CARYOLOGIA Vol. 60, no. 1-2: 141-145, 2007

Morphogenetic effects induced by pathogenic and non pathogenic *Rhizoctonia solani* Kühn strains on tomato roots

Sampò* Simonetta, Lorena Avidano and Graziella Berta

Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze dell'Ambiente e della Vita, Via Bellini 25G, 15100 Alessandria, Italy.

Abstract — The effects of two *Rhizoctonia solani* Kühn isolates on root colonization and morphology of *in vitro* grown tomato plants were compared. Pathogenic hyphae (R1556) penetrated and filled all the parenchyma cells of the cortex and of the central cylinder; the vessel elements underwent a significant degradation. Several brownish sclerotia and cushion formations were observed. Roots colonized by the non-pathogenic strain showed phytoplane non-pathogenic growth: hyphae occurred in the external root portion and in a few cortical parenchyma cells; a number of hyphal tips grew along the ridges of adjacent epidermal cells or in the intercellular spaces. Morphometric values were significantly lower in the roots of plants infected by the pathogenic isolate than in those infected by the non-pathogenic one compared to the controls. Root growth was described by a logistic model in infected plants and by a linear one in the controls.

Key words: *Lycopersicon esculentum*, morphology, *Rhizoctonia solani,* root colonization, root system.

INTRODUCTION

Rhizoctonia solani positively and/or negatively affects the growth and health of many plant species, promoting growth of vegetables and field crops (Sneh *et al.* 1986; Haselwontder and Read 1980) or causing damping-off, cankers, root rots, fruit decay and even foliage disease (Barker 1970). Re-planting diseases in orchards (Mazzola 1997) are also associated with this major class of soil-borne diseases. The pathogen reduces plant growth by rotting the roots, and thus reducing the ability of the plants to take up water and nutrients (Wallwork 1996); it may even lead to plant death, or at any rate to significant yield losses in field conditions (Sneh *et al.* 1996). *R. solani* occurs world-wide in most cultivated soils, and is indigenous in many uncultivated areas. The interactions between pathogenic *Rhizoctonia* spp. strains and several hosts have been described at an ultrastructural level (Dodman 1970, Armentrout and Downer 1987). A comparative study between virulent and hypovirulent isolates of *R. solani* colonizing cotton hypocotyls and roots pointed out many differences in the anatomy of colonization (Sneh *et al*. 1989), but the information in his regard is still scanty.

The aim of this study was to investigate the effects on root morphology and colonization induced by two *R. solani* isolates on *in vitro* - grown tomato plants.

MATERIALS AND METHODS

Fungal inoculum - A *Rhizoctonia solani* Kühn pathogenic isolate (R1556) isolated from tomato (kindly supplied by S. L. Woo, University of Naples "Federico II", Italy) and a non-pathogenic one, isolated from bean (kindly supplied by L. Gullino, University of Turin, DIVAPRA, Italy) were grown on Potato Dextrose Agar (PDA) (Fluka) at 28°C for 5 days. The inocula were obtained by placing 5-mm disks of PDA taken from the margins of 5-day old cultures of the *R. solani* isolates. To maintain virulence the pathogen was periodically re-isolated from infected plants. Each experiment contained twenty replicates and was repeated at least twice.

Treatments and plant growth conditions in vitro - Tomato seeds (*Lycopersicum esculentum* Mill. cv. Early Mech) were surface sterilized for 3 min in 5 % sodium hypochlorite and rinsed six times with

* Corresponding author: phone +39 0 131 360234; fax +39 0 131 360243; e-mail: simonetta.sampo@mfn.unipmn.it

sterile deionized water. These were aseptically put on moist sterile filter papers in plastic Petri dishes incubated in the dark at 24°C. Three-day old seedlings were aseptically transferred into Petri dishes, containing agar (1%) in modified Long Ashton nutrient solution (TROTTA *et al.* 1996). Some tomato seedlings were inoculated with the pathogenic *R. solani* strain, some with the non-pathogenic one, and some were maintained as controls. The Petri dishes were incubated in a growth chamber (16/8 h light/dark photoperiod and 24°/20°C thermoperiod, 150 µE m^{-2} s^{-1}, 60% relative humidity) with an inclination of 45° to favour geotropism and to avoid water condensation produced by plant respiration. Roots were maintained in the dark by wrapping with aluminium foil the lower half of the Petri dishes.

