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## Native soils with their microbiotas elicit a state of alert in tomato plants

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3 Native Soils with Their Microbiotas Elicit a State of Alert in Tomato Plants

4

5 Matteo Chialva<sup>1</sup>, Alessandra Salvioli di Fossalunga<sup>1</sup>, Stefania Daghino<sup>1</sup>, Stefano  
6 Ghignone<sup>2</sup>, Paolo Bagnaresi<sup>3</sup>, Marco Chiapello<sup>1</sup>, Mara Novero<sup>1</sup>, Davide Spadaro<sup>4</sup>,  
7 Silvia Perotto<sup>1</sup>, Paola Bonfante<sup>1\*</sup>

8

9 *Authors' affiliations:*

10 <sup>1</sup>*Department of Life Sciences and System Biology, University of Torino, Viale P.A.*  
11 *Mattioli 25, I-10125 Torino, Italy;* <sup>2</sup>*Department for Sustainable Plant Protection,*  
12 *Italian National Research Council (CNR), Viale P.A. Mattioli 25, I-10125 Torino, Italy;*  
13 <sup>3</sup>*Genomics Research Centre CRA-GPG, via S. Protaso, 302 I-29017 Fiorenzuola*  
14 *d'Arda, PC, Italy;* <sup>4</sup>*Department of Agricultural, Forestry and Food Sciences*  
15 *(Di.S.A.F.A.) and AGROINNOVA – Centre of Competence for the Innovation in the*  
16 *Agroenvironmental Sector, University of Torino, Largo Braccini 2, I-10095 Grugliasco,*  
17 *Italy.*

18

19 *\*Corresponding author:* Paola Bonfante, Department of Life Sciences and System  
20 Biology, University of Torino, Viale P.A. Mattioli 25, I-10125 Torino, Italy, +39 011  
21 6705965, paola.bonfante@unito.it.

22

23 Introduction, 621 words

24 Materials and Methods, 1528 words

25 Results, 2348 words

26 Discussion, 1443 words

27 Acknowledgement, 80 words

28

29 Main figures: 7 main figures (all in colour)

30 Supporting information: One file containing Supplementary figures and tables, Tables  
31 S3, S4, S5, S6 are in separate Excel files.

32

33 **Summary**

34 • Several studies have investigated soil microbial biodiversity, but comprehension  
35 of the mechanisms underlying plant responses to soil microbiota remains in its infancy.  
36 We focused on tomato (*Solanum lycopersicum*), testing the hypothesis that plants grown  
37 on native soils display different responses to soil microbiotas.

38 • Using transcriptomics, proteomics, and biochemistry, we describe the responses  
39 of two tomato genotypes (susceptible or resistant to *Fusarium oxysporum* f.sp  
40 *lycopersici*) grown on an artificial growth substrate and two native soils (conductive and  
41 suppressive to *Fusarium*).

42 • Native soils affected tomato responses by modulating pathways involved in  
43 responses to oxidative stress, phenols biosynthesis, lignin deposition, and innate  
44 immunity, particularly in the suppressive soil. In tomato plants grown on steam-  
45 disinfected soils, total phenols and lignin significantly decreased. The inoculation of a  
46 mycorrhizal fungus partly rescued this response locally and systemically. Plants  
47 inoculated with the fungal pathogen showed reduced disease symptoms in the resistant  
48 genotype in both soils, but the susceptible genotype was partially protected from the  
49 pathogen only when grown on the suppressive soil.

50 • The "state of alert" detected in tomatoes reveals novel mechanisms operating in  
51 plants in native soils and the soil microbiota appears to be one of the drivers of these  
52 plant responses.

53

54 **Key words**

55 Arbuscular mycorrhizal fungi, Defence responses, Lignin biosynthesis, Microbiota,  
56 Suppressive and conductive soils, Susceptible and resistant genotypes, Tomato.

57

58 **Introduction**

59 Crops, like their wild relatives, face many stresses, depending on the soil where they  
60 grow, the available nutrients, and other environmental conditions (Mundt, 2002). When  
61 grown as genetically homogeneous monocultures, crops are usually more susceptible to  
62 severe disease outbreaks than those grown in mixed cultivation. To decrease crop losses  
63 to disease, breeders have developed resistant varieties that have morphological and  
64 chemical barriers or activate defence responses to pathogens (Agrios, 2005).

65 Although much research has focused on the effects of plant genotype, the microbiota  
66 has recently emerged as an important factor in disease resistance. Plants, like animals,  
67 have their own microbiota, which can have a powerful effect on their health. Indeed,  
68 many physiological functions require the presence of these mostly benign microbes and  
69 the establishment of specific plant–microbe relationships (Ash & Mueller, 2016). In the  
70 plant microbiota, bacteria and fungi with beneficial functions, such as root symbionts  
71 and growth-promoting rhizobacteria, coexist with endophytes, saprotrophic microbes,  
72 and pathogens. Several studies on the plant microbiota have focused on identifying the  
73 extraordinary diversity of microbes present on both roots and epigeous organs  
74 (Bulgarelli *et al.*, 2012; Bai *et al.*, 2015; Coleman-Derr *et al.*, 2016), while others have  
75 examined the influence of the plant’s genotype on the composition of the microbiota  
76 (Lundberg *et al.*, 2012; Zgadzaj *et al.*, 2016). However, few plant studies have sought to  
77 understand how plants build up their microbiota (Lebeis *et al.*, 2015) or whether there is  
78 a relationship between microbiota, plant genetics, and nutrient availability (Horton *et*  
79 *al.*, 2014; Hacquard *et al.*, 2017; Castrillo *et al.*, 2017).

80 A complex interaction of biotic and abiotic factors, such as soil structure, nutrient and  
81 water availability, microbiota (including pathogens and symbionts), and plant genotype,  
82 affects plant productivity. To begin to untangle these complex interactions, we focused  
83 on tomato (*Solanum lycopersicum*), a relevant crop model plant, and tried to understand  
84 how plants respond when grown on native soils, as part of a larger project also covering  
85 microbial diversity (Poli *et al.*, 2016). Our hypotheses were: 1) microbiotas contained in  
86 different soils may trigger different plant responses and 2) different plant genotypes  
87 may respond differently to different soils and/or microbiotas. With the identification of  
88 the factors that govern plant responses, such hypotheses could allow us to better  
89 describe plant responses in conditions closer to those found in the field, rather than in

90 the lab (Poorter *et al.*, 2016).  
91 To test these hypotheses, we investigated the molecular responses of two tomato  
92 genotypes, one susceptible to the soil-borne pathogen *Fusarium oxysporum* f. sp  
93 *lycopersici* (FOL) and one resistant. We grew these two cultivars in two soils of  
94 different geographical origins, history, biological properties (suppressive or conducive  
95 to FOL) and cultivable fungal communities, but comparable textures and nutrient  
96 profiles (Poli *et al.*, 2016). As a control, we used a steam-disinfected growth substrate  
97 routinely used in tomato greenhouses. Transcript profiling by next-generation  
98 sequencing analyses showed that native soil components elicit an alert status in the plant  
99 by enhancing the induction of genes involved in defence responses, as compared with  
100 plants grown in a disinfected substrate. The disease-suppressive soil was indeed more  
101 effective in priming resistance supporting the hypothesis that microbiotas contained in  
102 different soils may trigger different plant responses. By contrast, the second hypothesis  
103 ( plant genotypes may respond differently to different soils and/or microbiotas) was not  
104 confirmed, since significant transcriptomics differences were not found between the two  
105 plant genotypes. Inoculation of an arbuscular mycorrhizal fungus (AMF) in the steam-  
106 disinfected soils induced similar responses, suggesting that the soil microbiota was one  
107 of the first drivers of the defence responses. Only under pathogen pressure did the plant  
108 genotype play a relevant role. These findings, indicate that biotic factors, more than  
109 abiotic, elicit specific responses in tomato grown in native soils.

