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Not Only Priming: Soil Microbiota May Protect Tomato from Root Pathogens

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Abstract

An increasing number of studies have investigated soil microbial biodiversity. However, the mechanisms regulating plant responses to soil microbiota are largely unknown. A previous work tested the hypothesis that tomato plants grown on native soils with their complex microbiotas

respond differently from tomato growing in a sterile substrate. Two soils, suppressive or conducive to *Fusarium oxysporum* f. sp. *lycopersici* (FOL), and two genotypes susceptible and resistant to the same pathogen were considered. The work highlighted that the two tested soil microbiotas, irrespectively of their taxonomic composition, elicit the PAMP-triggered Immunity Pathway, the first level of plant defence, as well as an increased lignin synthesis, leading to an active protection when FOL is present in the soil. Here, we tested the expression of a panel of genes involved in Effector-Triggered Immunity (ETI), demonstrating that soil microbiota, beside genotype, affects plant resistance to FOL also modulating this pathway.

TEXT

Next-generation sequencing (NGS) has enabled in-depth investigations of the microbial communities associated with animals, plants, and fungi. The awareness that multicellular eukaryotes host thousands of microbes, many beneficial, some essential and only a few deleterious has led to a paradigm shift in our knowledge of microbial–eukaryote interactions. NGS approaches helped us to reply to basic questions of traditional microbiology, as: 'Which are the microbes thriving in that niche?', and 'What are they doing?'. Focusing on the plant side and starting from the pioneering researches by Bulgarelli et al.[1] and Lundberg et al.[2], many other studies revealed the extraordinary diversity of microbes present on both roots, shoots, leaves, fruits[3,4], and demonstrated how different parameters affect the composition of the microbiota: plant genotype, soil features, environmental parameters[5,6]. Interestingly, the environment resulted to be the driving force also for human microbiota, where it dominates over host genetics in shaping human gut microbiota[7]. The strict relationship existing between microbiota and their eukaryotic host has also led to the development of the *holobiont* concept[8,9]. Host-microbial systems, being a complex assembly of diverse organisms, constitute unique biological entities, defined as 'meta-organisms' or holobionts[10]. However, metagenomic sequencing has only given indirect responses to the questions opened by

these new scenarios: ‘How the host responds to its extended microbiota, which represents its second genome?’.

Chialva et al.[11] focused on tomato (*Solanum lycopersicum*), testing the hypothesis that plants grown on native soils display different responses to soil microbiotas. Using transcriptomics, proteomics, and biochemistry, the study has described the responses of two tomato genotypes (susceptible or resistant to FOL) grown on two native soils (conducive and suppressive to FOL) and an artificial substrate. Results showed that native soils, particularly the suppressive one, affect tomato responses by modulating pathways involved in responses to oxidative stress, phenol biosynthesis, lignin deposition, and PAMP-triggered Immunity (PTI). By contrast, in tomato plants grown on steam-disinfected soils, total phenols and PTI responses significantly decreased, suggesting a crucial role of soil microbiota in eliciting a priming effect. To validate those observations, the mycorrhizal fungus *Funnelliformis mosseae,* was selected as one of the most abundant AM fungi in both soils, and inoculated in tomato growing on steam-disinfected soils: the fungal inoculation partly rescued some of the local and systemic responses, which were identified as a part of the priming response. Martinez-Medina et al. [12] have neatly identified different conditions where plant defence priming takes place and have acknowledged many beneficial microbes as a source for priming stimuli. Indeed, under the tested experimental conditions (native soils vs sterile substrate), tomato activates several genes involved in PTI, such as those encoding for PR proteins, WRKY transcription factors, ROS burst signalling and calcium signalling, which are involved in immune response[13]. To understand whether such an adaptive measure leads the plant to an enhanced defence readines[11] tomato plants were inoculated with FOL. As expected, reduced disease symptoms were detected in the resistant genotype ('Battito') in both soils; but surprisingly the susceptible genotype 'Cuore di Bue' was partially protected from FOL on the suppressive soil. However, it is still unknown whether

the Effector-Triggered Immunity (ETI), *i.e.* the second barrier against pathogens, responds to soil microbiota.

Here, we hypothesized that the priming status raised in tomato by soil microbiota could elicit the expression of genes directly involved in ETI in the presence of FOL. With this aim, we selected a panel of genes involved in the ETI pathway (Table 1) and tested their expression by using RT-qPCR in FOL-inoculated plant roots according to the set-up and methods described in Chialva et al.[11].