Quantification of plant growth and root morphogenesis - To quantify plant growth, shoot and root fresh weights, as well as shoot and root lengths were measured. Whole root systems were sampled at 14 days, put in a transparent container and digitised by using a Desk Scan II scanner (Régent Instrument Inc., Quebec, Canada) connected to a Power Macintosh 4400/200 computer (Apple Computer Inc., Cupertino, CA, USA). Digitised root images were analysed by a Mac Rhizo V 3.9 software (Régent Instrument Inc., Quebec, Canada) and specific root lenght (SRL), mean root diameter, number of root tips and root system branching (root tip number divided by total root length) were evaluated to characterize root architecture. The topological parameters that give an indication of the pattern of branching were calculated according to FITTER *et al.* (1988). To obtain growth curves, root length was measured daily.

Root infection by R. solani - Roots were gently dragged from the soft agar and cut. Root segments (1-2 mm long) from all the treatments were clarified (discoloured) in KOH water solution (10%) at 60° C for 30 min. The presence of the fungus was shown by microscopic observations on whole squashed roots stained with Cotton Blue. The degree of root infection by *R. solani* (I%) was evaluated by determining the total frequency of colonization (colonized root length/total root length x 100).

Root morphology as affected by the presence of the R. solani isolates - Root segments (1-2 mm long) from all the treatments were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7) for 3 h at room temperature, rinsed and post-fixed in 1% OsO_4 in the same buffer for 2 h at room temperature, dehydrated and embedded in EPON 812. Semi-thin sections were cut using a Sorvall MT-2 Porter-Blum ultra-microtome, stained with 1% toluidine blue in 1% tetraborate, and examined under an Axioscop Zeiss microscope connected to an AxioCam Zeiss camera.

Statistical analysis - Data on plant growth and root morphogenesis were analyzed by ANOVA and Fisher's least significant (P=0.05) test using Statview statistics package (SAS Institute Inc., Cary, NC, USA). Daily values of root length were submitted to the Systat 5.2.1 programme to obtain growth curves.

RESULTS

Quantification of plant growth and root morphogenesis - Shoot and root fresh weights, as well as shoot and root lengths of tomato plants infected with the *R. solani* non-pathogenic isolate did not significantly differ from the controls. On the contrary, the same parameters measured in tomato plants infected with the pathogenic isolate (R1556) were significantly lower (Table 1).

Specific root length (SRL) of tomato plants infected with the non-pathogenic isolate was significantly different from that of plants infected with pathogenic isolate R1556 and controls; degree of root branching of plants infected with the non-

Table 1 — Effects of non-pathogenic (R) and pathogenic (R1556) *Rhizoctonia solani* strains on shoot and root fresh weight, total shoot and root length, specific root length and root branching degree of *in vitro* grown tomato plants, 14 days after sowing. (C= control).

	Fresh shoot weight (g)	Fresh root weight (g)	Total shoot length (cm)	Total root length (cm)	Specific Root Length	Root branching degree
R	0.160±0.007 a*	0.032±0.001 a	7.821±0.195 a	54.716±1.136 a	1334.839±53.051 a	0.227±0.022 a
R1556	0.093±0.007 b	0.016±0.001 b	6.610±0.173 b	29.210±1.579 b	1816.410±72.369 b	0.460±0.033 b
C	0.201±0.014 a	0.038±0.008 a	8.733±0.504 a	57.567±4.938 a	1884.216±303.718 b	0.194±0.016 a

* Values followed by the same letters are not significantly different (P≤0.05) according to Fisher's least significant difference test.