110

## 111 **Materials and Methods**

### 112 **Plant, soils, and experimental design**

113 Two commercially relevant tomato genotypes, ‘Cuore di Bue’ and ‘Battito’ were  
114 selected. They are genetically related, being both ‘Oxheart’-type Heirloom varieties  
115 (Gioia *et al.*, 2010). Seeds were purchased from Ingegnoli (Milano, Italy). ‘Battito’ and  
116 ‘Cuore di Bue’ are resistant and susceptible, respectively, to FOL races 1 and 2, as  
117 stated by the producer and verified in a previous study (Poli *et al.*, 2016). To remove the  
118 seed microbiota (Shade *et al.*, 2017), in all experiments seeds were disinfected as detailed  
119 in Chialva *et al.* (2016), at least removing the seed ectosphere. Soils used were the same  
120 as those used by Poli *et al.* (2016). Albenga (AL) and Rosta (RO) soils were selected  
121 from two Italian regions on the basis of their comparable textures and nutrient profiles,

122 but different histories (agricultural *versus* meadow soil) and biological properties. Poli  
123 *et al.* (2016) have shown in fact that plants grown on AL displayed a moderate ability to  
124 suppress FOL growth whereas plants grown on RO allowed more FOL growth, leading  
125 to the conclusion that AL can be considered a suppressive soil and RO a conducive soil.  
126 In addition, Poli *et al.* (2016) characterized cultivable fungal communities of both soil  
127 revealing that the suppressive AL soil showed a higher load in *Fusarium* spp., *Phoma*  
128 spp., *Pyrenochaetopsis decipiens*, *Sarocladium strictum*, and *Trichoderma* spp., whilst  
129 the RO conducive one was richer in *Trichoderma* spp., *Penicillium* spp., *S. strictum*, and  
130 *Fusarium* spp.

131 In the current experiments, a control substrate (Pomix2, Evergreen, Moncalieri, Italy),  
132 which contains a mixture of peat and perlite, was used. This substrate was disinfected  
133 with fluent-steam at 100°C for 40 min, followed by 24 h at temperature higher than  
134 80°C, before use. After the disinfection protocol, no cultivable microbes were detected  
135 (data not show).

136 Three experiments were conducted. Experiments 1 and 3 were performed in the  
137 greenhouse and Experiment 2 was performed under controlled conditions in a growth  
138 chamber. Experiments 1 and 3 were set up in pots using the two tomato genotypes, and  
139 plant growing conditions were the same as those described in Poli *et al.* (2016). To  
140 investigate the plant transcriptomic response to native soils, microcosms were set-up  
141 under greenhouse condition (Experiment 1). ‘Battito’ and ‘Cuore di Bue’ tomato  
142 genotypes were used and plants were sampled after 30 days. Seedlings were grown in  
143 the two soils, AL and RO, plus the steam-disinfected growth substrate as a control  
144 (CONT) (Poli *et al.*, 2016). The roots from six plants for each substrate were pooled  
145 together, freeze-dried and three pools for each substrate used as replicates for RNA-seq  
146 analysis.

147 For Experiment 2, which aimed to validate transcript profilings data by performing  
148 molecular analysis and quantifying phenols and lignin, three subsets of plants  
149 maintained in a growth chamber were investigated: a) one set of ‘Cuore di Bue’ grown  
150 in the three substrates, as for Experiment 1, b) one set of seedlings maintained in the  
151 steam-disinfected native soils, processed as described for the CONT condition in  
152 Experiment 1, and c) a set grown as in conditions a and b with the addition of 30%  
153 diluted monospecific inoculum of the arbuscular mycorrhizal fungus (AMF)

154 *Funneliformis mosseae* (MycAgro Lab. Dijon, France). Seedlings were transferred into  
155 10x10x12 cm plastic pots, maintained under controlled temperature and light conditions  
156 [14-h light (24°C)/10-h dark (20°C)] and watered twice a week with tap water. Five  
157 replicates per condition were performed and plants sampled after 90 days.  
158 For Experiment 3, which aimed to understand the role of tomato genotypes, the virulent  
159 FOL strain MUT350 was added by mixing the soil with a talc powder containing FOL  
160 chlamydospores, at the final rate of  $3 \times 10^4$  chlamydospores mL<sup>-1</sup> of soil (Srinivasan *et*  
161 *al.*, 2009). Five replicates per condition were considered; plants were growing in a  
162 greenhouse and sampled after fruit set (120 days).

163 In Experiment 2, the presence of inoculated arbuscular mycorrhizal fungi (AMF)  
164 was assessed on fresh roots (Trouvelot *et al.*, 1986) as described in Chialva *et al.* (2016)  
165 observing 60 cm of roots per plant.

166

### 167 **RNA extraction and plant transcriptome analysis (Illumina RNA-seq)**

168 In order to study the plant transcriptome under the native soils and the control substrate  
169 conditions, material obtained in Experiment 1 was subjected to RNA-seq. The roots  
170 were washed in distilled water, blotted on filter paper, frozen in liquid nitrogen and  
171 freeze-dried overnight. Total RNA was extracted using a modified 'pine-tree' method  
172 (Chang *et al.*, 1993) with the addition of 2% PVPP to the extraction buffer (Guether *et*  
173 *al.*, 2009). RNA integrity (RIN) and concentration were double-checked (after  
174 extraction and before sequencing) using the 2100 Bioanalyzer system (Agilent  
175 Technologies) discarding samples with a RIN value <7. Details on library preparation,  
176 sequencing, and bioinformatics are provided in Methods S1.

177

### 178 **Proteome profiling**

179 To complement the transcriptomic data, proteome profiling was performed on the same  
180 material used for RNA-seq, but limited to the 'Cuore di Bue' genotype. Total proteins  
181 were extracted and analysed by LC-MS/MS on Q-Exactive Orbitrap (Bioproximity Inc.  
182 USA). Further details are provided in Methods S2.