Results indicate that soil microbiota promoted the ETI response of plants after FOL infection (Fig. 1): while in RNA-seq experiment, where FOL was not present, ETI genes were not differentially expressed, in FOL-inoculated plants RT-qPCR experiment detected gene modulation[11]. Both genotypes significantly upregulated the expression of *RIN4* ($p<0.05$) in both native soils compared to the control substrate. This protein is a target of type III pili effector proteins (virulence factors) from bacterial pathogens and interacts with RPS2 and RPM1 R protein leading to hypersensitive response[14,15]. Moreover, we tested the expression of two previously described ETI-marker genes[16] and found that one of them coding for a UDP-glucosyltransferase family 1 protein (UDP) is upregulated in both soils ($p<0.05$) with the exception of the susceptible cultivar in the conducive soil. However, the other marker gene tested (UDP1) did not show differential expression across conditions. By contrast, the expression of the *I-2* R gene, directly involved in FOL race 2[17], was upregulated only in the resistant genotype grown in the suppressive soil, while it remained consistent for the susceptible genotype in all the substrates. These results suggest a synergy between the genotype (presence of Resistance genes), the soil biological features, and – mechanistically – the ETI response. The 'Cuore di Bue' susceptible genotype has a more modulated response: FOL-suppressive soil with its microbiota activates the ETI response, while this action is not elicited in

the conducive soil. This well explains the modulation of *I-2* R gene: to be activated, plant defences require the suppressive soil microbiota acting on the resistant genotype, while the synergy between these two conditions is not satisfied in the susceptible genotype. The hypothesis may have an experimental validation by the presence of many bio-control Fusaria strains isolated in the Albenga soil[18].

Our previous experiments demonstrated that soil microbiota leads to a priming ('state of alert') in tomato eliciting the PTI, which represents the first level of plant defence. When challenged by a pathogen, the alerted plant activates a new set of more specific genes related to the ETI, which is the second specific defence level (Fig. 2). This mechanism leads to a partial protection from the pathogen attack, even in the absence of specific resistance genes (as for the cultivar 'Cuore di Bue'). The modulation of the ETI-related genes indicates that native soil microbiota also affects plant response to FOL via ETI, in addition to the crucial role played by the genotype.

In conclusion, the investigation of the mechanisms operating in plants in native soils and in the presence of complex soil microbiota has revealed new unexpected responses. It seems that - just like humans - the tomato plant living in non-sterile conditions can better activate its immunity defence via the interaction with its microbiota.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Figure Legends

Figure 1.

RT-qPCR relative expression levels of gene involved in ETI in tomato plants (*Solanum lycopersicum*) infected with *Fusarium oxysporum* f. sp. *lycopersici* (FOL).

Ubiquitin gene was used as reference for RT-qPCR. Letters indicate statistically supported differences (Kruskal–Wallis test at $P < 0.05$). Data are means ± SE ($n = 3$). AL, 'Albenga' suppressive soil; RO, 'Rosta' conducive soil; CONT, Control 'Neutral' soil. B, 'Battito' FOL-resistant genotype; C, 'Cuore di Bue' FOL-susceptible genotype. (A) *RIN4*, RPM1 interacting protein 4; (B) *I-2*, CC-NBS-LRR, resistance protein 1; (B,C) *UDP*, *UDP1*, UDP-glucosyltransferase family 1 proteins.

Figure 2

Scheme of defence responses activated by tomato (*Solanum lycopersicum*) in the presence of a complex native soil microbiota.

(1) According to the models proposed by Chialva et al.,[11] in native soils microbial-associated molecular patterns (MAMPs) such as flagellin (flg22) and chitin are perceived by tomato plant. Those events elicit the PTI pathway (Plant-triggered Immunity) as a first defence level with the activation of calcium signalling (CNGCs, cyclic nucleotide-gated channels; CaM/CaM-like (CML), calmodulin-like proteins; CDPKs, calcium-dependent protein kinases) and WRKY transcription factors. This brings to the downstream activation of pathogenesis-related proteins genes (PR), such

as PR1, and to cell-wall fortification and lignin synthesis. (2) Since PTI-related defence is elicited, a "continuative priming" by soil microbiota components occurs, maintaining plant defence active. (3) When plant is attacked by *Fusarium oxysporum* f. sp *lycopersici* (FOL) the plant is already primed and activates stronger ETI (Effector-triggered Immunity) defence. In both genotypes, effectors are strongly perceived (e.g. by *RIN4*): only in the FOL-resistant one a specific resistance mediated by *I-2* is activated leading to the activation of the downstream ETI responses (such as UDP upregulation). However, in the susceptible genotype even if *I-2* upregulation was not observed, FOL-suppressive soil induced the activation of downstream ETI pathway with the upregulation of a marker UDP gene.

