334 was resistant to *P. nicotianae*, while the cultivars Beldi and Baker were susceptible. Plant inoculations were performed with *P. nicotianae* zoospores, and severity of root rot was rated 30 days post-inoculation using a 0 (healthy plant) to 5 (dead plant) severity score. On SCM334 rootstock and with 'Beldi' or 'Baker' scions, the intensity of root rot was very low (mean score 0.1–0.2) and plants were healthy. However, with Baker or Beldi rootstocks and SCM334 scions, root rot was severe (mean score 3.1–4.6), leading to high numbers of wilting and dead plants. This severe root rot was similar to that observed on non-grafted plants of 'Baker' and 'Beldi' inoculated with the pathogen. Under greenhouse conditions, measurements of agronomic characters indicated non-consistent improvement of these features on the scion cultivar when SCM334 was the rootstock. Since plant foliage is not attacked by this pathogen, these results show that susceptible chili pepper scions grafted onto SCM334 rootstocks could be used for root rot management and improvement of pepper yields in *P. nicotianae* infested soils.

Key words: *Capsicum annuum*, tube-grafting, resistance.

Introduction

Serrano Criollo de Morelos 334 (SCM334, *Capsicum annuum* L.), considered as the most effective source of resistance to *Phytophthora capsici*, is widely used in pepper breeding programmes (Bonnet *et al.*, 2007). This Mexican wild-type pepper (Gil Ortega *et al.*, 1991; Egea-Gilabert *et al.*, 2008) is used by researchers around the world, and is also resistant to *P. nicotianae*, a pathogen that is causing appreciable damage to pepper production in Tunisia (Allagui and Lepoivre, 2000; Trabelsi *et al.*, 2007). Control of *P. nicotianae* by genetic manipulation or resistant cul-

tivars is not presently available. Pathogenic variability of isolates of *P. nicotianae* (Saadoun and Allagui, 2008) and presence of epistatic effects in SCM334 resistance (Bnejdi *et al.*, 2009) have complicated genetic studies. Selection of commercial varieties with durable resistance to this pathogen needs to be carried out. The current disease management strategies for *P. nicotianae* are usually restricted to the application of soil fungicides, crop rotation and use of drip instead of furrow irrigation. Grafting of susceptible scions onto resistant rootstocks has become popular in the Mediterranean region as a component of integrated management of several soilborne pathogens (Besri, 2001; Pavlou *et al.*, 2002; Bletsos, 2005). Grafting with tolerant rootstocks is also effective for overcoming abiotic stresses, including high salinity (Rivero *et al.*, 2003), thermal stress (Bulder *et al.*, 1991;

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Abdelmageed *et al.*, 2004), and excessive soil moisture (Black *et al.*, 2003).

This technique has traditionally been associated with perennial nut and fruit crops as well as in vegetable production of watermelon, eggplant, cucumber and tomato (Lee, 1994; Edelstein, 2004). Grafting of chili peppers (*C. annuum* L.) is a recent practice where *C. annuum* scions are grafted onto *C. annuum* rootstocks that have soilborne disease resistance to fungi and nematodes (Morra and Bilotto, 2006, in Rodriguez and Bosland, 2010). To promote graft-take, foliar applications of the phytohormone abscisic acid, to reduce defoliation, and the vitamin ascorbic acid, to accelerate callus formation at the cut stem surfaces, were suggested for grafting sweet peppers (*C. annuum* L.) (Johkan *et al.*, 2008, 2009). Pepper grafting may have positive (Colla *et al.*, 2008; Palada and Wu, 2008; López-Marín *et al.*, 2009; Gisbert *et al.*, 2010) or negative (M'hamdi *et al.*, 2010; García-Rodríguez *et al.*, 2010) influences on plant growth or yield traits.

The objectives of the present study were directed towards firstly, the management of root rot and plant wilting on chili pepper caused by *P. nicotianae* by means of grafting local susceptible cultivars onto SCM334 rootstock, and secondly to assess the effects of this technique on the agronomic performance of grafted plants.

Materials and methods

Plant material

Seeds of SCM334 and local cultivars 'Beldi' and 'Baker', were sown in 200 mL capacity pots contain-

ing a mixture of clay soil, sand and peat (2:1:1 v:v:v), heated at 100°C for 90 min. The pots containing the plants were placed in a growth chamber at 25±2°C, under 16 h daily photoperiod with light intensity of 122 mmol m⁻² s⁻¹. To obtain uniform stem diameters for scions and rootstocks at the graft unions, SCM334 was sown 4 days before the two other cultivars which both have thicker stems than SCM334 at the same age.