183

### 184 **Functional enrichment and KEGG pathway analysis**

185 GO terms overrepresented in differentially expressed gene (DEG) lists were identified

186 in R statistical programming (R Core Team, 2017) in RStudio GUI (RStudio Team,  
187 2016) using the GSeq R Bioconductor package v1.15 (Young *et al.*, 2010) (false  
188 discovery rate, FDR<0.1). InterPRO and KEGG pathway terms enriched among DEGs,  
189 differentially expressed proteins (DEPs), or gene ID subsets were identified using the  
190 'enricher' function in the clusterProfiler R package (Yu *et al.*, 2012) ( $P<0.05$ ). Mapping  
191 between gene ID (SL.2.4) and GO, InterPro, or KEGG entries was retrieved using  
192 BiomaRT queries on Ensembl Plants website (<http://plants.ensembl.org>). Z-score  
193 semantic space was calculated according to Walter *et al.* (2015). Expressed genes in  
194 different contrasts were mapped into the relevant KEGG pathway incorporating color-  
195 coded expression values using the pathview R package v1.23 (Luo *et al.*, 2013) as  
196 detailed in Matić *et al.* (2016).

197

### 198 **RT-qPCR analysis**

199 To validate RNA-seq data, and to test DEGs emerged from RNAseq in Experiment 2  
200 and 3, reverse transcription quantitative PCR (RT-qPCR) assays were performed on a  
201 set of genes listed in Table S1 together with the used primers. RNA was isolated using  
202 the modified 'pine-tree method' as described above. Material was quality-checked,  
203 processed to remove DNA, and retrotranscribed as described in Chialva *et al.* (2016).  
204 RT-qPCR amplifications and data analysis were performed as described in Methods S3.  
205

### 206 **Quantification of lignin and total phenols**

207 Since the KEGG pathway analysis and the proteomics profiling identified lignin and  
208 phenol metabolism as differentially expressed in tomatoes growing in the three  
209 substrates, these compounds were quantified by using plants from the Experiment 2.  
210 Lignin was measured in protein-free cell wall material using the acetyl bromide method  
211 by Hatfield *et al.* (1996) as described in Moreira Vilar *et al.* (2014). Ten mg of each  
212 cleaned cell-wall sample was digested in 0.5 ml of 25% acetyl bromide (v/v in glacial  
213 acetic acid) at 70°C for 30 min. Samples were then cooled on ice and 0.9 ml of 2 M  
214 NaOH and 0.1 ml of hydroxylamine-HCl was added. Four ml of glacial acetic acid was  
215 added to the reaction and after centrifugation (2000xg, 10 min) extracts were diluted 1:4  
216 and  $A_{280}$  measured using 10-mm quartz cuvettes. A standard curve was generated using  
217 Alkali Lignin (Sigma, 370959) ( $R^2>0.99$ ) and results expressed as mg g<sup>-1</sup> cell wall

218 (CW).

219 Total phenols (TPs) were extracted and quantified using a modified Folin-  
220 Ciocalteu (F-C) assay (Ainsworth & Gillespie, 2007) as described in Zouari et al.  
221 (2014). Results were expressed as mg gallic acid equivalents (GAE) g<sup>-1</sup> of tissue dry  
222 weight (DW) using serially diluted gallic acid (Sigma, #G7384) standard solutions from  
223 500 to 7.8 mg/l ( $R^2 > 0.99$ ). Spectrophotometric analyses were performed using a  
224 Beckman DU 530 UV/VIS spectrophotometer on three to five biological replicates with  
225 three technical replicates each.

226

### 227 **Statistical analysis**

228 Statistical tests were performed in the R statistical programming environment (R Core  
229 Team, 2017) using Rstudio GUI (RStudio Team, 2016). Data normality and  
230 homoscedasticity were tested using Shapiro-Wilk (Shapiro & Wilk, 1965) and  
231 Levene's test (Levene, 1960) in the 'stats' and 'car' (Fox & Weisberg, 2011) respectively  
232 ( $P < 0.05$ ). According to data distributions, ANOVA for normal homoschedastic data or  
233 Kruskal-Wallis test for non-normal homoschedastic data (Kruskal & Wallis, 1952) were  
234 adopted from the custom R package 'stats' at  $P < 0.05$ . Pairwise comparisons between  
235 treatments were performed when needed, using the appropriate post hoc tests. Tukey's  
236 test (Tukey, 1949) in the package 'agricolae' (Mendiburu, 2016) was adopted for  
237 ANOVA and Dunn's test (Dunn, 1964) in package 'FSA' (Ogle, 2016) for Kruskal-  
238 Wallis, both at  $P < 0.05$ .

239 Principal component analysis (PCA) on RNA-seq libraries was performed using  
240 DESeq2::plotPCA function in R (Love *et al.*, 2014) and *k-means* clustering analysis in  
241 'stats' R package using 'kmeans' function (R Core Team, 2017) Variance partitioning  
242 analyses on transcriptome data sets was performed using the 'varpar' function in the  
243 'vegan' package (Oksanen *et al.*, 2016). Genotype and soil factors were used as  
244 explanatory variables. Testable partitions were tested for significance using  
245 permutational ANOVA (999 permutation) on the RDA model ( $P < 0.05$ ).

246 Graphical elaborations were performed using ggplot2 (Wickham, 2009) or 'graphics'  
247 package in R environment (R Core Team, 2017).

248

## 249 **Data Availability**

250 Raw RNA-seq reads were submitted to the National Center for Biotechnology  
251 Information Sequence Read Archive (NCBI-GEO/SRA) under accession SRP126554.

252

## 253 **Results**

### 254 **Native soils shape the plant root transcriptome**

255 In Experiment 1, we grew tomato plants on the three substrates, looking for specific  
256 effects of AL and RO native soils *versus* the CONT substrate. After 3 months, the plants  
257 were healthy on all soils. To examine the plant transcriptome, we sampled tomato roots  
258 from the two genotypes and three substrate conditions, extracted total RNA, and  
259 produced 18 Illumina RNA-seq libraries, yielding 11–27 million filtered reads (Table  
260 S2). The mean mapping rate on the tomato reference genome (Sato *et al.*, 2012) was  
261 93% and we found that 23,759 genes out of 34,675 annotated in tomato were expressed  
262 in roots. Counts were further processed with DESeq2 for normalization and  
263 identification of differentially expressed genes (DEGs) among conditions. Clustering of  
264 normalized counts showed consistency among soil and genotype and all Pearson  
265 correlation coefficients for biological replicates were above 0.9 (Figure S1).

266 To investigate the role of genotype and soil factors, we first performed  
267 multivariate analyses on normalized counts. Principal Component Analysis (PCA) with  
268 *k*-means clustering (Liu & Si, 2014) split RNA-seq libraries into three clusters ( $k=3$ )  
269 corresponding to the different soil types (RO, AL, and CONT) (Fig. 1a). By contrast,  
270 the analysis produced no clusters for genotype. Variance partitioning of the whole  
271 normalized read counts data set (Fig. 1b) confirmed the PCA ordination showing that  
272 the soil type significantly explained a large part of transcriptome variability (22% of  
273 variance explained, ANOVA,  $P<0.001$ ), but the genotype, which explained 3% of  
274 variance, had no significant influence (ANOVA,  $P>0.05$ ).