Table 1.Table of primers used in RT-qPCR experiment.

Gene	Transcript ID	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
RPM1 interacting protein 4 (*RIN4)*	Solyc11g012010.1	TCCTTCTGTAGAGTCGGGCCA	TCTTCTTCGTCGTGTTGGTTGGT	[11]
CC-NBS-LRR, resistance protein 1 (*I-2*)	Solyc11g071430.1	TTTGAAAGGGTCCCAAATCC	TGCAGAGGGGTGTCAATTTC	This study
UDP-glucosyltransferase family 1 protein (UDP)	Solyc10g085880.1	CAAAGCTGAAAGAGGGAACG	TAACCCAAGCCCTAGCTCAAC	This study
UDP-	Solyc09g0925	GGTGCAACCCCATGTC	ATCAGAGAATGCCGCC	This

glucosyltransferase family 1 protein (UDP1)	00.1	CTATTG	AAGT	study

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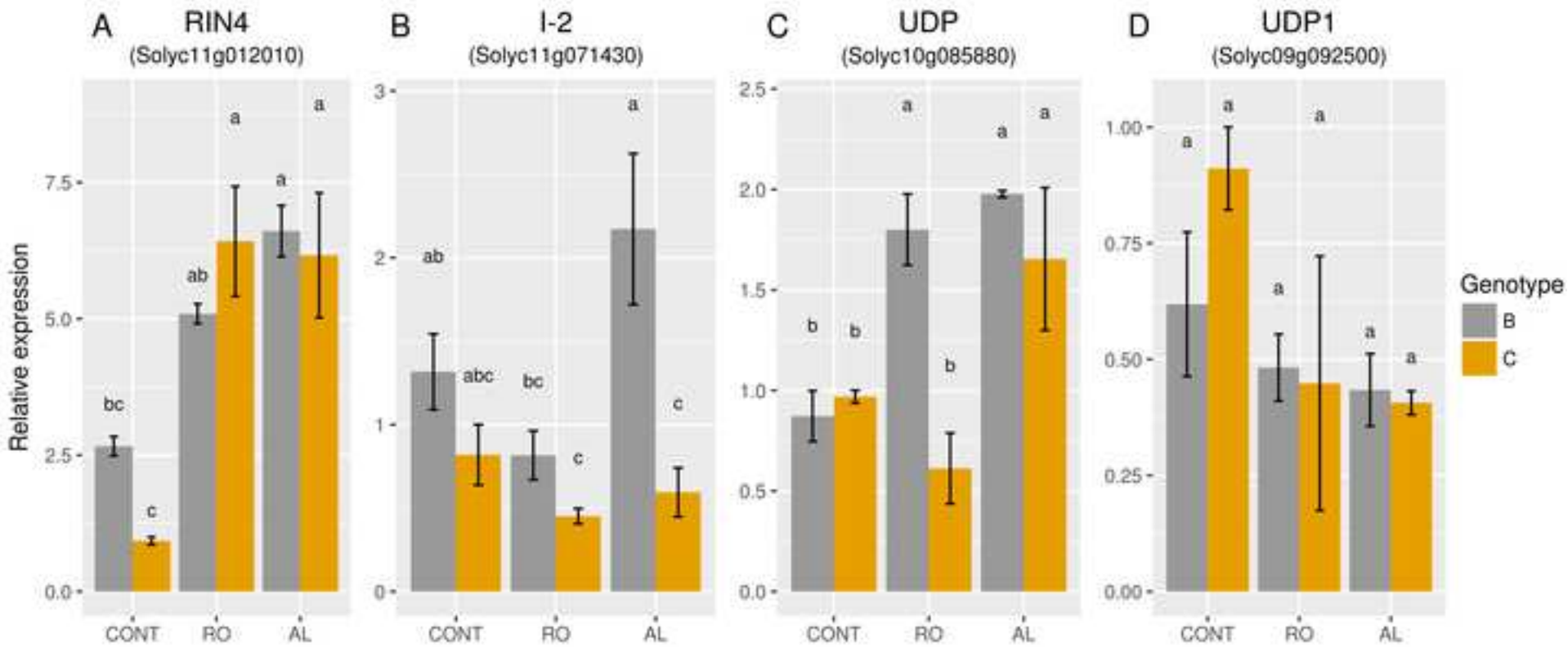
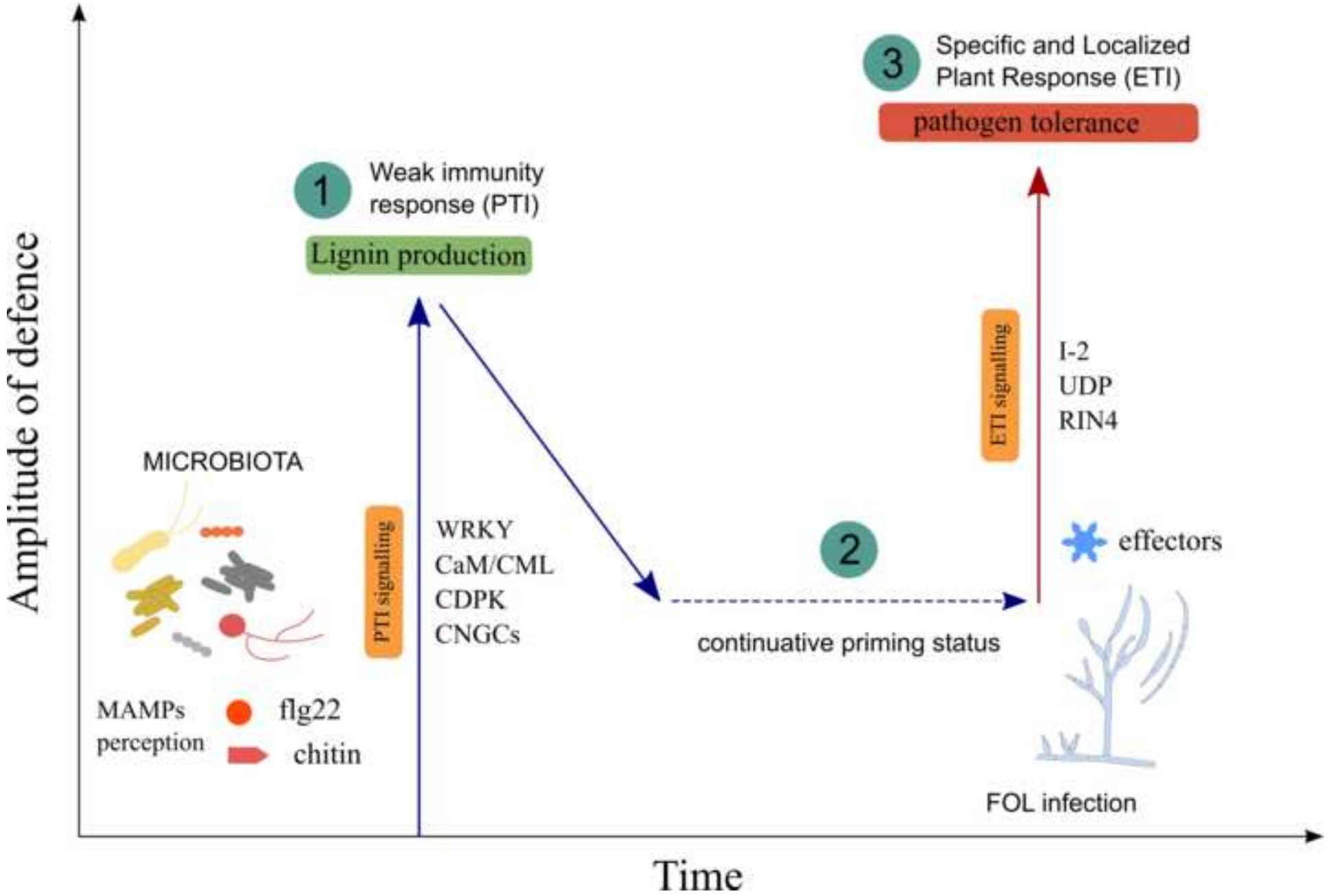
Figure


A
RIN4
(Solyc11g012010)
B
I-2
(Solyc11g071430)
C
UDP
(Solyc10g085880)
D
UDP1
(Solyc09g092500)
Relative expression
CONT
RO
AL
Genotype
B
C

Figure


Amplitude of defence
Time
1
Weak immunity response (PTI)
Lignin production
2
3
Specific and Localized Plant Response (ETI)
pathogen tolerance
MICROBIOTA
MAMPs perception
flg22
chitin
PTI signalling
WRKY
CaM/CML
CDPK
CNGCs
continuative priming status
ETI signalling
I-2
UDP
RIN4
effectors
FOL infection