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Abstract: Experimental studies investigated the effects of transgenic crops on the structure, function and diversity of soil and rhizosphere microbial communities playing key roles in belowground environments. Here, we review the available data on direct, indirect and pleiotropic effects of engineered plants on soil microbiota, considering both the technology and the genetic construct utilised. Plants modified to express phytopathogen/phytoparasite resistance, or traits beneficial to food industries and consumers differentially affected soil microorganisms, depending on transformation events, experimental conditions and taxa analysed. Future studies should address the development of harmonised methodologies considering the complex interactions governing soil life.

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3 **Belowground environmental effects of transgenic crops: a soil microbial perspective**

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26

Abstract

Experimental studies investigated the effects of transgenic crops on the structure, function and diversity of soil and rhizosphere microbial communities playing key roles in belowground environments. Here, we review the available data on direct, indirect and pleiotropic effects of engineered plants on soil microbiota, considering both the technology and the genetic construct utilised. Plants modified to express phytopathogen/phytoparasite resistance, or traits beneficial to food industries and consumers differentially affected soil microorganisms, depending on transformation events, experimental conditions and taxa analysed. Future studies should address the development of harmonised methodologies considering the complex interactions governing soil life.

Keywords

microbial communities; transgenic plants; pleiotropy; horizontal gene transfer; *Bt* plants.

1. Introduction

The cultivation of transgenic plants (or genetically modified plants, GMPs) has prompted scientists to seek greater understanding of their direct and indirect impact on natural and agricultural ecosystems. While GMPs have been assumed safe in terms of human health, unforeseen environmental effects have been observed in the field, varying according to the genetic traits of the modified plants, and in space and time, as a result of the complex network of interactions ruling aboveground and belowground ecosystem functioning [1]. Some of the effects reported in the available scientific literature may be directly ascribed to the technology utilised, while others are linked to the nature of the genes introduced in the transgenic plants.

52 Most transgenic events have been obtained by using the cauliflower mosaic virus (CaMV)
53 35S RNA promoter, which induces constitutive expression of transgenic proteins: some of
54 them act as toxins towards particular groups of organisms and are exuded by the roots [2-4].
55 This stresses the need to assess the effects of such genetic modification on microbes living in
56 the rhizosphere and in the soil. In such environments plants release up to 25% of the carbon
57 allocated to the roots as root exudates [5], and crop residues are incorporated at the end of
58 production cycles. Other outcomes of the technology used for the production of transgenic
59 plants may derive from pleiotropy, a phenomenon leading to the development of unexpected
60 phenotypes as a result of insertions of foreign genes in a new genomic context. For example,
61 some GMPs showed increases or decreases in the content of plant secondary metabolism
62 compounds or alterations in crop chemistry, not directly linked to the particular genes
63 introduced [6-8], which might affect, directly or indirectly, the soil microbiota.

64 With regard to the nature of the genes introduced in transgenic plants, the use of marker
65 genes for antibiotic resistance and their fate during and after cultivation in the field have been
66 considered critical issues by the World Health Organization [9], as antibiotic resistance genes
67 may be transferred to rhizosphere and soil microbes, and from them to pathogenic bacteria,
68 through horizontal gene transfer (HGT) [10]. Besides, crops modified to tolerate broad-
69 spectrum herbicides like glyphosate have also raised some concerns, as glyphosate inhibits
70 Class I EPSPS, a key enzyme in the synthesis of aromatic amino acids occurring in plants,
71 fungi and bacteria [11].

72 GMPs may directly or indirectly impact the structure, function and diversity of soil and
73 rhizosphere microbial communities, which play key roles in the belowground environment,
74 providing essential ecosystem services, e. g. decomposition of crop residues, completion of
75 biogeochemical cycles within the soil food web, and maintenance of environmental quality
76 and productivity [5]. Rhizosphere microorganisms may be affected by plant genotype [12]

77 and by changes in agricultural management inherent to the cultivation of transgenic plants,
78 such as herbicide application. Thus, they represent potential key nontarget organisms to be
79 monitored in studies on the environmental impact of transgenic crops (Fig. 1).

80 In this work we review the available data on direct, indirect and pleiotropic effects of
81 GMPs on the structure and function of soil microbial communities, considering both the
82 technology utilised for the production of engineered plants and the nature of the transgenes.

83

84 **2. Direct, indirect and pleiotropic effects of transgenic plants on soil microbes**

85 *2.1. Transgenic plants constitutively producing Bt toxins*

86

87 *Bt* plants are engineered with *cry* genes derived from the soil bacterium *Bacillus*
88 *thuringensis* Berliner to express insecticidal δ-endotoxins (called crystal proteins or Cry
89 proteins), conferring resistance to some insect pests from the orders Lepidoptera, Coleoptera
90 or Diptera [13]. The amounts of *Bt* toxins expressed in plant tissues and released into the
91 environment, ranging from 152 to 183 ng per gram in decomposing root residues, directly
92 derive from the technology utilised to produce transgenic plants constitutively expressing Cry
93 proteins. Such data will deserve attention in the years to come, in particular in multiple *Bt*
94 toxin stacked-trait lines [14]. Indeed, it has long been known that insecticidal *Bt* toxins are
95 exuded by *Bt* maize roots into the soil [2], where, together with those derived from plant
96 residues, are bound to humic acids and clay soil particles, and, protected from microbial
97 degradation, often maintain their activity [13]. Some authors reported that the Cry3Bb and the
98 Cry1Ac toxins may persist for 21 and 56 d in soil microcosm and laboratory experiments,
99 respectively [15, 16] and that no *Bt* toxin is retrieved from field soils for 3-6 consecutive
100 years of *Bt* cotton cultivation [17]. Variable persistence has been observed for Cry1Ab toxin,
101 which was not detected in a nine-year field trial of *Bt*-maize MON810 [18] or was shown to

102 be still detectable after 4 yrs in the field [13], maybe depending on soil chemical and physical
103 characteristics.