275 We then focused on the DEGs (fold-change  $>2$ , FDR $<0.05$ ) among soil  
276 conditions (Figure S2). In all the contrasts, the majority of DEG transcripts were  
277 upregulated in native soils, compared with CONT (Fig. 1c, Figure S2). We validated the  
278 relative expression estimates for 17 DEGs shared among contrasts by using RT-qPCR  
279 and found a high correlation with RNA-seq ( $r^2=0.85$ ,  $P<0.001$ ; Figure S3), validating  
280 our methodology. DEG analysis highlighted a similar number of DEGs when AL and

281 RO soils were compared with the CONT soil (969 and 847, respectively, Table S3)  
282 corresponding to around 4% of the tomato genes expressed in roots (Fig. 1d). However,  
283 the two contrasts shared only one-third of the DEGs (289), suggesting that each soil  
284 with its microbiota affects sets of tomato genes that only partly overlap. When the AL  
285 and RO data sets were directly compared (AL *versus* RO), the number of DEGs was  
286 lower (285 genes, corresponding to 1.2% of root transcripts) suggesting that in the two  
287 soils, the root transcription profile was largely shared. A similar trend was observed  
288 when the comparisons were performed to find enriched Gene Ontology (GO) (Fig. 1e,  
289 Table S4) and InterPro (Table S5) terms.

290

### 291 **Native soils modulate stress and defence responses in tomato roots**

292 To understand which tomato molecular components were affected by native soils, we  
293 focused on the shared set of 289 DEGs that responded in AL (suppressive) and RO  
294 (conductive) soils *versus* CONT substrate (FC>2, FDR<0.05) from Experiment 1 (Fig.  
295 1d, Table S6). This set contains mostly upregulated genes enriched in 19 GO and 38  
296 InterPro terms (FDR<0.05) (Fig. 1f). Both enrichment analyses detected the activation  
297 of general molecular defences against diverse environmental stresses in plants grown on  
298 native soil. The enriched GO terms (Figure S4a) included functions related to the  
299 apoplast (GO:0048046) and the response to oxidative stress (GO:0006979). Other  
300 enriched terms were related to the cell wall and nutrient transport, such as peroxidase  
301 activity (GO:0004601), metal ion binding (GO:0046872), metal ion transmembrane  
302 transporter activity (GO:0046873), acid phosphatase activity (GO:0003993), and  
303 transmembrane transport (GO:0055085). Similarly, analysis of enriched InterPro  
304 categories (Figure S4b) detected 24 categories, most of them related to cell-wall  
305 processes, such as laccases (IPR017761), type 1, 2, and 3 multicopper-oxidases  
306 (IPR011706, IPR011707, IPR001117), and plant peroxidases (IPR000823). Type III  
307 plant peroxidases, which are primarily involved in lignin biosynthesis (Weng &  
308 Chapple, 2010), were the most abundant class.

309 When comparing the GO and InterPro terms enriched in AL *versus* RO soil  
310 (Fig.1d), we found only 1 GO term ('apoplast', GO:0048046) shared with the terms  
311 enriched in AL or RO *versus* CONT (19 enriched GO terms) and none of the previously  
312 found InterPro terms. Some interesting GO categories emerged (Figure S5a), such as

313 those related to general stress responses. When analysing InterPro enriched domains  
314 (Figure S5b), functional categories related to central metabolism and plant-pathogen  
315 interactions emerged. Among the enriched domains, transcripts encoding peptidase  
316 domains (IPR000668, IPR013128) were upregulated in RO, while those encoding  
317 chitin-binding (IPR001002) and glutaredoxin (IPR002109) domains were upregulated  
318 in AL.

319 To support the transcriptomic data, we performed a proteome-profiling  
320 experiment analysing the same raw root material used for RNAseq. Since the genotype  
321 has a negligible role in shaping the plant response to soils (Fig.1b), we considered only  
322 the ‘Cuore di Bue’ genotype (FOL susceptible). The shared features between the  
323 proteome and transcriptome (Fig. 2) were either up- or down-regulated. The protein data  
324 sets obtained from the three contrasts were enriched in the ‘response to oxidative stress’  
325 GO term, and in related functions such as metal binding, heme binding, and peroxidase  
326 activity (Fig. 2). Proteins belonging to these categories were upregulated in the AL soil  
327 when compared to the other two substrates, and slightly upregulated in RO soil when  
328 compared with the disinfected substrate, suggesting that plants grown in native  
329 conditions (*i.e.* field-collected, non-disinfected substrates) respond to oxidative stress,  
330 and that different native soils might trigger responses of different intensities. Among the  
331 proteins upregulated in both AL and in RO samples, the peroxidase class was highly  
332 represented, including numerous enzymes involved in phenylpropanoid biosynthesis.

333

### 334 **Phenylpropanoid metabolism is induced in both native soils**

335 To analyse the Experiment 1 data sets at a deeper level, we mapped RNA-seq and  
336 proteome log<sub>2</sub> fold-change values onto KEGG pathways. When comparing gene  
337 expression in plants grown in native soils or in CONT, we found consistent regulation  
338 of several pathways involved in primary and secondary metabolism. However, when  
339 comparing soils with each other, we found only limited differences in the gene  
340 regulation in pathways dealing with central metabolism. With respect to primary  
341 metabolism, we found upregulation of the citrate cycle (sly00020) in both  
342 transcriptomic and proteomic data sets. Moreover, plants grown in AL soil, as compared  
343 with RO soil, showed differential regulation of some metabolic pathways in  
344 transcriptome data such as glycolysis (sly00010), starch and sucrose metabolism

345 (slyc00500) and amino acid metabolism (sly00260, sly00280, sly00290, sly00360).

346         With respect to secondary metabolism, phenylpropanoid biosynthesis (sly00940)  
347 was strongly regulated in both data sets in all considered contrasts (Figure S6-8).  
348 Several key enzymes were significantly upregulated in roots grown in native soils, as  
349 compared with the CONT substrate. The phenylpropanoid pathway originates from  
350 phenylalanine and leads to the synthesis of many diverse compounds, from insoluble  
351 lignin to soluble compounds (including salicylic acid) involved in defence against UV  
352 light, herbivores, or pathogens, as well as in the attraction of pollinators (Almagro *et al.*,  
353 2009; Fraser & Chapple, 2011). In addition to cell wall fortification, lignification is the  
354 first constitutive barrier against pathogen attack or abiotic stresses such as salinity  
355 (Neves *et al.*, 2010). In the phenylpropanoid pathway, 4 genes coding for beta-  
356 glucosidases (EC 3.2.1.21), which are involved in coumarin synthesis, and 14 genes  
357 coding for class III plant peroxidases (EC 1.11.1.7) were significantly induced,  
358 compared with their expression in plants grown on the CONT substrate. These genes  
359 were more induced in AL soil than in RO soil. Class III peroxidases were differentially  
360 expressed in both data sets.

361         To confirm the induction of phenylpropanoid biosynthesis, we conducted  
362 independent tests (Experiment 2 a) to quantify lignin and total phenolics in plants grown  
363 under controlled conditions in the same three substrates used for the RNA-seq  
364 experiment (Fig. 3a, c). We detected a significant increase in the contents of lignin and  
365 total phenolics in AL and RO root samples, as compared with CONT, confirming the  
366 RNA-seq and proteome profiling. Furthermore, we validated the increased lignin  
367 content at a systemic level in leaves, although total phenolics were not changed in  
368 leaves (Fig. 3b, d).

