Annals of Botany **124**: 933–946, 2019
doi: 10.1093/aob/mcy240, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

SPECIAL ISSUE ON ROOT TRAITS BENEFITTING CROP PRODUCTION IN ENVIRONMENTS WITH LIMITED WATER AND NUTRIENT AVAILABILITY

Genetic analysis of tomato root colonization by arbuscular mycorrhizal fungi

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Received: 20 June 2018 Returned for revision: 22 October 2018 Editorial decision: 19 December 2018 Accepted: 27 December 2018
Published electronically 9 February 2019

• **Background and Aims** Arbuscular mycorrhizal fungi (AMF) play an important role in plant nutrition and protection against pests and diseases, as well as in soil structuration, nutrient cycling and, generally speaking, in sustainable agriculture, particularly under drought, salinity and low input or organic agriculture. However, little is known about the genetics of the AMF–plant association in tomato. The aim of this study was the genetic analysis of root AMF colonization in tomato via the detection of the quantitative trait loci (QTLs) involved.
• **Methods** A population of 130 recombinant inbred lines derived from the wild species *Solanum pimpinellifolium*, genotyped for 1899 segregating, non-redundant single nucleotide polymorphisms (SNPs) from the SolCAP tomato panel, was characterized for intensity, frequency and arbuscular abundance of AMF colonization to detect the QTLs involved and to analyse the genes within their peaks (2–2.6 Mbp).
• **Key Results** The three AMF colonization parameters were highly correlated (0.78–0.97) and the best one, with the highest heritability (0.23), corresponded to colonization intensity. A total of eight QTLs in chromosomes 1, 3, 4, 5, 6, 8, 9 and 10 were detected. Seven of them simultaneously affected intensity and arbuscule abundance. The allele increasing the expression of the trait usually came from the wild parent in accordance with the parental means, and several epistatic interactions were found relevant for breeding purposes. SlCCaMK and SlLYK13 were found among the candidate genes. Carbohydrate transmembrane transporter activity, lipid metabolism and transport, metabolic processes related to nitrogen and phosphate-containing compounds, regulation of carbohydrates, and other biological processes involved in the plant defence were found to be over-represented within the QTL peaks.
• **Conclusions** Intensity is genetically the best morphological measure of tomato root AMF colonization. Wild alleles can improve AMF colonization, and the gene contents of AMF colonization QTLs might be important for explaining the establishment and functioning of the AMF–plant symbiosis.

Key words: AMF colonization, heritability, QTL analysis, candidate genes, SolCAP SNPs, *Solanum pimpinellifolium*, *S. lycopersicum*, biological process, underdominance, epistasis.

INTRODUCTION

About 177 Mt of fresh tomato fruits (*Solanum lycopersicum*) are produced yearly on 4.78 Mha in 144 countries, making tomato the second most important vegetable crop next to potato (FAOSTAT Database, 2016; http://www.fao.org/faostat/en/#data/QC). Tomato belongs to the *Solanaceae* family, genus *Solanum*. Its genome, with a predicted size of approx. 900 Mb and a chromosome number of $2n = 24$ has been fully sequenced (Tomato Genome Consortium, 2012).

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that form symbiotic associations with the vast majority of land plants (Smith and Read, 2008). These root symbionts provide the plant with mineral nutrients [in particular, phosphorus (P)] scavenged in the soil in exchange for photosynthates (i.e. sugars and lipids; Keymer *et al.*, 2017) provided by the plants. Carbon–mineral exchanges between host and fungus occur via highly branched fungal structures inside cortical root cells known as arbuscules (Parniske, 2008).

Recent studies have investigated mineral nutrient transport and signal exchange between AMF and host plants at the genetic and molecular level (Bravo *et al.*, 2016). Molecular dialogue between the two partners of the symbiosis is partially understood (Gobbato, 2015). Paszkowski (2006) described the pre-symbiotic responses that prepare the symbionts for a successful association as the 'anticipation programme'. An AMF diffusible signal (i.e. the Myc factor), inducing the expression of a plant gene *MtEnod11*, was detected in the model plant *Medicago truncatula* (Kosuta *et al.*, 2003). This fungal signal is a complex of lipochitooligosaccharides (Myc-LCOs) close to the Nod factors in the *Rhizobium*–legume association. Reciprocally, colonization by the AMF responds to strigolactones that are released by the plant and stimulate fungal metabolism and extraradical hyphal branching (Buee *et al.*, 2000; Gomez-Roldan, *et al.*, 2008; Parniske, 2008; Gobbato, 2015). Histologically, plants form a pre-penetration apparatus that allows intercellular colonization of the cortex (Genre *et al.*, 2008)

and, finally, the arbuscules developed in cortical cells are surrounded by a plant plasma membrane-derived periarbuscular membrane (Gutjahr and Parnike, 2013). Therefore, the process of root colonization by AMF is not simple, and has a strong impact on the metabolic profile on the tomato roots (Rivero *et al*., 2015).