104 In an experiment carried out on *Bt* maize plants in relation to soil biota, Saxena and
105 Stotzky found that the CryIAb toxin released into root exudates or directly incorporated into
106 soil exerted no adverse effects on culturable bacteria and saprophytic fungi (and also on
107 earthworms, nematodes, protozoa) [19]. Small or no changes in culturable microflora were
108 detected in the rhizosphere of Cry-expressing cotton and rice and in the composition of
109 microbial communities in the presence of Cry1Ab maize residues, compared with control
110 plants (Table 1). Accordingly, two long-term field studies found no consistent differences
111 among soil microbial communities between GMPs and controls and among successive years
112 [15, 20]. A significant temporary decrease in saprophytic fungal populations was observed 30
113 days after sowing *Bt* maize in comparison with the isogenic line [21], and variation in fungal
114 decomposer communities was detected in one out of 16 trials by Xue et al. [22] (Table 1).

115 Other works, using culture-independent methods, reported no significant or only small
116 effects of *Bt* maize plants on soil microbial communities, suggesting that plant age, soil type
117 and texture may represent the overriding factors affecting bacterial diversity (Table 1). By
118 contrast, different fingerprints of soil bacterial communities exposed to *Bt* maize were
119 reported by other authors [23-26]. Castaldini et al. [25] also observed that microbial activity,
120 assessed by measuring soil respiration, changed in soils amended with *Bt* plant residues, in
121 agreement with other reports [27, 28] (Table 1).

122 In the majority of the cited studies, it is impossible to distinguish between effects that can
123 be directly ascribed to the toxins and indirect and unspecific outcomes of the transgenic
124 events (pleiotropy). However, an interesting work highlighted the occurrence of pleiotropic
125 effects which were not linked to the products of the inserted genes but represented a result of
126 the transformation technology [16]: the cultivation of *Bt* cotton affected soil microbial

127 populations, while the purified *Bt* toxin showed no effect. These data were corroborated by
128 results detailed in Naef et al. [29], who found that purified Cry1Ab toxin did not inhibit the
129 growth of *Fusarium graminearum* and *Trichoderma atroviride*, while *Bt* and non-*Bt* maize
130 residues affected fungal growth *in vitro* (Table 1).

131 A pleiotropic effect of *cry1Ab* transgenic plants - alteration in the shikimic acid pathway
132 leading to a higher lignin content in the stem - was detected in several transformation events
133 of *Bt* maize lines [6, 8] and also in *Bt* canola, cotton, potato, rice and tobacco [27]. However,
134 the harm or benefit of the slower degradation rate of *Bt* plant residues and the putative
135 resulting shifts in microbial community composition remain to be verified. A field study [30]
136 found that *Bt* maize decomposed significantly faster than non-*Bt* maize in winter in bags with
137 20 and 125 μm mesh sizes, which excluded macrofauna but allowed microflora (bacteria,
138 fungi) and mesofauna activity. Such results were explained by the higher amount of proteins
139 in the plant matrix (20% of *Bt* toxin still present), which stimulated growth of soil microbial
140 populations. Conversely, no effects on the decomposition rate were detected by Hopkins and
141 Gregorich [31] either in microcosm experiments or when residues of some *Bt* hybrids were
142 ploughed into field soil.

143 A distinctive group of beneficial soil microorganisms, arbuscular mycorrhizal (AM)
144 symbionts, has been extensively investigated as potential key nontarget organisms in studies
145 on the environmental impact of GMPs, given their high responsiveness to agricultural
146 practices and environmental changes. A reduction in AM colonization was shown in *Bt*11,
147 *Bt*176 and MON810 maize lines expressing the Cry1Ab toxin, compared with non-*Bt* isogenic
148 lines (Fig. 2), whereas no effects were detected in other trials with different maize and cotton
149 lines expressing Cry1Ab, Cry1Ac and Cry2Ab (Table 1). A recent study on 14 *Bt* and non-*Bt*
150 maize lines expressing different numbers and types of engineered traits revealed that all the
151 various transgenic lines reduced mycorrhizal colonization by indigenous AM fungi occurring

152 in the greenhouse [32], while no changes in AM fungal colonization were found in the field
153 [33, 34], but AMF spore abundance was lower in field plots with a Bt maize cultivation
154 history than in control plots [35]. Such contrasting results might be explained by the different
155 nutrient status of soil or by the differential nutrient uptake of *Bt* and non-*Bt* plants [32, 36].

156 In general, great attention should be paid when discussing data obtained in short-term
157 experiments or single-point assessments, because microbial communities living in the soil and
158 in the rhizosphere are subject to seasonal shifts, which represent further factors affecting the
159 complex network of interactions characterising natural and agricultural ecosystems. Time-
160 course investigations based on large spatial and long temporal scales are needed to assess
161 putative long-term modifications occurring in microbial community structure and
162 composition during and after GMPs cultivation. Soil environment should continue to be
163 monitored after Bt crop use, as there are data showing that repeated cultivation of *Bt* corn over
164 many years resulted in greater microbial biomass, enzyme activity and functional diversity
165 than conventional corn grown in rotation [37].

166

167 *2.2. Transgenic plants resistant to phytopathogenic bacteria and fungi and to phytoparasites*
168 *other than insects*

169

170 Besides GMPs expressing *Bt* toxin, other transgenic plants have been developed to
171 control either invertebrate pests - by expressing lectin or proteinase inhibitors - or
172 phytopathogenic fungi - by expressing plant-derived defensins, lysozymes, cecropins,
173 pathogenesis-related proteins and systemic acquired resistance (Table 1).