369

### 370 **The two native soils have different effects on the expression of genes involved in** 371 **plant–microbe interactions**

372 KEGG pathway analysis highlighted the differential involvement of plant–microbe  
373 interaction signalling (sly04626) in tomato roots from native soils *versus* the control  
374 conditions (Figure S9–11). In this case, suppressive and conducive soils led to different  
375 responses: genes involved in pathogen-associated molecular pattern (PAMP)-triggered  
376 immunity (PTI) were differentially regulated, with an upregulation in the moderately

377 suppressive AL soil when compared to RO. By contrast, the differential regulation of  
378 genes involved in Effector-Triggered Immunity (ETI) was not statistically supported. In  
379 particular, we found transcriptional responses to both fungal PAMPs (e.g. regulation of  
380 genes encoding cyclic nucleotide-gated channels, which mediate cytosolic calcium  
381 signals), and bacterial PAMPs, with the regulation of a *Flagellin Sensing 2* gene  
382 (Solyc02g070890.2) belonging to the leucine-rich repeat receptor serine/threonine  
383 kinase (LRR-RLK) gene family (EC 2.7.11.1).

384 Pathogenesis-related proteins (PRs), which are well-characterized molecular  
385 markers for systemic acquired resistance in several herbaceous plants (Zhang *et al.*,  
386 2010) and for early plant responses to AMF (Pozo *et al.*, 2015), were differentially  
387 expressed in both the transcriptome and proteome data sets. Among them, two  
388 chitinases (Solyc06g053380.2, Solyc11g072760.1), were upregulated in both native  
389 soils when compared with the disinfected CONT substrate. Other PRs were more  
390 upregulated in the AL soil compared with RO (Figure S9); for example, a class III  
391 chitinase (Solyc02g082920.2.1) and a CEVI-1 peroxidase, both belonging to the PR-9  
392 class (Solyc01g006300.2.1) were specifically induced in AL soil. PR-9s are commonly  
393 involved in the deposition of phenolics into the plant cell wall during pathogenesis.  
394 Both PR-9 proteins were described as markers of tomato resistance to fungal soil-borne  
395 pathogens, including *Rhizoctonia solani* (Taheri & Tarighi, 2012).

396 Looking at other genes potentially involved in the response to biotic stresses, we  
397 found that expression of a polygalacturonase inhibitor protein (PGIP)  
398 (Solyc09g014590.2) was upregulated in both plants grown on native soils. PGIPs are  
399 extracellular leucine-rich repeat glycoproteins that can inhibit the activity of  
400 polygalacturonases produced by fungi and bacteria and trigger plant defence reactions  
401 (Federici *et al.*, 2006).

402

### 403 **Disinfection of the native soils shows that the microbiota elicits phenylpropanoid** 404 **and defence pathways**

405 Our transcriptomic and proteomic data, as also confirmed by biochemical data,  
406 indicated that tomatoes grown in both native soils have increased phenylpropanoid  
407 metabolism (Fig. 4, Figure S7-8). These plants also show activation of PTI and defence-  
408 related pathways, with stronger activation in the AL soil (Figure S9). To test whether

409 such events were caused by the biotic or abiotic soil components, we grew 'Cuore di  
410 Bue' tomatoes for 90 days on steam-disinfected native soils (Experiment 2b). Under  
411 these conditions, the plants showed reduced total phenolics ( $P<0.05$ ) in both root and  
412 leaf tissues, as well as less root lignin in AL soil (Fig. 4a, b).

413         Since disinfection dramatically decreases the microbial presence (Lau &  
414 Lennon, 2011; Panke-Buisse *et al.*, 2015), we tested whether this affected other  
415 molecular plant response. We took advantage of the RNA-seq results and selected a  
416 panel of genes that were related to phenylpropanoid metabolism, oxidative stress, and  
417 plant–pathogen interactions as markers of plant responses to native soils. The  
418 expression of these genes was investigated using RT-qPCR on plants growing on the  
419 two native soils before and after disinfection. We tested transcripts involved in PTI  
420 (Cyclic nucleotide-gated ion channel, Solyc01g095770.2, pathogenesis-related protein,  
421 Solyc01g106640.2, Respiratory burst oxidase homolog protein, Solyc01g099620.2),  
422 phenylpropanoid biosynthesis (Caffeoyl-CoA O-methyltransferase, Solyc02g093230.2),  
423 abiotic (Gibberellin 3-beta-hydroxylase 1, Solyc06g066820.2) and oxidative stress  
424 responses (Superoxide dismutase, Solyc11g066390.1). The RNA-seq data were  
425 confirmed by RT-qPCR in native soils, because the investigated genes were upregulated  
426 in the AL soil, as compared to the conducive RO soil ( $P<0.05$ ) (Fig. 5). By contrast,  
427 after disinfection, the transcripts did not reveal any significant difference. Only  
428 superoxide dismutase (SOD) expression remained higher in the disinfected AL than in  
429 the RO soil (Fig. 5).

430         As a further step, we tested whether the induction of total phenolics and lignin  
431 observed in native soils can be ascribed to specific microbiota components, such as  
432 AMF, as already described in the literature (Rivero *et al.*, 2015). To this end, we chose  
433 *Funneliformis mosseae*, the most-represented AMF species in both soils (unpublished  
434 results) and one of the best performing partners for tomato (Zouari *et al.*, 2014), and we  
435 inoculated it into the disinfected soils (Experiment 2c). At 90 days after inoculation, we  
436 found a recovery of total phenolics, similar to the values measured in plants sampled  
437 from native soils. No differences emerged in lignin content, with the exception of roots  
438 from plants growing in AL, which showed a full recovery after AM inoculation (Fig 4c,  
439 d).

440

441 **Introducing a pathogen: genes involved in PAMP-triggered immunity are**  
442 **activated in the suppressive soil**

443 To test whether putative priming defences induced by native soils and their associated  
444 microbiota could counteract FOL disease, we set up a long-term greenhouse experiment  
445 (Experiment 3). Tomato plants from both genotypes ('Cuore di Bue' and 'Battito') were  
446 grown in AL and RO soils in the presence and the absence of a virulent strain of FOL  
447 (MUT 350). Plants were grown for 120 days until fruit-set. 'Battito' showed the  
448 expected resistant phenotype (Figure S12), but the response of 'Cuore di Bue' depended  
449 on the soil type, since a better performance was detected on the weakly suppressive AL  
450 soil (Figure S13).

451 To test the expression profile of some DEGs identified as being involved in the  
452 PTI response (Figure S9), we conducted RT-qPCR on the 'Cuore di Bue' genotype with  
453 or without pathogen inoculation (FOL+ and FOL-, Fig. 6). In AL versus RO samples, 6  
454 out of seven tested PTI genes were upregulated ( $P < 0.05$ ) in the absence of FOL (Fig.  
455 6a), confirming the RNA-seq results. Two out of seven genes (*CML* and *WRKY22*) were  
456 further upregulated in response to FOL inoculation (Fig. 6b). This trend was more  
457 evident when comparing FOL+ vs FOL- for each individual soil: in the suppressive AL  
458 soil, only one transcript, encoding a Plant Respiratory Burst Oxidase Homolog  
459 (Solyc01g099620.2), was induced by FOL inoculation (Fig. 6c). Three out of seven  
460 genes were upregulated in the permissive RO soil, revealing that the 'Cuore di Bue'  
461 genotype reacted more to the pathogen in RO (Fig. 6d) under conducive conditions.