The effect of AMF on tomato growth, yield and resistance to biotic and abiotic stresses has been reported in a number of studies. Increased nutrient acquisition under salt (Al-Karaki, 2000; Al-Karaki *et al.*, 2001) and drought stress (Chitarra *et al.*, 2016; Ruiz-Lozano *et al*., 2016) as well as increased metabolic plasticity of mycorrhized tomato plants to cope with both types of stresses (Rivero *et al.*, 2018) were demonstrated. Improved tomato yield and faster berry ripening were also noticed following AMF inoculation (Bowles *et al.*, 2016; Chialva *et al.*, 2016). Finally, the resistance of AMF-colonized tomato plants against diseases caused by, for instance, *Phytophthora parasitica* (Cordier *et al.*, 1998) via mechanisms such as priming (Song *et al.*, 2015) and the role of soil microbiota in eliciting a 'state of alert' in tomato plants challenged by a fungal pathogen (Chialva *et al.*, 2018) were also reported. Thus, the level of root colonization is part of a general approach considered in breeding programmes to develop plants able to select particular beneficial microbes from a large community that includes many members without beneficial function or for broad microbiome characteristics (e.g. the microbes that promote plant health) (Bakker *et al.*, 2012; Schlaeppi and Bulgarelli, 2015). However, several studies (reviewed by Hohmann and Messer, 2017) found no correlation between the response (defined as the difference in performance between colonized and non-colonized plants) of the AMF-colonized plant and its percentage of AMF-colonized root. Therefore, knowledge of the genetics of AMF root colonization is of great interest as a first step in this field to compare among species.

Since AMF could be responsible for plant growth reduction in cultivation systems where nutrients are not limiting (i.e. in current fertilized soils where the cost of maintaining the symbiosis exceeds the benefit to the host), it may be logical that breeding under adequate fertilizer levels has selected for less AMF-responsive genotypes (Kaeppler *et al.*, 2000). It is noteworthy that Martín-Robles *et al.* (2018) found that, in general, domestication reduced mycorrhizal benefits for crops (including tomato) under high P supply. Beyond domestication, decades of varietal selection based mostly on yield criteria have led to a limited repertoire of crops used intensively, as new varieties correspond to mass agricultural production practices. The comparison of seed varieties sold by commercial US seed houses in 1903 with those sold in 1983 (Rural Advancement Foundation International; https://rafiusa.org/issues/seeds/) highlighted the suppression of about 93 % of the varieties of 66 crops included in the study, reducing the range of tomatoes, for instance, from 408 to 79. Interestingly, in wheat, previous results have demonstrated that old varieties developed before 1900 showed a higher mycorrhizal response than varieties developed more recently (Hetrick *et al.*, 1992). This brings the challenge of broadening the genetic variation regarding the AMF–plant interaction in breeding programmes to develop cultivars (or rootstocks) highly responsive to AMF, particularly for lower input agriculture, and including AMF-mediated disease resistance as suggested by Hohmann and Messemer (2017).

The improvement of crop yield has been possible through the indirect manipulation of quantitative trait loci (QTLs) that control heritable variability of the traits and physiological mechanisms that determine biomass production and its partitioning (Collins *et al.*, 2008). Although AMF symbiosis shows little host specificity (Parniske, 2008), root colonization and plant response may vary widely between plant species (Klironomos, 2003) and within species amongst cultivars (Declerck *et al.*, 1995; Tawaraya, 2001). Genetic variation has been reported among maize inbred lines (Kaeppler, 2000), wheat (Lehnert *et al.*, 2017) and poplar (Labbè *et al.*, 2011), leading to the detection of genetic factors or QTLs controlling root AMF colonization in these studies, but none in a panel of Western Africa sorghum genotypes (Leiser *et al.*, 2016).

The aim of this work was the genetic analysis of root colonization by AMF (*Rhizophagus irregularis* MUCL 41833) in tomato making use of a recombinant inbred line (RIL) population derived from the wild species *Solanum pimpinellifolium.* Specifically, the objectives were (1) to estimate the proportion of genetic variation (heritability) of the main AMF root colonization parameters in this population; (2) to investigate these colonization parameters by QTL analysis; and (3) to study candidate genes within detected AMF colonization QTLs.

MATERIALS AND METHODS

Biological material

Tomato. A total of 130 F_{10} lines (P population) derived by single seed descent from the hybrid between a salt-sensitive genotype of *S. lycopersicum* 'Cerasiforme' (E9) and a salt-tolerant line from *S. pimpinellifolium* L. (L5) (Monforte *et al.*, 1997) were used in the experiment including the parental lines as reference genotypes. The seeds were surface-disinfected for 10 min in a solution of H_2O_2 (8° active chloride) then rinsed with sterile (121 °C for 15 min) deionized water (three consecutive baths of 5 min each) and placed on wet paper (Whatman™) in Petri plates (Greiner Bioone GmbH, Germany) before incubation in the dark at room temperature (approx. 20 °C). Germination occurred within 7 d.