174 Engineered potatoes producing *Galanthus nivalis* agglutinin, conferring resistance to
175 nematodes, showed a reduction in microbial activity and different physiological profiles of
176 rhizosphere microbial communities [38]. Transgenic potato plants expressing the cysteine

177 proteinase inhibitors able to control potato-cyst nematode, tested in the field for two growing
178 seasons, showed a reduction in bacterial and fungal abundance after one year's growth [39].
179 On the other hand, the impact of transgenic potato plants expressing a phage T4 lysozyme
180 gene on bacterial communities was comparable with the effects of plant genotype, vegetation
181 stage, soil type and pathogen infection [40-42]. Accordingly, other transgenic events
182 expressing lytic peptides able to control phytopathogenic bacteria, like cecropin B- and
183 quorum quenching lactonase AttM-expressing plants, did not cause significant changes in soil
184 bacterial communities [43, 44] (Table 1).

185 Several transgenic plants resistant to pathogenic fungi were obtained by inserting genes
186 encoding pathogenesis-related proteins such as chitinases. A chitinase-expressing transgenic
187 rice showed a reduction in root colonisation by endophytic and mycorrhizal fungi and an
188 increase in intraradical bacteria [45]. Symbiotic fungi and bacteria represent a very important
189 group of nontarget microbes to be monitored in impact studies of this particular kind of
190 GMPs, since they can be affected by plant antimicrobials . The establishment of mycorrhizal
191 symbiosis by *Funneliformis mosseae* was not affected in the roots of *Nicotiana* spp.
192 expressing chitinases and pathogenesis-related proteins, although a delay in mycorrhizal
193 colonisation was observed in plants expressing the PR-2 protein [46-48]. Other reports
194 confirmed such results, showing delayed root colonisation by AMF in tobacco plants
195 modified for the expression of enhanced systemic acquired resistance, compared with non
196 transgenic lines [49]. The low sensitivity of AMF symbionts to antifungal enzymes may be
197 ascribed to their differential expression in root tissues: thus in transgenic tobacco roots
198 chitinase levels were only 2-4 times higher than in controls, whereas their content increased
199 23-44 times in the leaves [50]. Differences in AMF root colonisation and *Pseudomonas*
200 population dynamics were observed among wheat plants expressing the *pm3b* mildew
201 resistance transgene and parental lines. Conversely, no differences were detected between GM

202 and non-GM sister lines, obtained through the same tissue culture and regeneration process,
203 demonstrating that the differences in root colonisation may be ascribed to the transformation
204 technology [51].

205 Defensins are antimicrobial proteins able to inhibit the growth of phytopathogenic fungi
206 by reducing hyphal elongation through specific binding to sphingolipid sites in hyphal
207 membranes. Defensin-engineered *Solanum melongena* (aubergine) plants expressing the gene
208 for the Dm-AMP1 protein in all tissues were not affected in their ability to establish
209 mycorrhizal symbiosis by *F. mosseae*. Interestingly, the antimicrobial protein was exuded
210 from the roots into the surrounding environment, where it maintained the ability to control
211 phytopathogenic fungal growth [4]. Unfortunately, no information is available on the
212 persistence of the Dm-AMP1 protein released from transgenic roots in the rhizosphere and in
213 the nearby soil.

214 Some of the antimicrobial compounds produced by transgenic plants do not accumulate
215 in the rhizosphere, since they are degraded by proteases, as in the case of cecropin B [52], or
216 are bound to plant residues, as in the case of lysozyme [53]. However, only limited
217 information about the persistence of other, protease-resistant compounds, such as chitinases
218 and vacuolar PR proteins, is available [54]. *In vitro* assays on transgenic tobacco plants
219 expressing hen egg lysozyme detected release of the active enzyme through roots [53],
220 whereas the enzymes β-1,3-glucanase and chitinase remained bound on the root surface of
221 transgenic barley [55]. In addition, biological and chemical degradation of antimicrobial
222 proteins may be hindered by adsorption of enzymes to soil inorganic or organic colloids, as
223 reported above for Cry proteins: retention of root-bound β-1,3-glucanase and chitinase
224 activity in silty loam soil was detected, even in the presence of rhizosphere microorganisms
225 [56].

Although the available studies provided some data on the impact of transgenic plants on soil microbial communities and beneficial symbionts, the experiments performed failed to discriminate between pleiotropic effects and effects due to the transgene products. Further research should devote particular attention to other groups of non-target beneficial microorganisms and to the development of highly specific systems for phytopathogenic control.

2.3. Transgenic plants expressing antibiotic resistance genes

The production of transgenic plant varieties is generally obtained by engineering a genetic construct which includes not only the gene of interest and the relevant promoter for protein constitutive expression, but also an antibiotic resistant gene. Such a gene is introduced exclusively for technical reasons, as it represents an optimal selectable marker, allowing easy detection of transformed cells incorporating the transgenes. One of the most widely used antibiotic resistance genes is *nptII*, deriving from a bacterial transposon (Tn5 from *Escherichia coli*) whose product inactivates aminoglycoside antibiotics, such as kanamycin and neomycin. Other antibiotic markers have been utilised, often in combination: as an example, a cassette containing *nptII*, *Gent* and *Tet* genes, conferring resistance to neomycine, gentamycin and tetracycline antibiotics, has been used to develop papaya plants resistant to Papaya ringspot virus (PRSV) infection.