462

463 **Discussion**

464 Notwithstanding the emerging appreciation of the biological importance of the  
465 microbiome concept, plant responses to complex microbial communities have rarely  
466 been examined: many reports have carefully investigated the microbial biodiversity  
467 associated with plants by using metabarcoding or metagenomics approaches (Bulgarelli  
468 *et al.*, 2015), but have generally neglected the effect on plant functions. Our  
469 experiments, on two tomato genotypes growing in two native soils with different  
470 physiochemical and biological properties (Poli *et al.*, 2016), have revealed some novel  
471 plant responses, thus helping us to understand how crops respond to the stimuli that  
472 originate from the biotic and abiotic components of soils.  
473 Transcriptomics and proteomics demonstrated that the overall characteristics of the  
474 substrate contribute more than plant genotype to shaping the molecular responses in  
475 tomato roots, and that only few genes respond differently in tomato plants grown in the  
476 two different native soils. This means that, notwithstanding the significant abiotic and  
477 biotic differences of the soils (Poli *et al.*, 2016), tomato roots seem to display a broadly  
478 similar expression profile when grown in native soils, as compared with roots grown in  
479 the control substrate. Soil is considered the primary force driving plant–microbiota  
480 diversity (Jeanbille *et al.*, 2016); our present data reveal that soil is also a key factor that  
481 shapes the molecular profile in tomato.

482

### 483 **Soil microbiota has a crucial role in the elicitation of phenylpropanoid pathways**

484 Transcriptomics and proteomics data from Experiment 1 led to a second novel result:  
485 many of the soil-responsive genes that are similarly modulated in the two native soils  
486 compared to an artificial, disinfected substrate, have biological relevance. These genes  
487 are mainly involved in the activation of phenylpropanoid metabolism and other defence  
488 responses, suggesting that tomato plants activate a pre-alert status, which can be  
489 correlated with the biotic and abiotic components of the native soils. Similar responses  
490 were also detected on *Arabidopsis* leaves inoculated with non-pathogenic phyllosphere  
491 commensals (Vogel *et al.*, 2016).

492 Phenylpropanoid metabolism is at the intersection of some of the most crucial  
493 pathways in plants, from the construction of structural barriers (cell wall and  
494 lignification) to the activation of many defence responses (Fraser & Chapple, 2011;  
495 Yogendra *et al.*, 2015). Some studies have linked plant–microbiota interactions (or even

496 selected components of the microbiota, such as AMF) with an increase in the  
497 production of lignin and phenolics (Rivero *et al.*, 2015; Beckers *et al.*, 2016). Here, we  
498 quantified these compounds and found that tomatoes grown in native soils produce  
499 more phenols and lignin in their roots and leaves.

500 In an attempt to disentangle the effect of the soil physiochemical features from  
501 the effect of the microbiota on local and systemic responses, in Experiment 2 we  
502 measured total phenolics and lignin content of plants grown in native soils, where a  
503 previous disinfection treatment led to an important reduction of endogenous microbes  
504 (Lau & Lennon, 2011; Panke-Buisse *et al.*, 2015). Here, we observed a significant  
505 decrease in total phenolics, while the decrease in the lignin content was significant only  
506 in the AL soil. The inoculation of the AM fungus *Funneliformis mosseae* led to an  
507 increase of phenolics back to the original values in the roots. Taken as a whole, the  
508 experiments provide evidence that the microbiota, more than the chemico-physical soil  
509 features, has a crucial role in the elicitation of phenylpropanoid pathway, and that the  
510 AM fungus *F. mosseae* alone largely rescues the activation of this metabolism. Indeed,  
511 previous papers have reported that AM fungi activate different steps of the  
512 phenylpropanoid pathway (Rivero *et al.*, 2015; Beckers *et al.*, 2016; Bruisson *et al.*,  
513 2016)

514

### 515 **The efficiency of the PTI response elicited by soil microbiota depends on soil** 516 **features and plant genotypes**

517 Plants have developed a complex immune system to protect themselves against  
518 pathogen attack (Jones & Dangl, 2006). In addition to pathogen-associated molecular  
519 pattern (PAMP)-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI),  
520 multiple pathways, including those involving salicylic acid signalling and MAP kinase  
521 cascades, form a robust network for plant immunity (Tsuda *et al.*, 2013). To date,  
522 however, our understanding of the mechanisms governing plant immunity comes from  
523 experiments conducted under controlled conditions, where specific, known microbes are  
524 added and their effects on plant immunity are determined as a result of gene–gene  
525 interactions (Thomma *et al.*, 2011; Pieterse *et al.*, 2014). Our data, which considered the  
526 plant response to the whole microbiota, only revealed the elicitation of PTI, the first and  
527 weaker form of defence in response to a microbial pathogen. However, our results did

528 not show elicitation of other pathways by native soils. The activation of the PTI-related  
529 genes likely reflects the plant response to multiple signals from the microbial  
530 communities thriving in the diverse substrates. The specific transcriptomic/proteomic  
531 picture we obtained might reflect the plant response to long-lasting stimuli produced by  
532 a complex soil microbiota. Interestingly, the differential expression of marker genes for  
533 plant–microbe interactions, including those for PTI, were detected in plants growing in  
534 AL vs RO soil, but disappeared in the plants grown in the two disinfected soils  
535 (Experiment 2 b). This strongly suggests that the components of the plant microbiota  
536 are directly involved in eliciting priming responses. Taken as a whole, these findings  
537 validated our first hypothesis, i.e., that different soils with their diverse microbiotas  
538 trigger differential plant responses.

539 Many recent reports have revealed that plant genotypes affect the establishment of  
540 different microbiotas (Bulgarelli *et al.*, 2015; Zgadzaj *et al.*, 2016), but the opposite  
541 question (do different plant genotypes respond differently to the same soil microbiota?)  
542 remains to be answered. Our experiments did not reveal any change in the  
543 transcriptomic profiles of ‘Cuore di Bue’ and ‘Battito’ genotypes, which differ in being  
544 susceptible and resistant to FOL, respectively. A previous detailed mycoflora analysis  
545 revealed that many *Fusaria* were present in both AL and RO soils, but pathogenic  
546 strains were not isolated (Poli *et al.*, 2016), providing a functional explanation for the  
547 overlap in the transcriptomic profiles; in the absence of the pathogen, the plant  
548 responses are very similar. With Experiment 3, we directly tested the relevance of the  
549 two genotypes by introducing the pathogen, and by looking at the phenotype in a long-  
550 lasting experiment. The resistant ‘Battito’ performed better in the presence of the  
551 pathogen in all soil conditions, as expected, and confirming previous tests (Poli *et al.*,  
552 2016). Also, the susceptible ‘Cuore di Bue’ elaborated defences, which allowed it to  
553 produce fruits in both the soils. However, measuring the expression of PTI-related  
554 genes after 120 days of FOL exposure revealed a surprising result: PTI-related genes  
555 were mostly upregulated in “Cuore di Bue” plants grown in the conducive RO soil in  
556 the presence of the pathogen. By contrast, in the suppressive AL soil, where the PTI  
557 genes were already activated in the absence of the pathogen, important modifications  
558 were not detected. The data reveal that the two genotypes respond differently to the  
559 pathogen, as expected, but, in addition, the susceptible genotype modulates its defense

560 responses depending on the soil. The protective effect, elicited by the soil microbiota, is  
561 not sufficient, however, to efficiently defeat the pathogen and to guarantee the health of  
562 ‘Cuore di Bue’ plants growing in the conducive RO soil.