Arbuscular mycorrhizal fungi. Rhizophagus irregularis (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler comb. nov. MUCL 41833 was provided by INOQ GmbH (Schnega, Germany) after it was mass-produced from a starter culture supplied by the Glomeromycota *in vitro* collection (GINCO; http://www.mycorrhiza.be/ginco-bel). Inoculum consisted of spores, root fragments containing intraradical spores/vesicles and hyphae. According to the most probable number method (Porter, 1979), 37 000 infective mycorrhizal propagules per litre were enumerated.

Experimental design

Seven-day-old pre-germinated tomato seeds were transferred to a greenhouse for the experiment. Plants were grown

in 1.5 L plastic pots (Modiform, The Netherlands) containing a mix of sand with a granulometry of 0.4–0.8 mm (Euroquartz, Belgium) and vermiculite (Biobest, Belgium) in a 2:1 volume. A 20 mL aliquot of AMF inoculum was applied to each pot directly below the seedling. The experiment was initiated in two contiguous greenhouse chambers (for reasons of space) at the Katholiek Universiteit Leuven (Leuven, Belgium). The pots were arranged in a fully randomized design. Each of the 130 lines and parents was replicated six times. Replicates for each genotype were evenly distributed between the two chambers. Watering was conducted using an individual water dripping system with modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) decreased in P (i.e. 90 % P-impoverished solution; P = 6.245 mg L^{-1}). The Hoagland's modified solution (pH 5.6 ± 0.2) consisted of deionized water with minerals at the following concentrations (mg L^{-1}): NH_4NO_3, 80; $Ca(NO_3)_2{\cdot}4H_2O$, 826; KNO_3, 357; KCl, 45.1; K_2SO_4 105.4; KNO_3, 50; KH_2PO_4, 27.4; $MgSO_4$, 120.4; $MnSO_4{\cdot}H_2O$, 0.5; H_3BO_3, 1.4; $CuSO_4{\cdot}5H_2O$, 0.2; $(NH_4)_6Mo7O_2{\cdot}4H_2O$, 0.1; $ZnSO_4{\cdot}7H_2O$, 0.6; and Fe-EDTA, 19.

Plants were grown under greenhouse conditions at 25 °C/18 °C (day/night), a relative humidity of 65 %, a photoperiod of 16 h d^{-1} and a photosynthetic photon flux density of 120 µmol m^{-2} s^{-1}.

Root colonization

After a growth period of 8 weeks, the root system was harvested to determine the AMF colonization following staining with the ink–vinegar method of Vierheilig *et al.* (1998). The roots were chopped in fragments of approx. 1 cm. A 25 mL aliquot of a solution of KOH (10 %) was added to the roots in a Falcon tube before incubation at 70 °C in a water bath for 1 h. The KOH solution was then removed and the roots were washed with a solution of HCl (1 %). Then 25 mL of 2 % ink (Parker blue ink, USA) in a solution of HCl (1 %) was used to stain the roots. The root samples were incubated in a water bath (70 °C) for 1 h, rinsed and stored in deionized water before being observed under a dissecting microscope (Olympus BH2-RFCA, Japan) at ×200 magnification. Frequency (F%), intensity (M%) and abundance of arbuscules (A%) were evaluated following the method of Trouvelot *et al.* (1986). Thirty root fragments (1 cm long) of each root system (i.e. plant) and six plants (i.e. replicates) per genotype were considered to estimate genotypic means. The frequency of root colonization (F%) is the percentage of root fragments that contains either hyphae, arbuscules or vesicles/spores. The intensity of root colonization (M%) is the abundance of hyphae, arbuscules or vesicles/spores in the root system, while the arbuscule abundance (A%) is the percentage of arbuscules in the root system.

Molecular marker and QTL analysis

Broad-sense heritability (H^2) was calculated for the three measures of root AMF colonization (F%, M% and A%) in the RIL population assuming that the individuals from the ninth self-pollinated generation were nearly homozygous for all loci. Heritability was calculated as reported previously by Villalta *et al.* (2007), using the formula: $H^2 = V_g/(V_g + V_e)$ where V_g and V_e are the estimates of genotype and environmental variance, respectively, by restricted maximum likelihood (REML). These estimates were obtained by considering genotypes as random effects.

Genotypes from the recombinant population (130 P-RILs) at F_{10} were genotyped for 7720 single nucleotide polymorphisms (SNPs) from the SolCAP tomato panel (Illumina BeadXhip WG-401–1004), and 4370 of them were segregating. A linkage map based on 1899 non-redundant SolCAP SNPs, covering 1326.37 cM of genetic length, was used for QTL analysis (Asins *et al.*, 2015).

Quantitative trait locus analysis of root AMF colonization traits (F%, M% and A%) was carried out using interval mapping (IM), multiple QTL mapping (MQM) and Kruskal–Wallis (KW) procedures in MapQTL® 6 (Van Ooijen, 2009). A 5 % experiment-wise significance level controlling for dependent markers was assessed by permutation tests. These LOD (logarithm of odds) critical values ranged from 1.9 to 2.3 depending on the trait and chromosome. Significant QTLs were named as AMF_Col followed by the number of the chromosome where they are located.

