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# Influence of plant genotype on the cultivable fungi associated to tomato rhizosphere and roots in different soils

*Anna POLI*[a], *Alexandra LAZZARI*[a], *Valeria PRIGIONE*[a], *Samuele VOYRON*[a], *Davide SPADARO*[b], *Giovanna Cristina VARESE*[a,*]

[a]*Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10100 Torino, Italy*
[b]*Department of Agricultural, Forestry and Food Sciences (DISAFA) and AGROINNOVA, Centre of Competence for the Innovation in the Agro-environmental Sector, University of Torino, Largo Braccini 2, Grugliasco, 10095 TO, Italy*

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ABSTRACT

Rhizosphere and root-associated microbiota are crucial in determining plant health and in increasing productivity of agricultural crops. To date, research has mainly focused on the bacterial dimension of the microbiota. However, interest in the mycobiota is increasing, since fungi play a key role in soil ecosystems. We examined the effect of plant genotype, soil, and of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) on the cultivable component of rhizosphere and root-associated mycobiota of tomato. Resistant and susceptible varieties were cultivated on two different soils (A and B), under glasshouse conditions. Isolated fungi were identified by morphological and molecular approaches. Differences were found between the rhizosphere and the roots, which in general displayed a lower number of species. The structure of the mycobiota was significantly affected by the soil type in the rhizosphere as well as by the plant genotype within the roots (NPERMANOVA, $p < 0.05$). The addition of *Fol* changed the community structure, particularly in soil A, where *Penicillium* spp. and *Fusarium* spp. were the dominant responding fungi. Overall, the results indicated that i) soil type and plant genotype affect the fungal communities; ii) plant roots select few species from the rhizosphere; and iii) the fungal community structure is influenced by *Fol*.



## Introduction

In plants, a microbiota is an interactive microorganism community associated with the plant rhizosphere and roots, which plays a crucial role in influencing plant health (Abd-Elsalam *et al.* 2010; Mendes *et al.* 2011). Likewise, the microbial community is affected by both plant and soil types; specific members of the microbiota are stimulated or repressed by chemical exudates released in the rhizosphere, the root-surrounding soil region (Berendsen *et al.* 2012).

* *Corresponding author.*
E-mail address: cristina.varese@unito.it (G. C. Varese).
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Vegetable and ornamental crops are often attacked by several soilborne pathogens, resulting in economic losses. Tomato (*Lycopersicum esculetum*) is a popular and economically relevant culture and has been proposed as a model for studying plant-pathogens interactions, since its productivity can be limited by a number of diseases caused by viruses, bacteria, and fungi (Arie *et al.* 2007). One of the major soilborne pathogens that endangers tomato crops worldwide is *Fusarium oxysporum*, the causal agent of Fusarium wilt, which is capable of affecting a variety of crops species. *Fusarium oxysporum* has been subdivided in over 120 morphologically undistinguishable *formae speciales*, depending on the host plant (Michielse & Rep 2009), further classified into physiological races on the basis of cultivar specificity (Di Pietro *et al.* 2003). To date, management of wilt disease relies mainly on soil disinfestation and use of resistant cultivars. However, several compounds have been banned or limited in their use. As for the use of resistant cultivars, new more virulent races frequently arise to overcome the host resistance (Kinkel *et al.* 2011). Therefore, due to the possible alternatives in disease control (Fravel *et al.* 2003; Mazzola 2002, 2004), the search for potential biocontrol agents is intensifying.

The microbial community *in toto* (bacteria, fungi, pseudofungi, and protozoa) is considered to be crucial for plant protection and novel discoveries are necessary to improve crop quality and yield. As supported by a number of studies, several factors including the plant species, the plant genotype, and the soil type are capable of shaping the rhizosphere microbiota (Hardoim *et al.* 2011; Inceoglu *et al.* 2012; Philippot *et al.* 2013). Considering the fact that plant resistance represents one of the strategies to overcome vascular diseases, several studies have been conducted on a number of crops in order to clarify the effects of resistant and susceptible cultivars on microbial communities (An *et al.* 2011; Azad *et al.* 1987; Nallanchakravarthula *et al.* 2014; Yao & Wu 2010). The soil microbial community has been demonstrated to be significantly affected by the plant genotype, indicating a role of the rhizosphere microorganisms in conferring resistance to pathogens (An *et al.* 2011; Inceoglu *et al.* 2012; Nallanchakravarthula *et al.* 2014).