The release of antibiotic resistance genes in the field and in the soil by transgenic crops raised concerns on the possibility of their uptake by native soil bacteria through horizontal gene transfer (HGT), a fundamental mechanism of genetic recombination and evolution in many bacterial species. In particular, the rhizosphere represents a “hot spot” for bacterial recombination, given its high nutrient content and water/exudate fluxes, as compared with

251 bulk soil. For example, a high transfer frequency of plasmids with antibiotic resistance has
252 been reported to occur in wheat rhizosphere [57]. In such a peculiar ecological niche, various
253 events of HGT occurring by conjugation, transformation and transduction have been
254 described, including the acquisition of plant-derived genes by rhizosphere bacteria [58, 59].
255 Transformation was found to be active in the transfer of kanamycin resistant genes from
256 transgenic plant DNA to the rhizosphere bacterium *Acinetobacter* sp. in soil microcosms [60,
257 61], confirming previous data on the possibility of bacterial natural transformation [62].

258 The possible spread of antibiotic resistance genes in agricultural and natural ecosystems
259 through competent soil bacteria carrying homologous sequences and their HGT represents a
260 potential risk to be taken into account when assessing the environmental impact of GMPs. For
261 this reason the World Health Organisation recommended the development of new
262 technologies to obtain GMPs while avoiding the use of antibiotic resistance genes [9]. Indeed,
263 some transgenic events have been developed using, as a selection marker, the
264 phosphinothricin N-acetyltransferase (PAT) enzyme that confers resistance to herbicidal
265 activity of glufosinate. GMPs could also be produced by removing antibiotic resistance
266 selection markers, using either co-transformation followed by the segregation of relevant
267 genes or site-specific recombination with excision of marker genes [63]. Other promising
268 strategies involve the use of positive selection markers based on hormone, saccharide and
269 aminoacid metabolism [64] or of modified tubulin genes [65].

270

271 *2.4. Herbicide tolerant transgenic plants*

272

273 Many crop species, including the widely cultivated beet (*Beta vulgaris* L.), maize (*Zea*
274 *mays* L.) and oilseed rape (canola) (*Brassica napus* L.), have been genetically engineered to
275 express bacterial genes that confer herbicide tolerance. The aim of this technology is to

276 improve pre-emergence and post-emergence control of many different weeds by using
277 herbicides (e.g., glyphosate, glufosinate-ammonium) without harming the cultivated crops. So
278 far, contrasting effects have been described on the composition and diversity of soil and
279 microbial communities living in the rhizosphere of herbicide tolerant (HT) *B. napus,* maize
280 and soybean [66-72] (Table 1). Interestingly, pleiotropic effects of genetic transformation
281 were reported for glyphosate-resistant (GR) soybean, which exuded higher amounts of
282 carbohydrates and amino acids at a rate higher than non transgenic plants. Such exudates
283 could significantly enhance the growth of *Fusarium* regardless of glyphosate treatment [73].
284 Moreover, significant effects of GR crops and the relevant herbicides were observed for some
285 important functional groups of microbes, such as nitrogen fixing bacteria, pseudomonads, and
286 rhizobacteria [71].

287 As the cultivation of such plants entails the use of herbicides, such as glyphosate, which
288 inhibits key enzymes occurring not only in plants, but also in fungi and bacteria [71], further
289 work should focus on the effects of HT plant cultivation on microbial groups (e.g.,
290 mycorrhizal fungi, antagonists, nitrogen fixing bacteria), either associated with the rate and
291 time of herbicide application or linked to the relevant management practices, eg. altered
292 rotations, land use, tillage system.

293

294 *2.5. Transgenic plants modified to express different traits for the benefit of the food industry*
295 *and consumers*

296

297 Certain plant species have been engineered with the aim of modifying a range of
298 properties in order to reduce processing costs and offer greater benefit for consumers.
299 Examples include potatoes, which have been genetically modified to contain more starch and
300 accumulate less sugar, thus reducing processing costs, or tomatoes, which have been modified

301 to increase their shelf life by delaying ripening; the content and type of sugars and starch has
302 been modified in a number of target crops, to obtain more uniform starches, or starches with
303 altered branching or degree of polymerisation. Only a few studies investigated the impact of
304 such GMPs on soil microbes.

305 Transgenic potato plants with altered starch content affected ammonia oxidizer
306 communities [74], rhizosphere bacteria [75, 76] and mycorrhizal fungi [77]. As other studies
307 carried out at earlier growth development did not find any differences in fungal biomass and
308 plant exudation [78], further works should consider plant growth stages when assessing
309 GMPs impact, by using time-course assessments. In addition, since soil showed a short-term
310 ability to restore the original rhizosphere and rhizoplane communities in transgenic tobacco
311 [79], revealing a high potentiality to act as a strong buffering agent, different soil types should
312 be included in the analysis of GMPs effects in the years to come.

313

314 **3. Concluding remarks**

315

316 The available experimental data investigating the effects of transgenic plants on the
317 different components of the soil microbiota, have generally overlooked the natural variability
318 which may occur among different varieties of crop plants, as they evaluated the potential
319 impact on soil microbial communities comparing transgenic with parental lines. This
320 approach is scientifically sound, but it fails to answer to the interesting question as to whether
321 the effects of a specific transgenic crop are clearly beyond the differences that would be found
322 between a range of conventional cultivars. Interestingly, a recent quantitative assessment of
323 soil functioning was devised to detect the natural variation (or normal operating range, NOR)
324 of soil function, allowing the discrimination of soil critical parameters. Such a method could
325 be used to understand the relevance of changes induced by GMPs [80]. On the other hand,

326 scarce information is available on microbiological changes resulting from agricultural
327 practices inherent to transgenic crops, such as broad spectrum herbicide applications rates and
328 timing, altered rotations and production schemes, land-use forms and tillage systems, which
329 may affect belowground microbial biodiversity and food webs. Accordingly, such questions
330 should be tackled in future assessments, in order to achieve a better understanding of the
331 realistic effects of transgenic crops on soil microbes.