A two-way analysis of variance (ANOVA) was used to study the interaction (epistasis) between markers corresponding to QTLs controlling the traits (M%, A% and F%), and the co-factors used for their MQM analyses.

Genes covering between 2 and 2.6 Mbp around the SNP(s) showing a maximum LOD score at each QTL governing AMF colonization traits were downloaded from the Sol Genomics Network (https://solgenomics.net/) and studied for the presence of frameshift InDels (insertions/deletions) in the parental genomes using data reported by Kevei *et al.* (2015). Root expression of candidate genes was inferred from the Heinz cultivar using the tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi?dataSource=Rose_Lab_Atlas_Renormalized). Over-representation tests (released 3 February 2018) of all downloaded genes, or only mutated genes, within the QTLs were carried out by means of the PANTHER Classification System (http://www.pantherdb.org/) (Mi *et al.*, 2013, 2016) using Fisher's exact test with a false discovery rate (FDR) multiple test correction or the Binomial test, respectively.

RESULTS

All the tomato plants were colonized by the AMF, and no statistically significant difference was detected between the parents of the RIL population. Typical fungal structures (i.e. hyphae, vesicles/spores and arbuscules) were detected within the roots. However, root colonization differed widely among the lines (Fig. 1). The vast majority of RILs (95 %) showed an F% comprised between 40 and 90 %, while only 2 and 3 % of the RILs showed a frequency of colonization below 40 % and above 90 %, respectively. The F% of parental lines E9 and L5 was 68.8 ± 7.2 % and 59.2 ± 14.5 %, respectively. Within the RIL population, the mean F% was 67.6 %, ranging from 15.3 to 92.2 %. Intensity of root colonization (M%) varied between 2.3 and 50.8 %, and 73.84% of the RILs had an M% comprised between 10 and 30 %. The M% of parental lines was 15.0 ±3.7 % and

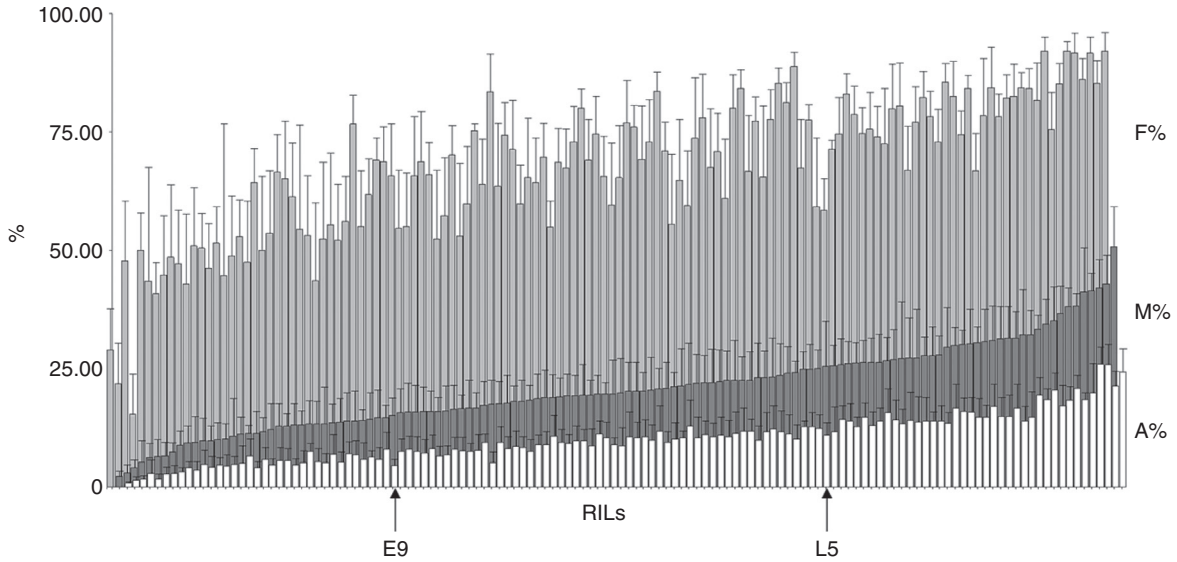

FIG. 1. Recombinant inbred lines and parents, E9 and L5 (genotypes/RILs on the *x*-axis), ordered by their means (% on the *y*-axis) for M%. Means and standard errors of each genotype for A% and F% are also shown. The position of parents E9 and L5 is indicated.

25.4 ± 9.6 % for line E9 and line L5, respectively. According to arbuscule abundance (A%), 86.15 % of RILs showed an A% below 15 %. A% ranged from 0.7 to 25.9%. The A% of parental lines E9 and L5 was 7.5 ± 2.6 % and 11.7 ± 5.2 %, respectively. F%, M% and A% were significantly correlated ($P < 0.0001$), and the most similar AMF colonization traits were M% and A% ($r = 0.97$) while the most different ones were F% and A% ($r = 0.78$). Regarding genetic variation, the highest broad-sense heritability corresponded to M% (0.2351). Heritability estimates for A% and F% were 0.2043 and 0.1921, respectively.