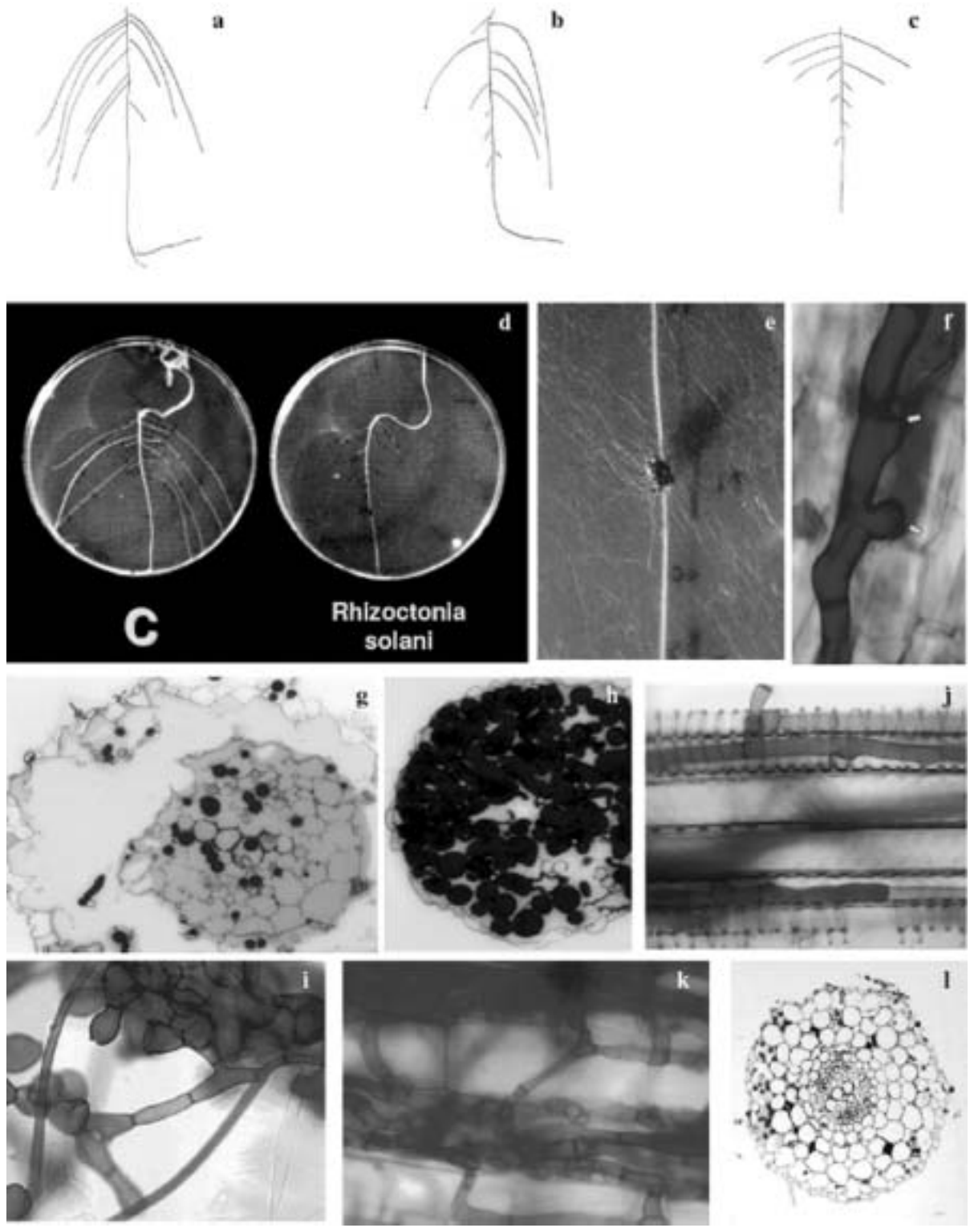

Fig. 1 — Digitised images of tomato roots non infected (a), or infected with a non-pathogenic (b) or a pathogenic (c) *Rhizoctonia solani* isolate. Petri dishes containing or not fungal inoculum (d). Pathogenic isolate (R1556) of *R. solani*: brownish sclerotia produced *in vitro* (e); appressoria formation on cell walls (f); root cortical cells (g) and central cylinder cells (h, j) clearly damaged and also totally colonized (844 x). Non- pathogenic *R. solani* isolate: monilioid cells (i); hyphae growing in parenchyma cells (k) and in the intercellular spaces of the cortical parenchyma (l) (578 x).

pathogenic isolate was not significantly different compared to the controls, whereas in plants infected with R1556 it was significantly higher than in the controls (Table 1). Topological indices showed a herringbone-like model for all the treatments (Figs. 1a, b, c, d). Data on root growth of control plants and of plants infected with the non-pathogenic isolate fitted linear curves with different slopes which changed on the 8th day, whereas those of the plants infected with R1556 fitted a logistic curve (Table 2).

Table 2 — Growth curve parameters. Data on root growth fitted a linear model (y= a+bx) in plants infected with the non-pathogenic isolate and in the controls; roots of tomato infected with the pathogenic strain showed a logistic growth (y= a/1 + e $^{b+cx}$).

	a	b	c
C	-5.46 ± 0.64	4.27 ± 0.15	-
R	-7.38 ± 0.65	3.74 ± 0.14	-
R1556	16.40 ± 0.26	2.61 ± 0.06	-0.59 ± 0.02

Root infection by pathogenic and non-pathogenic isolates of R. solani - Percentage infection by the pathogenic isolate in 14-day old tomato plants was 13.27 in roots analysed *in toto* and was higher in the primary root than in the laterals (Table 3). Pathogenic hyphae (R1556) formed appressoria (Fig. 1f), penetrated