Along with the rhizosphere microorganisms, the so-called 'endophytes' which are associated to the plant tissues, are a relevant component of the root microbiome. The endophytic community, as the rhizospheric community, is important for plant growth and is influenced by plant and soil factors, and microbial features responsible for the survival of endophytes within the roots (Gaiero *et al.* 2013; Turner *et al.* 2013).

Understanding the rules that drive formation of a plant microbiome and identifying its components is a crucial point to increase productivity and reduce pathogen attacks. To date, several studies have mainly focused on the bacterial microbiota (Bulgarelli *et al.* 2013; Chaparro *et al.* 2014; Inceoglu *et al.* 2012; Spence *et al.* 2014; Turner *et al.* 2013), while a void has still to be filled on the fungal community and its function, although research on this topic is rapidly increasing (Nallanchakravarthula *et al.* 2014; Nam *et al.* 2015; Yao & Wu 2010).

Tomato is known to differentially respond to beneficial (Salvioli *et al.* 2012), pathogenic and biocontrol fungi (Spadaro & Gullino 2005), and genotypes with different features provide an unprecedented model to investigate the network of interactions taking place belowground. With the present work, we intended to shed a light on the cultivable component of the mycobiota associated to tomato plant, clarifying how the soil and the plant genotype can determine its shaping. In addition, we aimed to assess whether the presence of a fungal pathogen could modify the structure of the rhizosphere and root associated fungal community. Finally, the availability of fungal cultures (both from rhizosphere and roots) would offer valuable tools to investigate the functionality of the fungal communities with the intent of reconstructing specific tomato microbiomes; to this aim, cultivable fungi only were considered in this work.

## Materials and methods

### *Plant cultivars, experimental soils and plant growth*

Two cultivars of tomato and two different soils were used in this study. The cultivars Heinz 1706 and Moneymaker, were selected as resistant (R) and susceptible (S) to *Fusarium oxysporum* f. sp. *lycopersici*, respectively (Huang & Lindhout 1997; Ozminkowski 2004). Two soils, A and B, were collected in Northern Italy and chosen on the basis of their different history, physical and chemical characteristics which were determined by AgroBio Lab (Rutigliano, Italy) with accredited methods for pH, structure, organic carbon, total nitrogen, mineral composition, and conductivity (Table 1). Soil A was cultivated with vegetables since 1980 while soil B was taken from a field where wheat was cultivated for 15 y and later the soil was set aside for 10 y (no crops were grown).

Tomato seeds of both cultivars were sown in plug trays (80 plugs/tray) containing peat–perlite substrate and were watered daily. Following, three 14 d old tomato seedlings were transplanted in 2 L pots containing either soil A or soil B. Three pots were prepared for each treatment. Plants were maintained for 4 weeks under glasshouse conditions (temperature ranging between 26 °C and 28 °C; automatic watering and shading).

**Table 1 – Physical and chemical characteristics of the soils considered in this study.**

| | Soil A | Soil B |
|---|---|---|
| Origin | Albenga (SV), Liguria, northern Italy | Rosta (TO), Piedmont, northern Italy |
| Geographical coordinates | 44.067171 N, 8.212949 E | 45.074190 N, 7.461910 E |
| pH | 7.22 | 7.60 |
| Sand:silt:clay (%) | 60.0:10.7:29.3 | 60.0:16.7:23.3 |
| C organic (%) | 3.18 | 0.60 |
| N total (‰) | 1.68 | 1.54 |
| Ca (mg $kg^{-1}$) | 3903.80 | 4036.90 |
| Mg (mg $kg^{-1}$) | 726.00 | 469.40 |
| K (mg $kg^{-1}$) | 834.20 | 116.00 |
| Na (mg $kg^{-1}$) | 895.20 | 149.40 |
| P (mg $kg^{-1}$) | 16.90 | 10.20 |
| B (mg $kg^{-1}$) | 0.70 | 1.00 |
| Fe (mg $kg^{-1}$) | 93.70 | 19.60 |
| Conductivity (mS $cm^{-1}$) | 9.90 | 0.46 |