Grafting

Plants were grafted using the tube technique (Oda, 1995). When the stem diameters of the scion plants at the graft zones were about 2 mm, the stems were cut obliquely at a 45° angle below the third leaf from the top and grafted onto the rootstocks at the epicotyl level of 2 mm in diameter (Figure 1A, B, C). Immediately after grafting, the seedlings were sprayed with a solution containing 100 mg L⁻¹ ascorbic acid and placed into sealed transparent plastic containers to maintain the relative humidity above 95%. All the containers were kept in the growth chamber for 1 d without light at 25±2°C. Plants were then maintained for an additional 14 d under 16 h daily photoperiods with white fluorescent tube lights (Philips TLD 58W/830). The light intensity was increased gradually by switching on a new fluorescent tube each day until the seventh day of acclimation at 122 mmol m⁻² s⁻¹, after which the light intensity was maintained at this level. The relative humidity of 95% or more was decreased gradually on the 5th day after grafting when the plastic containers were removed.

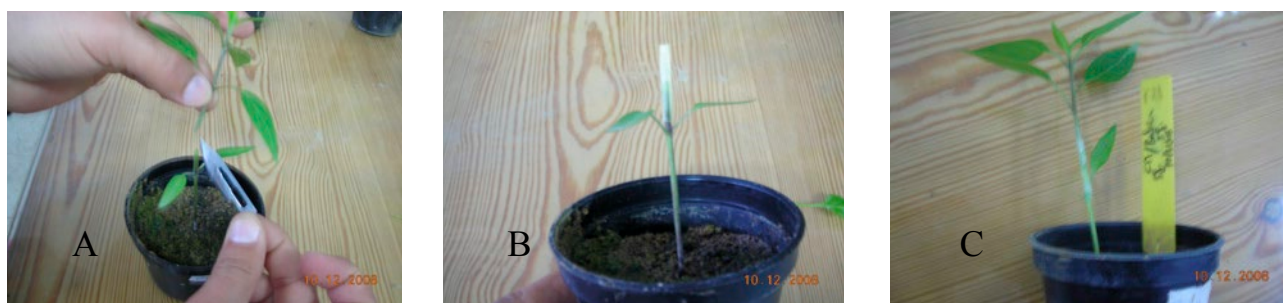


Figure 1. The process of pepper grafting starts by cutting the stem of the rootstock at the epicotyl level (A) followed by adjusting the plastic tube (B) in which the scion is carefully introduced (C) before finally placing the grafted plants in a growth chamber.

Inoculum

A highly pathogenic isolate of *P. nicotianae* recovered from infected pepper at Korba, Tunisia, was used for inoculum production. This isolate, *Pnt* 374, was maintained at room temperature (20–25°C) on potato dextrose agar. To obtain zoospores for inoculation, mycelium plugs excised from the edge of 7-d-old colonies were transferred onto pea broth agar (Allagui and Lepoivre, 2000) and maintained on this medium in a growth chamber for 9 d at 25±2°C under a 16 h photoperiod. When the mycelium had completely colonized the plates, agar was cut into rectangular portions of 4 × 2 cm, and covered with sterile distilled water. Plates were incubated for 3 d at 25±2°C under a 16 h photoperiod. Plates were then cooled to 4°C for 30 min and left at room temperature for 20 min to stimulate zoospore release from zoosporangia. Zoospores were counted using a hemacytometer and adjusted to the concentration 8 × 10⁴ zoospores mL⁻¹.

Inoculation and disease assessment

Fifteen days after grafting, each potted plant was inoculated individually with 4 mL of zoospore suspension (8 × 10⁴ zoospores mL⁻¹). Inoculated plants were maintained in a growth chamber at 25±2°C, with 122 mmol m⁻² s⁻¹ and a 16 h daily photoperiod, and watered every 2–3 d. Thirty d after inoculation, the root system of each plant was carefully removed, and the soil was washed off with tap water. Severity of root rot was assessed using the following scale (Allagui and Lepoivre, 2000): 0, healthy plant; 0.5, necrosis limited at the extremity of radicals; 1, rot only on the lower half of primary roots; 2, rot on all the primary roots; 3, rot reaching the crown, secondary roots and adventive root radicals; 4, hypocotyls rotten, or 5, dead plant caused by severe root rot and general wilting. To confirm that the root rot was caused by the inoculated pathogen, re-isolations were made in the semi-selective medium 'BNPRA' (Fujisawa and Masago, 1975). *Phytophthora nicotianae* was re-isolated from diseased root tissue.