In total, eight QTLs were detected for the AMF colonization traits (Table 1; Supplementary Data Fig. S1). Five of them in chromosomes 1, 3, 5, 9 and 10 were significant by both procedures (MQM and KW). The most relevant one, governing the three traits and explaining 8.7 % of total variance for M%, is AMF_Col_10. AMF colonization QTLs generally corresponded to both A% and M%. Only two QTLs were detected for F%, in chromosomes 10 (also affecting M% and A%); and 6, AMF_Col_6 affecting exclusively F%. QTLs on chromosomes 6 and 8 were not significant by KW but appeared involved in significant epistasis like AMF_Col_3 (Fig. 2). The wild allele showed increasing AMF colonization intensity at five out of the eight QTLs, and underdominace was observed at AMF_Col_9 and AMF_Col_3 (Fig. 3).

TABLE 1. *List of QTLs that were detected by using the MQM procedure and corresponding SNPs (SolCAP SNPs named by number) at the LOD peak*

QTL	Chr	cM	SNP	Trait	LOD	*LL*	*PP*	PEV	a	Sig.	KW	NSML
AMF_Col_1	P1	103.126	43895	A%	3.01	9.813	7.206	5.3	1.304	2.3	0.050	4
				M%	3.32	20.131	15.285	5.8	2.423	2.3	0.050	
AMF_Col_3	P3	77.434	65890	A%	4.12	10.164	6.855	7.3	1.654	2.3	0.005	3
				M%	4.41	20.741	14.675	7.9	3.033	2.2	0.005	
AMF_Col_4	P4	21.29	43558	A%	4.38	6.982	10.037	7.8	–1.528	2.1	ns	1
				M%	4.47	14.978	20.438	8	–2.730	2.2	ns	
AMF_Col_5	P5	32.291	23832-	**A%**	3.77	7.106	9.913	6.7	–1.403	2.1	0.005	2
			23831	**M%**	3.77	15.230	20.186	6.7	–2.478	2.2	0.005	
AMF_Col_6	P6	95.035	54417	F%	2.33	59.464	67.251	5.7	–3.894	2.1	ns	1
AMF_Col_8	P8	53.942	48469-	A%	2.56	9.728	7.270	4.4	1.229	2.1	ns	2
			29418	M%	2.37	19.785	15.607	4.1	2.089	2.2	ns	
AMF_Col_9	P9	24.128	26692	**A%**	3.23	7.137	9.882	5.7	–1.372	2.2	0.001	2
				M%	2.21	15.722	19.693	3.8	–1.986	2.2	0.005	
AMF_Col_10	P10	48.882	30331	**A%**	3.77	7.111	9.909	6.7	–1.399	1.9	0.050	1
				M%	4.82	14.886	20.530	8.7	–2.822	2.1	0.005	
				F%	3.19	58.966	67.749	7.9	–4.392	2.0	0.010	

The 5 % experiment-wise significant LOD scores (Sig.) for each trait–linkage group combination estimated from 1000 permutation tests each are included.

Traits for which QTLs were detected by interval mapping are given in bold.

The map position (cM) of QTL peaks in the tomato chromosomes (Chr) and the means for both homozygous genotypes, *LL* and *PP*, are indicated.

The estimated additive value is a, and the percentage of explained variance, PEV. NSML is the number of SNPs associated with the maximum LOD score.

P-values of significant QTLs by the Kruskal–Wallis procedure (KW) are also indicated.