332 The development of effective and integrated methodologies for the assessment of the
333 impact of transgenic crops on soil microorganisms remains a major scientific challenge. New
334 data should be produced using adequately designed and standardised tests, sampling methods
335 and statistical analyses, not only in short-term, small-scale laboratory or glasshouse
336 experiments, but also in long-term systematic and continued field trials, during and after crop
337 removal. Special attention should be paid to the monitoring of key and sensitive microbial
338 functional groups fundamental for soil fertility and plant nutrition and governing the most
339 important soil ecological functions, e.g. nitrification, nitrogen fixation, phosphate
340 mobilization, organic carbon cycling and sink. Finally, further studies should be focused not
341 only on punctiform effects on single organisms, but also on all the other possible outcomes,
342 such as combinatorial and cumulative effects which characterise the complex network of
343 interactions ruling soil life.

344

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346

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349

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570 **FIGURE LEGENDS**

571

572 **Figure 1**. Schematic drawing representing direct and indirect impact of transgenic crops
573 on soil microbial communities and microbe-mediated processes and functions. Red: sources
574 of potential impact; blue: microbe-mediated processes; black: soil microbial functional
575 groups. PGP, Plant Growth Promoters; HGT, Horizontal Gene Transfer.

576 **Figure 2**. Light micrographs showing fungal development in transgenic and non
577 transgenic maize plants (*Zea mays*) during the different stages of mycorrhizal symbiosis
578 establishment. (a, b) Appressoria developed by *Funneliformis mosseae* on *Bt* 176 maize roots
579 producing infection pegs which become septate and devoid of protoplasm (arrows). (a) Scale
580 bar = 35 µm; (b) Scale bar = 25 µm. (c) *F. mosseae* appressorium and entry point successfully
581 colonising a non transgenic maize root. Scale bar = 50 µm. (d) Mycorrhizal colonisation of a
582 non transgenic maize root by *F. mosseae*. Scale bar = 80 µm.

583

584

Table 1

Table1. Pleiotropic (P) and undetermined (pleiotropic or direct, U) effects of transgenes expressed in different plant species on soil microorganims, as revealed by culture-dependent and independent methods. Consistent (+), transient (+/-) and no effects (-) are reported.

Protein or *gene*	**Plant**	**Impact occurrence**	**Methods**	**Organism**	**References**
			Expression of *Bt* toxins		
Cry1Ab	corn	+ (U)	ARISA[a]	Rhizosphere bacterial community	[24]
		+ (U)	DGGE	Rhizosphere bacterial community	[25]
		+ (U)	AMF colonization	Glomeromycota	[25, 32], [1s[b], 2s]
		+ (U)	AMF spore count	Glomeromycota	[35]
		+ (U)	Soil respiration	Soil and rhizosphere bacterial communities	[24], [3s]
		+ (U)	Quantitative PCR	Ammonia oxidizing archaea and bacteria, nitrogen fixing bacteria	[23]
		+ (U)	Enzymes activities, microbial biomass,CLPP	Soil microbial communities	[37]
		+ (U)	N e C cycle activities, DGGE	Rhizosphere microbial communities	[26]
		+/- (U)	CLPP, plate counts, soil respiration	Soil bacterial communities	[4s]
		+/- (P)	Fungal growth measurement	*Fusarium graminearum* and *Trichoderma atroviride*	[29]
		+/- (U)	plate counts	Rhizosphere and soil culturable bacterial and fungal communities or specific functional bacterial groups	[15, 21]
		+/- (U)	DGGE	Soil bacterial community	[15]
		-	plate counts	Rhizosphere bacterial and fungal community	[19, 24, 27], [5s]
		-	CLCP, CLPP	Rhizosphere bacterial and fungal community	[24], [6s, 7s]
		-	DGGE	Rhizosphere and soil bacterial and AMF communities	[6s, 8s, 9s]
		-	DGGE	Bacterial communities	[23], [10s]
		-	PFLA	Rhizosphere and soil bacterial communities	[7s, 11s]
		-	microarrays	*Bacillus subtilis* and *Streptomyces coelicolor*-related bacteria	[12s]
		-	SSCP	Rhizosphere bacterial community	[13]

		-	454 pyrosequencing, T-RFLP	Fungi	[13s]
		-	T-RFLP, cloning and sequencing	Glomeromycota	[34]
		-	454 pyrosequencing	Rhizobacterial communities	[20]
		-	Soil respiration	Soil microbial communities	[14s, 15s]
		-	Soil enzymatic activities	Rhizosphere microbial communities	[15, 21]
		-	Mineralizable C, total soil C and N, lignin	Rhizosphere microbial communities	[14s, 16s, 17s]
		-	AMF colonization	Glomeromycota	[33], [18s]
Cry1Ab	cotton	+ (U)	Soil enzymatic activities	Soil bacterial communities	[19s]
		+/- (P)	PCR-RFLP	Rhizosphere bacterial and fungal communities	[16]
		+/- (P)	Plate counts	Soil bacterial and fungal communities	[16]
		-	Soil enzymatic activities	Soil bacterial communities	[20s]
		-	CLPP	Soil microbial community, specific functional bacterial groups	[27], [20s, 21s]
		-	Plate counts	Soil microbial communities	[22s]
Cry1Ab	rice	+ (U)	T-RFLP	Fungal communities	[23s]
		+ (U)	RNA-SIP, clone libraries	Rhizosphere methanogenic archaeal communities	[24s]
		+/- (U)	plate counts	Aerobic bacteria, actinomycetes and fungi	[25s]
		+/- (U)	Soil enzymatic activities	Soil bacterial communities	[25s, 26s]
		-	TGGE	Soil bacterial communities	[26s]
		-	T-RFLP	Soil bacterial and fungal communities	[27s]
		-	PLFA	Rhizosphere bacterial communities	[28s]
Cry1Ab/ CrylAc	rice	+ (U)	DGGE, quantitative PCR	Methanogenic archaeal and methanotrophic bacterial communities	[29s]
Cry1A.105	corn	-	T-RFLP	Bacterial endophytes	[30s]
Cry1Ac	brinjal	+/-	Quantitative PCR, biomass, RFLP	Microbial communities	[31s]
CrylAc	cotton	+ (U)	Soil enzymatic activities	Soil bacterial communities	[19s, 32s]
		+/- (P)	PCR-RFLP	Soil bacterial and fungal communities	[16]
		+/- (P)	Plate counts	Soil bacterial and fungal communities	[16]