Evaluations were made for the scion/rootstock combinations indicated in Table 1. Five replicate plants were used for each combination and the experiment was repeated three times, from September 2008 to February 2009. The experiments were of randomized complete block design, included five non-grafted plants each for SCM334, 'Beldi' and 'Baker'

in each experiment to serve as non-inoculated experimental controls.

Agronomic performance

The plants of 'Beldi' and 'Baker' grafted on SCM334 and of self-rooted SCM334 that were resistant at young stages were kept for assessment of agronomic performance. The susceptible non-inoculated self-rooted 'Beldi' and 'Baker' (experimental controls) were also maintained. These grafted and non-grafted plants were maintained during 2 months (March and April 2009) in the growth chamber, in pots containing the soil described above.

To evaluate the effect of SCM334 on the agronomic performance of the local cultivars 'Beldi' and 'Baker', an experiment was conducted in a greenhouse from May to December 2009, where mean temperature ranged between 12 and 27°C. Five plants per recovered cultivar ('Beldi', 'Baker', SCM334 self-rooted, 'Beldi'/SCM334 and 'Baker'/SCM334) were randomly transplanted from the pots in the growth chamber to the soil of the greenhouse at 40 cm spacings within rows and 70 cm between rows. Local cultural practices (irrigation, fertilization and insecticide sprays) were used. Self-rooted 'Beldi' and 'Baker' were not inoculated in the soil of the plantation because they would be killed by the

Table 1. Mean root rot severity scores for different pepper rootstock/scion combinations observed 30 d after inoculation with *Phytophthora nicotianae*.

Rootstock	Scion	Severity of root rot ^c
SCM334 ^a	SCM334	0.1 a (0–0.5)
SCM334 ^b	SCM334	0.0 a (0)
SCM334	Baker	0.1 a (0–0.5)
SCM334	Beldi	0.2 a (0–0.5)
'Baker'	SCM334	3.1 b (3–4)
'Baker' ^a	Baker	3.2 b (2–5)
'Baker' ^b	Baker	0.0 a (0)
'Beldi' ^a	Beldi	4.0 c (0–5)
'Beldi' ^b	Beldi	0.0 a (0)
'Beldi'	SCM334	4.6 d (3–5)

^a Non-grafted control plants.

^b Non-inoculated control plants.

^c Each value is the mean for 15 plants. Ranges in scores are in parentheses. Root rot severity scale was from 0 (healthy plant) to 5 (dead plant). Means followed by different letters are significantly different according to FLS test ($P \leq 0.05$).

pathogen, so their agronomic performance could not be measured. If plants are inoculated at the seedling stage and kept till adult stage, root necrosis and plant wilting will develop in susceptible plants. Therefore, plant inoculation at adult stages was not examined in this experiment.

Parameters of growth and yield of the different pepper plants were assessed during the plant vegetative growth period. These were plant height, stem diameter, leaf length, leaf width, number of fruit per plant, and fruit weight per plant. Plant height and stem diameter at 2 cm above the grafting joint, were measured on each plant at the full fructification stage. Leaf length and width were also measured on nine leaves per plant (three at the bottom, three at the middle and three at the top). Fruits changing from green to red were harvested each 2–3 days during the fructification period. Weight, length, width and pericarp thickness of each harvested fruit were determined immediately after harvest, using a vernier caliper.

Statistical analyses

Analysis of variance of disease severity using GLM procedures (SPSS for Windows Version 17) indicated that the replication and combination \times replication effects were not significant. Therefore, combination means analysis was conducted without considering the replication effect. Data of plant growth and fruit parameters were subjected to one-way analysis of variance, and means were separated using FLSD tests at $P \leq 0.05$.

Results and discussion

Spraying grafted pepper plants with ascorbic acid strongly enhanced the healing at graft unions via callus formation, and significantly reduced scion defoliation (data not shown). This positive result was obtained after numerous laboratory trials using grafted plants that were either sprayed or not sprayed with ascorbic acid. Moreover, tube grafting with appropriate acclimation during the five first 5 d after grafting was crucial for a high grafting success rate of almost 100%.

When SCM334 rootstocks and 'Beldi' or 'Baker' scions were used, the severity of root rot was very low, with mean severity score ranging from 0.1–0.2, and grafted plants were healthy (Table 1 and Figure

2A, B, C). However, when 'Baker' or 'Beldi' rootstocks were used with SCM334 scions, the severity of root rot was high, with mean severity score ranging from 3.1–4.6. This resulted in a high number of wilting and dead plants due to root rot (Table 1 and Figure 2D, E, F). Such high severity in root rot was similar to that observed on the control non-grafted plants of 'Baker' and 'Beldi' that were inoculated with *P. nicotianae*. These results suggest that SCM334 used as a scion might not confer resistance to the susceptible rootstock cultivars tested. To our knowledge, this suggestion has not been previously observed in other research on pepper graft combinations.