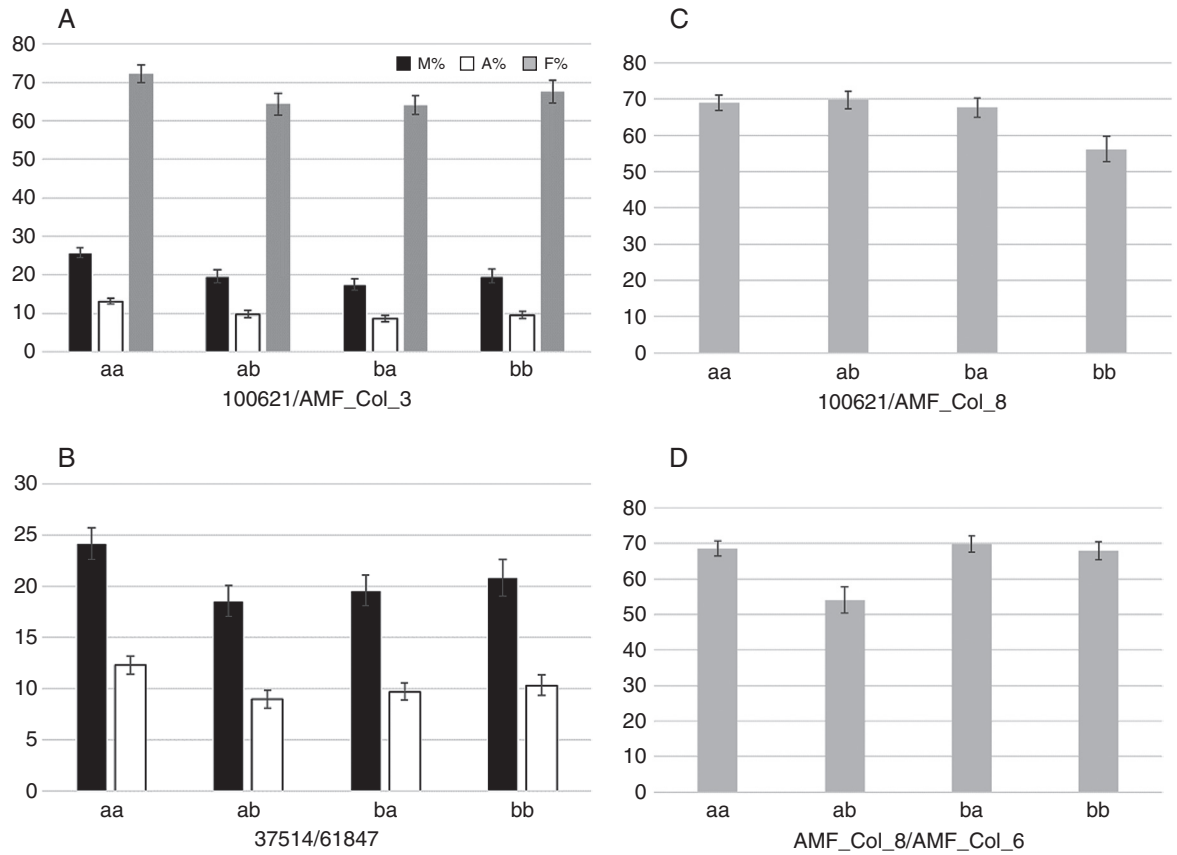

FIG. 2. Means and standard errors for significant epistatic interactions between QTLs governing AMF colonization and co-factors of MQM analysis. Homozygotes for the *lycopersicum* or the *pimpinellifolium* allele are coded as a or b, respectively. (A) Epistasis between co-factor 100621 (SolCAP SNP) and AMF_Col_3 for M% ($P \leq 0.009$), A% ($P \leq 0.019$) and F% ($P \leq 0.034$). (B) Epistasis between co-factors 37514 and 61847 for M% ($P \leq 0.032$) and A% ($P \leq 0.027$). (C) Epistasis between co-factor 100621 and AMF_Col_8 for F% ($P \leq 0.027$). (D) Epistasis between AMF_Col_8 and AMF_Col_6 for F% ($P \leq 0.022$).

The complete list of genes found around (2–2.6 Mbp) the peaks of the eight QTLs is shown in Supplementary data Table S2. From these 2062 genes/loci, only 233 showed a frame-shift mutation at one of the parental genomes (E9 or, mostly, L5). A summary list of candidate genes underlying AMF colonization intensity QTLs taking into account these mutations (enabling segregation in the RIL population), and discarding 71 cases where the protein function was unknown, is presented in Table 2. Two genes previously reported to be relevant for the establishment of the AMF–tomato symbiosis (Buendia *et al.*, 2016), SlCCaMK (Solyc01g096820) and SlLYK13 (Solyc01g0984410), were found among candidates within AMF_Col_1 and are included in Table 2.

PANTHER over-representation test using all genes in the eight QTLs (Supplementary data Table S2) provided significant results for some biological processes (Fig. 4) such as regulation of carbohydrate metabolic process and glycolysis, lipid metabolic process, nitrogen (N) compound metabolic process, nucleobase- and phosphate-containing compound metabolic processes, protein folding and targeting. These biological

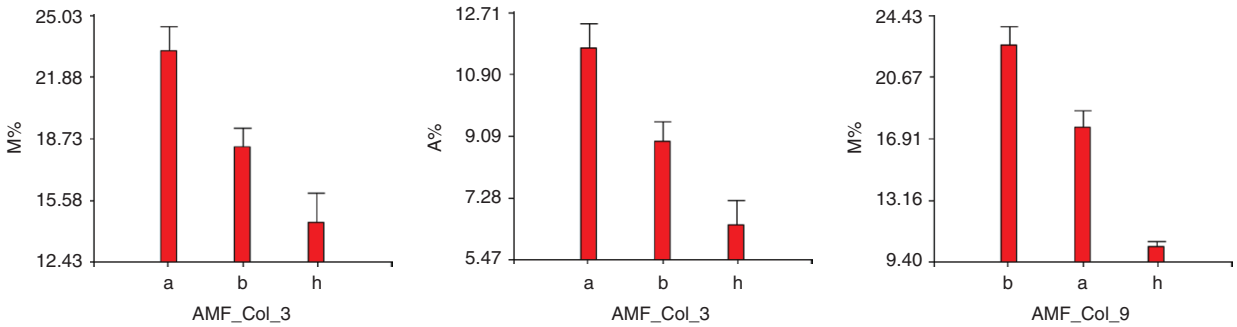

FIG. 3. Genotypic means and standard errors of underdominant QTLs. Homozygotes for the *lycopersicum* or the *pimpinellifolium* allele are coded as a or b, respectively, while h indicates the heterozygote.