and filled all the cortical cells, the parenchyma cells of the central cylinder (Figs. 1g, h), and even the vessel elements (Fig. 1j). Roots colonized by the non - pathogenic strain showed a phytoplane non-pathogenic growth. More explicitly, fungal hyphae were observed on the plant surface without inner penetration. Some non - pathogenic hyphae occurred in the external root portion and in a few cortical parenchyma cells; hyphal tips grew along the ridges of adjacent epidermal cells or in the intercellular spaces of the epidermis (Figs. 1k, l). R1556 produced melanized hyphae and several brownish sclerotia and cushion formations (Fig. 1e), whilst the non-pathogenic isolate produced several monilioid cells (Fig. 1i), but no sclerotia were observed.

Table 3 — Percentage of root infection by the *Rhizoctonia solani* pathogenic isolate (R1556) in 14-day old tomato plants.

Root order	Percentage of root infection
Lateral roots	8.84±3.61
Primary roots	20.90±5.18
Roots *in toto*	13.27±4.23

Root morphology as affected by the presence of R. solani - Morphological modifications induced by the pathogenic and non pathogenic isolates of *R. solani* were observed in semi-thin sections of the primary root in 3-, 7- and 14-day old tomato plants. Significant differences in fungus-host interactions were observed: the pathogenic isolate produced a denser hyphal mantle on the outer root surface than the non-pathogenic one. The non-pathogenic hyphae were appressed to the apparently intact epidermal outer cell walls, but not where the epidermal layer was damaged; plant tissues were weakly infected and no appressoria or infection cushions were observed. In the penetration stages of the pathogenic isolate (72 h), the epidermis was separated and disintegrated. During colonization, pathogenic fungal hyphae reached and penetrated the central cylinder.