SCM334 was reported by the AVRDC as a suitable rootstock to manage *P. capsici* on sweet pepper (Anonymous, 2007). *Phytophthora capsici* is able to attack roots, stems, leaves and fruit (Leonian, 1922; Thompkins *et al.*, 1941). The benefit of using SCM334 rootstock in this case is limited to the control of root and crown rot without conferring any advantage to managing *P. capsici* that develops on the aerial parts of the susceptible scions. Since *P. nicotianae* is usually restricted to host root systems and does not affect aerial plant parts, SCM334 is likely to be better suited to provide resistance against *Phytophthora* diseases that target host roots.

Grafting has been reported to increase crop vigor and yield of sweet pepper, and may be useful for low-input sustainable horticulture (Colla *et al.*, 2008; Palada and Wu, 2008; López-Marín *et al.*, 2009). Grafting chili pepper varieties onto SCM334 might solve the problem of *P. nicotianae*, but other fungal or non-fungal diseases must not be serious problems on SCM334 rootstock. Recent results (Allagui and Lepoivre, 1996; Saadoun and Allagui, 2008) have showed that SCM334, although inoculated by several isolates, has maintained resistance to *P. nicotianae* since 1996.

There was a significant reduction in plant height (11%); leaf length (12%) and leaf width (10%) when 'Beldi' was grafted onto SCM334 in comparison to self-rooted 'Beldi'. No difference was observed in the growth characters of 'Baker' / SCM334 compared to 'Baker' self-rooted plants, however (Table 2). The rootstock SCM334 induced a small increase in some growth parameters and decreased others. This could be the result of scion-rootstock interactions. García-Rodríguez *et al.* (2010) reported significant vigour reduction of 'Rebelde' grafted onto SCM334. However, Gisbert *et al.* (2010) did not find significant differenc-