TABLE 2. *Summary list of candidate genes for AMF colonization QTLs, mostly segregating for frameshift InDels from Kevei et al. (2015) in parental genomes, E9 or L5 (M.), and discarding 71 genes encoding proteins with unknown function*

QTL	Exp.	M.	Annotated gene	mRNA	Order	Reference
AMF_Col_1	Max	L5	Cysteine-type endopeptidase/ ubiquitin thiolesterase	Solyc01g096300.2.1	151	3
AMF_Col_1	VL	L5	Homeobox leucine zipper protein	Solyc01g096320.2.1	149	
AMF_Col_1	M	L5	Sigma factor-binding protein 1	Solyc01g096510.2.1	130	
AMF_Col_1	Max	L5	Peptidyl-prolyl cis-trans isomerase (FKBP)	Solyc01g096520.2.1	129	1
AMF_Col_1	Max		Calcium-dependent protein kinase 1 (SlCCaMK)	Solyc01g096820.2.1	99	8
AMF_Col_1	M	L5	Ankyrin repeat protein-like	Solyc01g097200.1.1	61	7, 3
AMF_Col_1	M	L5	Ankyrin-like protein	Solyc01g097210.2.1	60	7, 3
AMF_Col_1	M	L5	Ankyrin repeat protein-like	Solyc01g097220.1.1	59	7, 3
AMF_Col_1	L	L5	**Pathogenesis-related protein 4B (fragment)**	Solyc01g097240.2.1	57	5
AMF_Col_1	N	L5	Ammonium transporter	Solyc01g097370.1.1	44	3
AMF_Col_1	VL	L5	Aldo/keto reductase family protein	Solyc01g097380.1.1	43	
AMF_Col_1	VL	L5	Aldo/keto reductase family protein	Solyc01g097390.2.1	42	
AMF_Col_1	M	L5	Pseudouridine synthase	Solyc01g097590.2.1	22	
AMF_Col_1	N	L5	Inositol monophosphatase family protein	Solyc01g097780.1.1	3	
AMF_Col_1	M	L5	Deoxyuridine triphosphate nucleotidohydrolase	Solyc01g097970.2.1	16	7
AMF_Col_1	H	L5	Kinase family protein	Solyc01g097980.2.1	17	
AMF_Col_1	VL	L5	RNA polymerase Rpb1	Solyc01g098240.1.1	43	
AMF_Col_1	Max	L5	Cyclin-dependent protein kinase	Solyc01g098350.2.1	54	
AMF_Col_1	N	L5	LRR receptor-like serine/threonine-protein kinase	Solyc01g098370.1.1	56	2, 3
AMF_Col_1	L	L5	Dihydrodipicolinate reductase family protein	Solyc01g098380.2.1	57	
AMF_Col_1	L		Receptor-like kinase (SlLYK13)	Solyc01g098410.2.1	60	8
AMF_Col_1	M	L5	GDSL esterase/lipase At1g54790	Solyc01g098650.2.1	84	3
AMF_Col_1	VL	L5	Kinesin 1-like	Solyc01g098670.1.1	86	
AMF_Col_1	Max	L5	LRR receptor-like serine/threonine-protein kinase RLP	Solyc01g098680.2.1	87	2
AMF_Col_1	M	L5	Heavy metal-associated domain containing protein expressed	Solyc01g098760.2.1	95	
AMF_Col_1	VL	L5	**Xylanase inhibitor (fragment)**	Solyc01g098770.1.1	96	
AMF_Col_1	VL	L5	Lipoxygenase	Solyc01g099150.2.1	134	
AMF_Col_1	M	E9	U4/U6.U5 tri-snRNP-associated protein 1	Solyc01g099380.2.1	157	
AMF_Col_1	VL	E9	SRC2-like protein	Solyc01g099390.2.1	158	
AMF_Col_1	M	E9	WD-repeat domain phosphoinositide-interacting protein 3	Solyc01g099400.2.1	159	
AMF_Col_1	H	L5	Rapid alkalinization factor 2	Solyc01g099520.2.1	171	2, 3
AMF_Col_3	M	L5	D-Cysteine desulphhydrase	Solyc03g098230.2.1	54	
AMF_Col_3	VL	L5	**Glutamate decarboxylase**	Solyc03g098240.2.1	53	
AMF_Col_3	L	L5	DNA polymerase epsilon subunit 2	Solyc03g098250.2.1	52	
AMF_Col_3	N	L5	F-box protein interaction domain-containing protein	Solyc03g098350.1.1	42	3
AMF_Col_3	Max	L5	LRR receptor-like serine/threonine-protein kinase	Solyc03g098360.1.1	41	2, 3
AMF_Col_3	N	L5	F-box protein interaction domain-containing protein	Solyc03g098370.1.1	40	3
AMF_Col_3	VL	L5	F-box family protein	Solyc03g098570.1.1	22	3
AMF_Col_3	Max	L5	Kunitz-type trypsin inhibitor alpha chain	Solyc03g020010.1.1	10	1, 5
AMF_Col_3	M	L5	Pentatricopeptide repeat-containing protein	Solyc03g020000.2.1	11	4, 3
AMF_Col_3	M	L5	Pentatricopeptide repeat-containing protein	Solyc03g019990.1.1	12	4, 3
AMF_Col_3	Max	L5	Receptor-like kinase RLK	Solyc03g019980.1.1	13	2, 9
AMF_Col_3	L	L5	Uncharacterized PH domain-containing protein	Solyc03g019770.2.1	34	
AMF_Col_3	H	L5	3-Oxoacyl-reductase	Solyc03g111060.2.1	71	
AMF_Col_3	M	L5	3-Oxoacyl-reductase	Solyc03g111070.2.1	72	
AMF_Col_3	H	L5	3-Oxoacyl-reductase	Solyc03g111080.2.1	73	
AMF_Col_3	Max	L5	Bromodomain factor	Solyc03g111090.2.1	74	
AMF_Col_3	H	L5	60S ribosomal protein L17	Solyc03g111230.2.1	88	
AMF_Col_3	M	L5	SWI/SNF-related matrix-associated actin-dependent regulator	Solyc03g111250.2.1	90	7
AMF_Col_3	N	L5	Cysteine-rich receptor-like protein kinase	Solyc03g111530.2.1	118	2
AMF_Col_4	L	L5	Lanosterol synthase	Solyc04g070980.2.1	37	
AMF_Col_4	H	L5	PHD-finger family protein expressed	Solyc04g070990.2.1	36	
AMF_Col_4	N	L5	Ycf2	Solyc04g071020.1.1	33	
AMF_Col_4	Max	L5	U-box domain-containing protein	Solyc04g071030.1.1	32	
AMF_Col_4	Max	L5	Mitochondrial substrate carrier (fragment)	Solyc04g071290.2.1	6	3
AMF_Col_4	N	L5	Ulp1 protease	Solyc04g071380.1.1	3	
AMF_Col_4	Max		Cellulose synthase	Solyc04g071650.2.1	30	9
AMF_Col_4	VL	L5	Xanthoxin dehydrogenase	Solyc04g071960.2.1	58	
AMF_Col_4	Max	L5	F8A5.7 protein (K4BTJ5)	Solyc04g072080.1.1	70	
AMF_Col_4	H	L5	Acid phosphatase-like	Solyc04g072190.2.1	81	
AMF_Col_4	Max	L5	FRIGIDA	Solyc04g072200.1.1	82	
AMF_Col_4	N	L5	F-box family protein	Solyc04g072330.1.1	95	
AMF_Col_4	L	L5	Metal ion-binding protein	Solyc04g072700.2.1	132	
AMF_Col_5	M	L5	Mitochondrial carrier protein	Solyc05g007730.2.1	157	3
AMF_Col_5	H	L5	Pentatricopeptide repeat-containing protein	Solyc05g007740.1.1	156	4, 3
AMF_Col_5	L	L5	F-box family protein	Solyc05g008470.1.1	83	3