		-	AMF colonization	Glomeromycota	[33s]
	rice	+/- (U)	CLPP, DGGE	Rhizosphere microbial communities	[34s]
		-	Enzymatic activities	Rhizosphere microbial communities	[34s]
Cry1Ac	turnip	-	DGGE	Rhizosphere bacterial community	[35s]
Cry1F	corn	+ (U)	AMF colonization	Glomeromycota	[32]
		+ (U)	AMF spore count	Glomeromycota	[35]
		+ (U)	Quantitative PCR	Ammonia oxidizing archaea and bacteria, nitrogen fixing bacteria	[23]
		-	CLPP, plate counts	Rhizosphere bacterial community	[11s]
		-	DGGE	Bacterial communities	[23], [10s]
		-	AMF colonization	Glomeromycota	[33]
Cry2Ab2	corn	-	T-RFLP	Bacterial endophytes	[30s]
Cry2Ab	cotton	-	AMF colonization	Glomeromycota	[33s]
Cry3Bb1	corn	+ (U)	AMF colonization	Glomeromycota	[32]
		+ (U)	AMF spore count	Glomeromycota	[35]
		+/- (U)	plate counts	Rhizosphere and soil culturable bacterial and fungal communities or specific functional bacterial groups	[15, 21]
		-	T-RFLP	Soil decomposer community	[22]
		-	ARISA, DGGE, microarrays, SSCP, T-RFLP	Rhizosphere and soil bacterial community or specific functional bacterial groups or bacterial endophytes	[15], [30s, 36s, 37s]
		-	AMF colonization	Glomeromycota	[33]
		-	N-mineralization	Soil microbial communities	[36s]
		-	Soil enzymatic activities, soil respiration	Soil microbial communities	[15]
		-	Soil enzymatic activities, lignin content	Rhizosphere microbial communities	[38s]
Cry34/35Ab1	corn	+ (U)	AMF colonization	Glomeromycota	[32]
		+ (U)	AMF spore count	Glomeromycota	[35]
		-	AMF colonization	Glomeromycota	[33]
			Resistance to pathogens other than insects		
Agglutinin	potato	+/- (P)	Soil enzymatic activities	Rhizosphere microbial communities	[38]

		-	CLPP	Rhizosphere microbial communities	[38]
Cecropin B	potato	+/- (U)	PCR-RFLP	rhizosphere Bacillus spp.	[43]
Cecropin B/attacin		+/- (U)	T-RFLP, clone libraries, soil enzymatic activities	Rhizosphere bacterial communities	[41]
Chicken egg white cystatin	potato	+ (U)	PLFA	Rhizosphere bacterial communities	[39]
Chitinase	rice	+ (U)	AMF colonization	Glomeromycota	[45]
Chitinase	*Nicotiana sylvestris*	-	AMF colonization	Glomeromycota	[48]
Concanavalin A	potato	+/- (P)	CLPP	Soil bacterial communities	[38]
CSA synthesis genes	tobacco	+ (U)	AMF colonization	Glomeromycota	[49]
Defensin Dm-AMP1	aubergine	-	AMF colonization	Glomeromycota	[5]
ESF39A	elm	-	AMF colonization	Glomeromycota	[39s]
Lactonase AttM	tobacco	-	Plate count	Specific microbial groups	[44]
		-	DGGE	Rhizosphere bacterial communities	[44]
NahG gene	tobacco	+ (U)	AMF colonization	Glomeromycota	[49]
pm3b gene	wheat	+/- (P)	DGGE	Pseudomonads,	[51]
		+/- (P)	AMF colonization	Glomeromycota	[51]
PR-2	tobacco	+ (U)	AMF colonization	Glomeromycota	[47]
T4 lysozyme	potato	+	T-RFLP	Bacterial communities,	[42]
		+/- (U)	T-RFLP, clone libraries, enzymatic activities	Rhizosphere bacterial communities	[41]
		-	plate counts, CLCP, PCR-RFLP, DGGE	Rhizosphere bacteria,	[40s] [42]
		-	plate counts, FAME	Beneficial plant-associated bacteria	[40s-42s]
			Resistance to herbicides		
Ahas gene	soybean	-	DGGE	Soil microbial community	[43s]
Cp4-epsps gene	canola	+ (U)	CLPP	Rhizosphere bacterial communities	[44s]
		+ (U)	FAME	Rhizosphere bacterial communities	[68]; [44s]
		+/- (U)	CLPP, T-ARDRA, FAME	Rhizosphere bacterial communities	[67]
		+/- (U)	Microbial biomass, CLPP, soil	Soil bacteria	[45s]