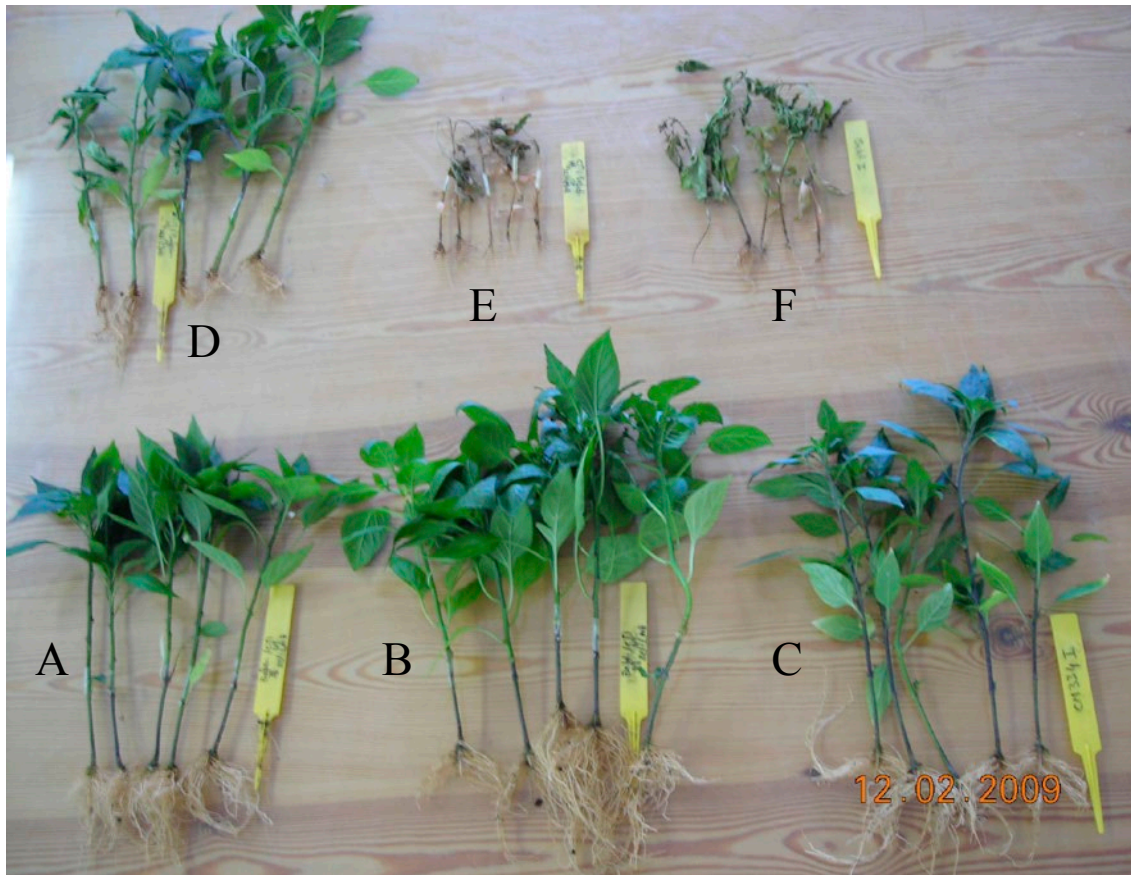


Figure 2. Healthy and diseased grafted plants 30 d after roots were inoculated with *Phytophthora nicotianae*. Note that the plants in Figure 2A, 2B and 2C, are healthy because they had SCM334 as rootstock material. 'Baker' /SCM334 (A), 'Beldi' /SCM334 (B) and SCM334/SCM334 (C). The plants in the row above had as rootstock the susceptible cultivars 'Beldi' or 'Baker': SCM334/'Baker' (D), SCM334/'Beldi' (E) and 'Beldi'/'Beldi' (F).

Table 2. Mean plant parameters in grafted and non-grafted pepper plants measured at full fructification^a.

Combination	Plant height (cm)	Stem diameter (cm)	Leaf length (cm) (LL)	Leaf width (cm) (LW)	LL/LW
'Beldi' /SCM334	175.4 b	1.5 c	14.6 b	7.1 c	2.1 a
'Beldi'	197.6 c	1.6 c	16.5 c	7.9 d	2.1 a
'Baker' /SCM334	154.2 ab	1.1 a	14.6 b	5.9 b	2.5 b
'Baker'	154.0 ab	1.2 ab	14.8 b	6.2 b	2.4 b
SCM334	139.4 a	1.4 bc	12.4 a	4.4 a	2.8 c

^a Means in the same column followed by different letters are significantly different according to FLS test at $P \leq 0.05$. Each value is the mean on five plants.

Table 3. Mean yield and fruit parameters^a in grafted and non-grafted pepper plants.

Combination	Yield/plant (g)	Fruit number / plant	Pericarp thickness (mm)	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	FL/FW
'Beldi' /SCM334	821.3 b	51.4 a	4.8 c	16.4 b	10.3 b	2.5 c	4.1 bc
'Beldi'	764.0 b	39.8 a	4.9 c	20.2 c	12.4 c	2.5 c	5.0 d
'Baker' /SCM334	673.5 b	45.0 a	4.7 c	15.0 b	10.6 b	2.4 b	4.4 c
'Baker'	763.2 b	50.0 a	4.5 b	15.3 b	9.5 b	2.4 b	4.0 b
SCM334	150.5 a	52.4 a	3.0 a	2.9 a	2.8 a	1.5 a	1.9 a

^a Means in the same column followed by different letters are significantly different according to FLSD test at $P \leq 0.05$. Each value is the mean of measurements on five plants per combination.

es in the growth of the pepper cultivars 'Coyote' or 'Almuden' grafted onto rootstocks of 'Foc' or 'Charlot'. Concerning fruit characters, SCM334 induced a decrease in weight, length and shape of 'Beldi' fruits. However, total weight per plant, fruit width and pericarp thickness were unchanged (Table 3). No significant differences in these characters were detected on 'Baker' /SCM334 plants in comparison to 'Baker' self-rooted plants, except for fruit shape and pericarp thickness which increased, respectively, by 4 and 10% (Table 3).

As plant foliage is not attacked by *P. nicotianae*, our results show that susceptible chilli pepper grafted on SCM334 rootstock is a promising alternative method for improving pepper yields by taking advantage its resistance to root rot caused by *P. nicotianae*. Other pepper rootstocks, such as 'Brutus' (M'hamdi *et al.*, 2010) or the tomato cultivar 'Celebrity' (Rodríguez and Bosland, 2010) used for pepper scions could also be of potential interest for future testing. *Phytophthora nicotianae* is becoming an increasingly important pathogen on pepper in other countries such as Spain (Andrés-Ares *et al.*, 2003; Rodríguez-Molina *et al.*, 2010) and the countries cited by Erwin and Ribeiro (1996). Grafting susceptible cultivars on the rootstock SCM334 could represent an additional disease management approach where pepper root rot caused by *P. nicotianae* may, or has, become an issue in pepper production.

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