563 All together, these data suggest that in native soils and in the presence of heterogeneous  
564 microbial communities living in the plant ectosphere and endosphere, tomato plants  
565 modulate some of their metabolic pathways, among which phenylpropanoid metabolism  
566 and PTI pathway (Fig. 7). Tomato plants respond to both native soils by activating a  
567 first level of defences based on cell wall fortification. However, the PTI pathway is  
568 regulated differently accordingly to the disease-suppressiveness of the two soils. In the  
569 suppressive soil, these PTI-like responses are induced to higher levels, providing a  
570 protective shield when a pathogen such as FOL is added to the system. This result is in  
571 agreement with the concept of ‘general suppression’ suggested by Raaijmakers &  
572 Mazzola (2016). Our results agree with those of Vogel *et al.* (2016), who described a  
573 comparable defence response elicited by phyllosphere commensals on *Arabidopsis*  
574 *thaliana*. This confirms that the rules governing the responses to the microbiota are  
575 similar in different plant compartments.

576         Next-generation sequencing techniques and the resulting data sets have provided  
577 us with many pieces of a complex puzzle, *i.e.* identification of tomato metabolic  
578 processes related to plant immunity, production of compounds related to cell-wall  
579 fortification and lignin. In conclusion, even if the puzzle is far from complete, we have  
580 started to reveal the multi-level mechanisms that operate in plants living in realistic  
581 conditions that are closer to those experienced by field-grown plants, rather than by  
582 plants grown in artificial growth substrates. These mechanisms bring into play multiple  
583 factors (soils, microbiotas, genotypes) that affect plant health, overcoming the  
584 reductionist approach of one-to-one interactions.

585

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594

#### 595 **Author contributions**

596 MChial., SP, AS and P.Bonfante designed research; MChial performed research with  
597 the contribution of SD and MC for proteomics, AS for molecular analysis, PBag and SG  
598 for bioinformatics. MN performed AMF analysis and manuscript editing; DS set up the  
599 greenhouse experiments; MChial and P.Bonfante analyzed the data and wrote the paper.

600

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## 602 **Figure legends**

603 **Fig 1. Analysis of the root transcriptome of tomato (*Solanum lycopersicum*) plants**  
604 **grown in native and artificial soils. (a)** PCA plot with *k*-means clustering of RNA-seq  
605 libraries showing the two principal components (PC1 and PC2), which separated the  
606 samples by soil type. *K*-means clusters (*k*=3) are represented by ellipses and group  
607 sequencing libraries by soils. In the legend, the first letter indicates genotype ('Cuore di  
608 Bue' or 'Battito') and the following letters represent the substrate (RO, AL, or CONT).  
609 **(b)** Donut plot showing the amount of transcriptome variability explained from soil  
610 (22%) and genotype (3%) factors. Data were tested using permutational ANOVA (999  
611 permutations, \**P*<0.001; ns = not significant). Collinearity between genotype and soils  
612 explained none of the variance. **(c)** Heatmap of DEGs (differentially expressed genes)  
613 across the three soil contrasts analysed with DESeq2 (*FC*>1, *P*<0.05) shows that native  
614 soils mostly upregulate transcription when compared to the control transcriptome from  
615 plants grown in disinfected soil. **(d)** Venn diagrams showing the number of shared  
616 DEGs categories (*FDR*<0.05) across the three main contrasts. **(e)** The 20 most-enriched  
617 GO categories shared between AL *versus* CONT (red bars) and RO *versus* CONT (blue  
618 bars).

619 AL='Albenga' suppressive soil; RO='Rosta' conducive soil; CONT=neutral control soil.

620

621 **Fig. 2 Overlap of GO-categories enriched in both proteome and transcriptome**  
622 **experiments in tomato (*Solanum lycopersicum*) 'Cuore di Bue' genotype.** The y-axis  
623 in the bubble plot represents the  $-\log_2$  of adjusted *P*-value of proteome enrichment; the

624 x-axis shows the z-score computed on DEPs contained in each GO category (z-score >  
625 0 prevalence of upregulation, z-score < 0 prevalence of down-regulation). Categories  
626 from each of the three GO domains are indicated by colours (BP='biological process',  
627 CC='cellular component', MF='molecular function'). Categories with  $\log_2$  adj. *P*-value  
628 > 25 or z-score < -1 are labelled. The size of each bubble represents the number of  
629 differentially expressed proteins for each term. AL='Albenga' suppressive soil;  
630 RO='Rosta' conducive soil; CONT=neutral control soil.

631

632 **Fig. 3 Total phenols and lignin concentrations in tomato (*Solanum lycopersicum*)**  
633 **plants grown in native and artificial substrates. (a, b)** Total phenols (TPs) in roots  
634 and leaves, respectively. **(c, d)** Lignin content in root and leaves, respectively. Letters  
635 indicate significant differences among treatments (ANOVA, Tukey's post-hoc test,  
636  $P < 0.05$ ). TPs are expressed as mg of gallic acid equivalents (GAE) per grams of dry  
637 weight (DW) material. Lignin amount is expressed as mg per grams of cell-wall (CW)  
638 material.  $N=5$ . AL='Albenga' suppressive soil; RO='Rosta' conducive soil;  
639 CONT=neutral control soil. Boxplots display the median (horizontal line), the quartiles  
640 (boxes) and the 1.5-times interquartile range (whiskers).

641

642 **Fig. 4 Levels of total phenols and lignin in tomato (*Solanum lycopersicum*) 'Cuore**  
643 **di Bue' genotype grown in disinfected native soils and disinfected soils inoculated**  
644 **with *F. mosseae*. (a and b)** Total phenols (TPs) in roots and leaves, respectively. **(c and**  
645 **d)** Lignin content in root and leaves, respectively. Letters indicate significant differences  
646 among treatments (ANOVA, Tukey's post-hoc test,  $P < 0.05$ ). TPs are expressed as mg of  
647 gallic acid equivalents (GAE) per grams of dry weight (DW) material. Lignin amount is  
648 expressed as mg per grams of cell-wall (CW) material.  $N=5$ . AL='Albenga' suppressive  
649 soil; RO='Rosta' conducive soil; CONT=neutral control soil. Boxplots display the  
650 median (horizontal line), the quartiles (boxes) and the 1.5-times interquartile range  
651 (whiskers).