			enzymatic activities		
Cp4-epsps gene	cotton	-	AMF colonization	Glomeromycota	[33s]
Cp4-epsps gene	corn	-	T-RFLP, quantitative PCR	Denitryfying and fungal communities	[70]
Cp4-epsps gene	soybean	+ (U)	FAME	Microbial community	[46s]
		+/- (U)	Microbial biomass, soil enzymatic activities	Microbial community	[72]
		+ (P)	Plate count and specific test	Specific microbial groups	[71]
		-	AMF colonization	Glomeromycota	[47s]
Cp4-epsps gene	wheat	+/- (U)	Microbial biomass, CLPP, soil enzymatic activities	Soil bacteria	[45s]
pat gene	canola	+ (U)	DGGE	Rhizosphere bacterial community	[69]
		+ (U)	Soil enzymatic activities	Rhizosphere bacterial communities	[48s]
		-	DGGE	Rhizosphere bacterial community	[48s]
pat gene	corn	-	SSCP	Rhizosphere bacterial communities	[66], [49s]
		+/- (U)	PFLA	Microbial community	[50s]
		-	ELFA, CLPP, basal respiration, decomposition	Rhizosphere bacterial communities	[51s]
ppo gene	rice	-	T-RFLP	Rhizosphere bacterial and fungal communities	[52s]
			Traits for the benefit of the food industry and consumers		
Alpha-amylase	alfalfa	+ (U)	ERIC-PCR	Rhizosphere bacterial community	[53s]
		+ (U)	plate counts	Rhizosphere and soil bacterial communities	[53s, 54s]
		+ (U)	CLPP, soil respiration	Soil bacterial communities	[54s]
		-	PCR-RFLP	Soil bacterial and fungal communities	[54s]
RNAi of *gbss* gene	potato	+ (U)	T-RFLP, PFLA-SIP	Rhizosphere fungal communities	[77]
		+ (U)	PFLA-SIP, clone libraries	Rhizosphere bacterial communities	[76]
		+ (U)	DGGE	Rhizosphere bacterial communities	[74, 76]
		+/- (U)	T-RFLP, soil enzymatic activities	Rhizosphere fungal communities	[55s, 56s]
		+/- (U)	DGGE	Rhizosphere bacterial communities	[75]
		-	PFLA	Rhizosphere bacterial communities	[78]
		-	T-RFLP	Fungal communities; Actinomycetales, α and β	[55s]

				Proteobacteria	
Lignin metabolism (suppressed cynnomyl alcohol dehydrogenase activity)	poplar	-	454 pyrosequencing, DGGE	Fungal communities	[57s, 58s]
lignin peroxidase	alfalfa	+ (U)	plate counts, CLPP, soil respiration	Soil bacterial communities	[54s]
		-	PCR-RFLP	Soil bacterial communities	[54s]
Mn-dep. lignin peroxidase	alfalfa	+ (U)	CLPP, ERIC-PCR	Rhizosphere bacterial communities	[53s]
malate dehydrogenase	alfalfa	+ (U)	CLPP, PCR-RFLP	Rhizosphere bacterial communities	[59s]
Lhcb1-2 genes	eucalyptus	+/- (U)	Plate counts, ARDRA, DGGE	Rhizosphere bacterial community	[60s]
Phytase	tobacco	-	T-RFLP	Surface and endophytic bacteria; Glomeromycota	[61s]

[a] ARISA (Automated Ribosomal Spacer Analysis), CLCP/CLPP (Community-Level Catabolic/Physiological Profiling), DGGE (Denaturing Gradient Gel Electrophoresis), ERIC-PCR(Enterobacterial Repetitive Intergenic Consensus Sequence-PCR), ELFA (Ester linked Fatty Acid Analysis), FAME (Fatty Acid Methyl Ester profiles), MPN (Most Probable Number), PCR-RFLP (PCR-Restriction Fragment Length Polimorphisms), PLFA(Phospholipid Fatty Acid Analysis), PFLA-SIP (Phospholipid Fatty Acid Analysis-Stable Isotope Probing, SSCP (Single Strand Conformational Polymorphisms), T-ARDRA (Terminal-Amplified Ribosomal DNA Restriction Analysis), T-RFLP (Terminal-Restriction Fragment Length Polimorphisms). [b] s (References in Supplementary Material).

Figure 1
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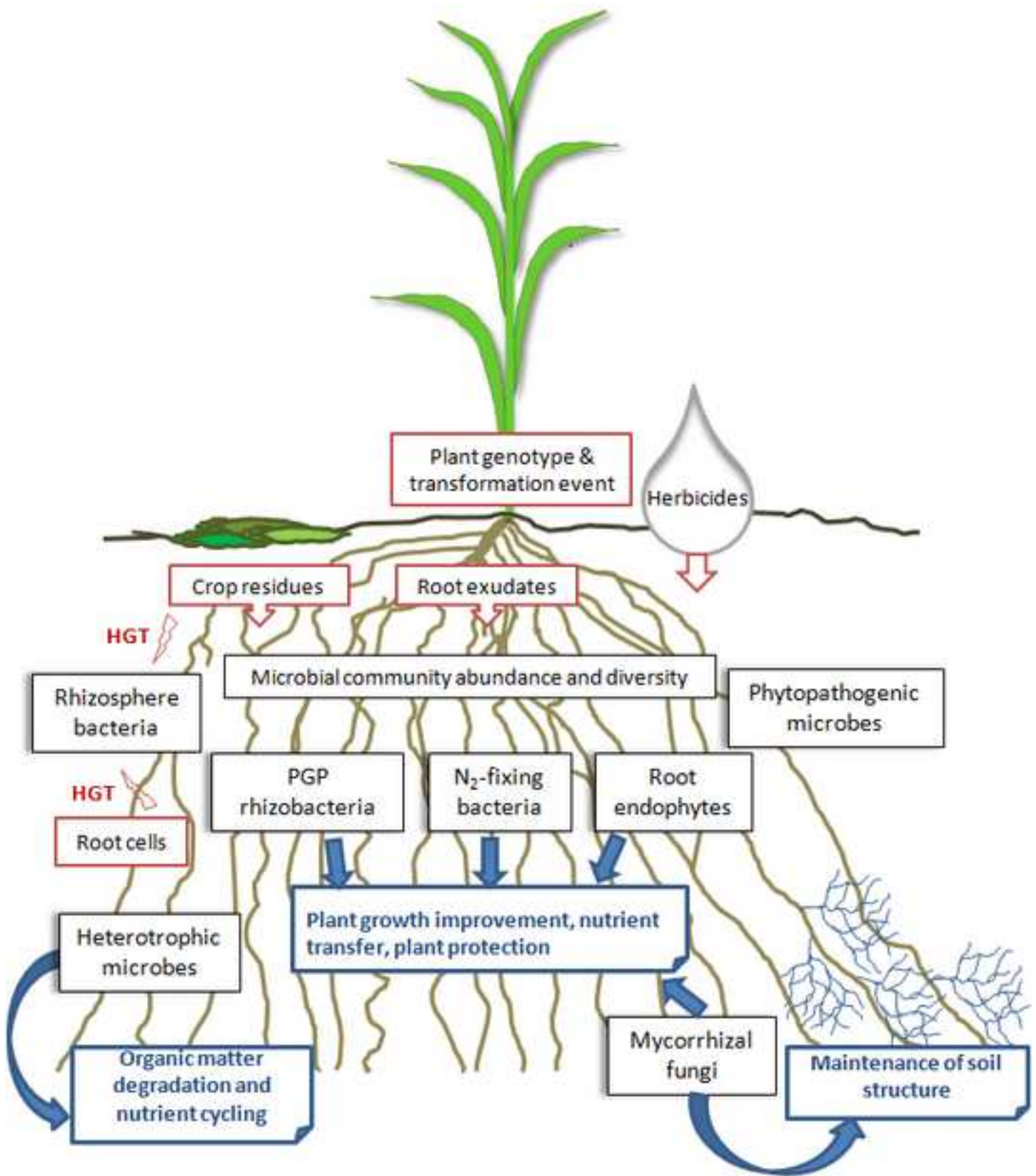