CONCLUSIONS

The two *R. solani* isolates showed, in the phase of sterile mycelium, some typical features, including a distinctive right-angle or near-right-angle branching pattern, the presence of dolipore septa, the absence of clamp connections, and the constriction of hyphae near their origin points as previously described (SNEH *et al.* 1991). The hyphae of both isolates were attached to the epidermal surface, but in a different way. This stage was of large importance for the subsequent stages of infection, including cushion formation and penetration by the pathogen. Such a contact induced the formation of short hyphal internodes and short perpendicular lateral branches in the pathogenic isolate before appressorium formation. These branches frequently formed feet along the grooves (ARMENTROUT *et al.* 1987). The pathogenic isolate also degraded outer epidermal cell walls in the pre-penetration stages, as previously observed (WEINHOLD and MOTTA 1973), though other authors (CHRISTOU 1962) suggest that penetration can be induced by mechanical pressure.

The hyphae grew in parallel lines, reached the central cylinder and, during vessel penetration, cell walls underwent significant degradation. Topological indices showed a herringbone-like model probably related to the *in vitro* conditions; however, root system morphology as well as the growth curves pointed out the effective pathogenic vocation of R1556, with determinate growth for the R1556-infected roots and indeterminate growth for the other treatments.

REFERENCES

ARMENTROUT V.N., DOWNER A.J., 1987 — *Infection cushion development by Rhizoctonia solani on cotton.* Phytopathology, 77: 619-623.

BARKER K.F., 1970 — *Types of Rhizoctonia diseases and their occurrence.* In J.R. Parmeter Jr (Eds.) "Biology and Pathology of *Rhizoctonia solani*", p. 125-148. University of California Press, Berkeley.

CHRISTOU T., 1962 — *Penetration and host parasite relationship of Rhizoctonia solani in the bean plant.* Phytopathology, 52: 381-389.

DODMAN R. L., 1970 — *Factors affecting the penetration phase of infection by Rhizoctonia solani.* In T.A. Toussoun, V. Vega, P.E. Nelson (Eds) "Root disease and soilborne pathogens", p. 116-121. University of California Press, Berkley.

FITTER A.H., NICHOLS M.L., HARVEY, M.L., 1988 — *Root system architecture in relation to life history and nutrient supply*. Functional Ecology, 2: 345-351.

HASELWONTDER K. and READ D.J., 1980 — *Fungal association of roots of dominant and subdominant plants in high alpine vegetation systems with special reference to mycorrhiza*. Oecologia, 45: 57- 62.

MAZZOLA M., 1997 — *Identification and pathogenicity of Rhizoctonia spp. isolated from apple roots and orchard soils.* Phytopathology, 87: 582-587.

SNEH B., BURPEE L., OGOSHI A., 1991 — *Identification of Rhizoctonia species*. APS Press, St. Paul, MN.

SNEH B., ICHIELEVICH-AUSTER M., BARASH I., SHOMER I, 1989 — *Comparative anatomy of colonization of cotton hypocotyls and roots by virulent and hypovirulent isolates of Rhizoctonia solani.* Canadian Journal of Botany, 67: 2142-2149.

SNEH B., JABAJI-HARE S., NEATE S., DJIST G., 1996 — *Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control*. Chapter V (p 247-404) and Chapter VIB (p. 445-559) Kluwer Academic Publishiers, Dordrecht, The Netherlands.

SNEH B., ZEIDMAN B.M., ICHIELEVICH-AUSTER M., BARASH I., KOLTIN Y., 1986 — *Increased growth responses induced by a non-pathogenic Rhizoctonia solani*. Canadian Journal of Botany, 64: 2372-2378.

TROTTA A., VARESE G.C., GNAVI E., FUSCONI E., SAMPÒ S., BERTA G., 1996 — *Interaction between the soilborne pathogen Phytophthora parasitica var. parasitica and the arbuscular mycorrhizal fungus Glomus mosseae in tomato plants*. Plant Soil, 185:199-209.

WALLWORK H., 1996 — *Cereal root and crown diseases*. Kondinin Group, Perth, Australia.

WEINHOLD A.R., MOTTA J., 1973 — *Initial host responses in cotton to infection by Rhizoctonia solani.* Phytopathology, 63: 157-162.

Received November 30th 2006; accepted January 30th 2007