652

653 **Fig. 5 RT-qPCR of native-soil induced gene expression in tomato (*Solanum***  
654 ***lycopersicum*) plants under native and steam-disinfected treatments in suppressive**  
655 **versus conducive soils. Dotted red lines indicate the threshold at fold change=1.**  
656 Asterisks indicate statistically supported differences (Kruskall-Wallis test at  $P < 0.05$ ).

657 Data are mean  $\pm$  SD,  $n=3$ . CCoAOMT=Caffeoyl-CoA O-methyltransferase  
658 (Solyc02g093230.2); G3B=Gibberellin 3-beta-hydroxylase 1 (Solyc06g066820.2);  
659 SOD=superoxide dismutase (Solyc11g066390.1); Rboh=Respiratory burst oxidase  
660 homolog protein (Solyc01g099620.2); PR=Pathogenesis-related protein  
661 (Solyc01g106640.2); CNG=Cyclic nucleotide-gated ion channel (Solyc01g095770.2).

662

663 **Fig. 6 RT-qPCR of PTI-related gene expression in FOL-inoculated (FOL+) and**  
664 **non-inoculated (FOL-) tomato (*Solanum lycopersicum*) plants in both soils. (a, b)**  
665 Relative expression of genes in AL *versus* RO under FOL- and FOL+ treatments; (c, d)  
666 Relative expression of genes in FOL+ versus FOL in AL and RO native soils. Dotted  
667 red lines indicate the threshold at fold change=1. Asterisks indicate statistically  
668 supported differences (Kruskal-Wallis test at  $P<0.05$ ). Data are mean  $\pm$  SD,  $n=3$ ;  
669 FOL=*Fusarium oxysporum* f.sp. *lycopersici*. AL='Albenga' suppressive soil;  
670 RO='Rosta' conducive soil.

671

672 **Fig. 7 Proposed model of tomato (*Solanum lycopersicum*) plant response to soil**  
673 **microbiota.** The scheme illustrates the main pathways which are differentially  
674 regulated between the suppressive vs. conducive soil on the basis of transcriptomic and  
675 proteomics analysis. The highlighted pathways were validated with chemical  
676 quantification of total phenols and lignin, as well as RT-qPCR of genes involved on  
677 tomato defence on both native and disinfected soils. In this model, irrespectively of their  
678 genotype, tomato would perceive microbial MAMPS (as flagellin, flg22, and chitin)  
679 through specific receptors (FLS2 and CERK1, respectively) whose transcripts were  
680 upregulated. The signalling cascade would activate a PTI-like mechanism upregulating  
681 Cyclic nucleotide-gated channels (CNGCs) which increase the amount of cytosolic  
682 calcium, inducing in turn the expression of calmodulin (CaM)/CaM-like (CML)  
683 proteins and calcium-dependent protein kinases (CDPKs). Transcript profiling revealed  
684 other downstream events: an *Rboh* (Respiratory burst oxidase homolog) gene leads to a  
685 ROS burst, and to the activation of transcription factors as WRKY 22 and 33. We  
686 suggest that the latter elicits plant defences through the presence of pathogenesis-related  
687 proteins (PRs) and of the phenylpropanoid pathway. It would start with the up-  
688 regulation of the phenylalanine ammonia lyase (PAL) enzyme, followed by the up-

689 regulation of the caffeoyl-CoA O-methyltransferase (CCoAOMT) gene and of class III  
690 peroxidases (PRX (III)) which are directly involved in lignin and total phenolics (TPs)  
691 biosynthesis. All these pathways may confer an increased resistance against *Fusarium*  
692 *oxysporum* f.sp. *lycopersici* not only in the resistant genotype, but also in the susceptible  
693 one (long-term greenhouse Experiment 3).

694 Rectangular splitted boxes represent the expression ratio (log<sub>2</sub>fold-change) in  
695 suppressive versus conducive soils contrast in ‘Cuore di Bue’ genotype for both  
696 transcripts (left box) and proteins (right box). Red colour represents upregulation, blue  
697 downregulation.

698 **Supporting Information**

699 **Figure S1** Correlation heatmap of RNA-seq libraries using Euclidean distances between  
700 samples calculated on RLD (regularized log transformation) normalized reads counts.

701 **Figure S2** MA plots obtained from DESeq2 Differential Expression analysis using all  
702 considered contrasts with both separated or pooled genotype.

703 **Figure S3** Correlation plot of DEGs between RT-qPCR and RNA-seq analysis.

704 **Figure S4** GO and InterPro domain enrichment analysis on the DEG set shared in  
705 native *versus* control substrate contrast.

706 **Figure S5** GO and InterPro enrichment analysis of DEGs in AL *versus* RO soil contrast.

707 **Figure S6** Phenylpropanoid biosynthesis KEGG pathway (sly00940) in AL *versus* RO  
708 and in 'Cuore di Bue' genotype

709 **Figure S7** Phenylpropanoid biosynthesis KEGG pathway (sly00940) in AL *versus*  
710 CONTROL and in 'Cuore di Bue' genotype

711 **Figure S8** Phenylpropanoid biosynthesis KEGG pathway (sly00940) in RO *versus*  
712 CONTROL and in 'Cuore di Bue' genotype. Regulated transcripts and proteins are  
713 displayed in left and right box, respectively. Up-regulated IDs are in red, down-  
714 regulated in green.

715 **Figure S9** Plant–pathogen interaction KEGG pathway (sly04626) in AL *versus* RO and  
716 in 'Cuore di Bue' genotype.

717 **Figure S10** Plant–pathogen interaction KEGG pathway (sly04626) in AL *versus*  
718 CONTROL and in 'Cuore di Bue' genotype.

719 **Figure S11** Plant–pathogen interaction KEGG pathway (sly04626) in RO *versus*  
720 CONTROL and in 'Cuore di Bue' genotype. Transcripts and proteins regulation is  
721 displayed in left and right box respectively. Up-regulated IDs are in red, down-regulated  
722 in green.

723 **Figure S12** *S. lycopersicum* cv 'Battito' plants grown in native soils with and without  
724 FOL inoculation.

725 **Figure S13** *S. lycopersicum* cv 'Cuore di Bue' plants grown in native soils with and  
726 without FOL inoculation. (a, b) plants growing in RO, and AL (c, d) native soils  
727 inoculated (b, d) or not (a, c) with FOL pathogen.

728

729 **Table S1.** RT-qPCR primers used in this study.

730 **Table S2.** Sequencing and genome mapping statistics.

731 **Table S3.** Differentially expressed transcripts in the three soil contrasts (FDR<0.05,  
732 FC>2).

733 **Table S4.** GO enrichment analysis ( $P<0.1$ ) on DEGs from Table S3 by soil contrasts.

734 **Table S5.** InterPro domains enrichment analysis ( $P<0.1$ ) on DEGs from Table S3 by  
735 soil contrasts.

736 **Table S6.** Shared DEGs between AL versus CONT and RO *versus* CONT soil contrasts  
737 from Table S3.

738

739 **Methods S1.** Illumina RNA sequencing and bioinformatic analysis.

740 **Methods S2.** Proteome Profiling

741 **Methods S3.** Real-Time PCR protocol used in the study.