Figure 2

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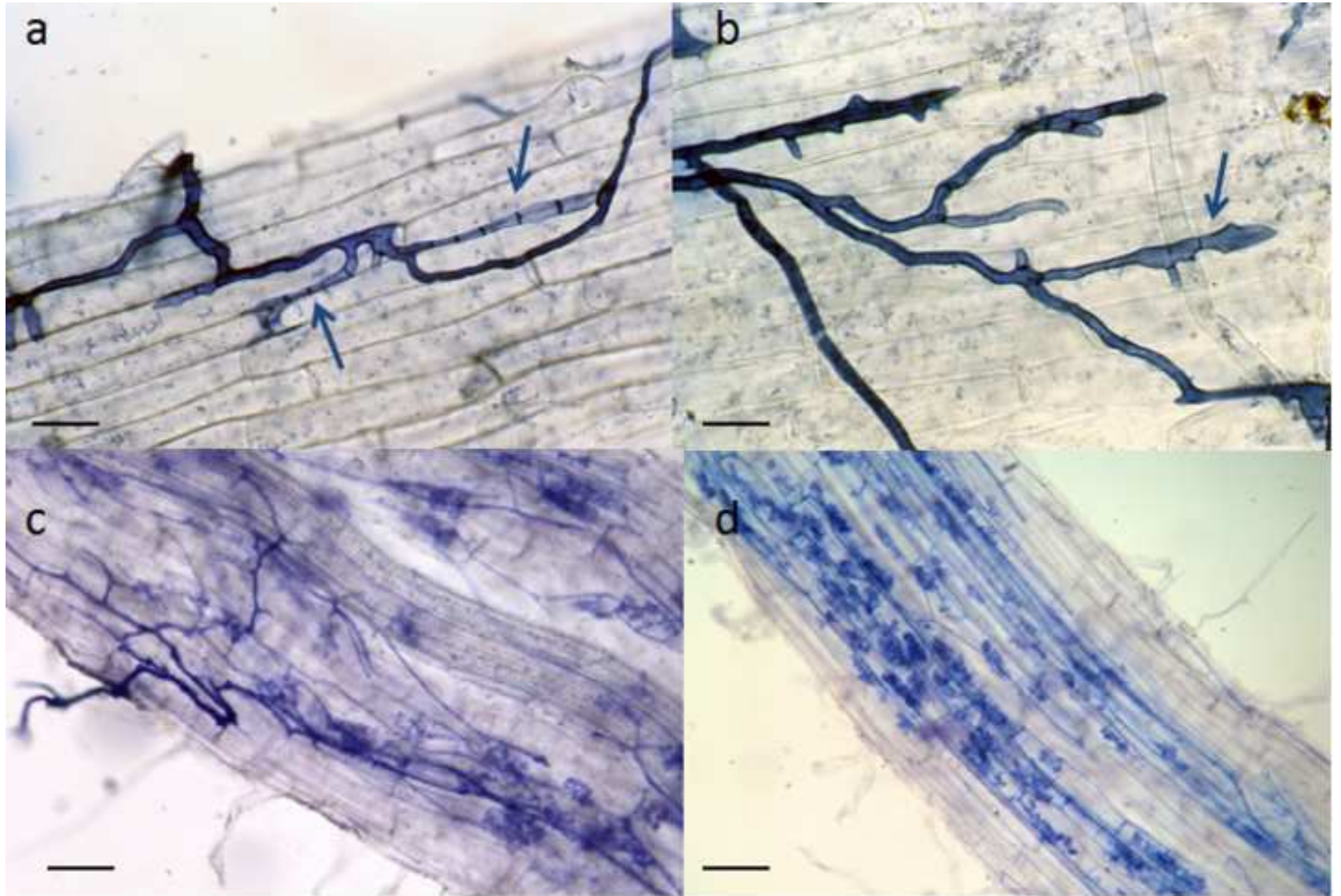

e-component

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