TABLE 2. *Continued*

QTL	Exp.	M.	Annotated gene	mRNA	Order	Reference
AMF_Col_5	M	L5	Mate efflux family protein	Solyc05g008500.1.1	80	4
AMF_Col_5	L	L5	Multidrug resistance protein mdtK	Solyc05g008510.2.1	79	6
AMF_Col_5	Max	L5	Fructose-bisphosphate aldolase	Solyc05g008600.2.1	70	
AMF_Col_5	H	L5	2-Oxoglutarate and iron-dependent oxygenase	Solyc05g008610.2.1	69	
AMF_Col_5	L	L5	Glutamine amidotransferase	Solyc05g008910.2.1	39	
AMF_Col_5	VL	L5	Receptor-like kinase	Solyc05g008950.2.1	35	2
AMF_Col_5	Max	L5	Receptor-like protein kinase	Solyc05g008960.2.1	34	2
AMF_Col_5	L	L5	Serine/threonine protein kinase family protein	Solyc05g008970.1.1	33	2
AMF_Col_5	M	L5	Receptor-like protein kinase	Solyc05g008980.2.1	32	2
AMF_Col_5	M	L5	Transport inhibitor response 1 (fragment)	Solyc05g009260.1.1	4	
AMF_Col_5	M	L5	Acetyl esterase	Solyc05g009610.1.1	30	
AMF_Col_5	Max	L5	Choline transporter-like protein	Solyc05g009670.2.1	36	
AMF_Col_5	H	L5	Predicted membrane protein (fragment)	Solyc05g009690.2.1	38	
AMF_Col_5	N	L5	Zinc finger family protein	Solyc05g009770.1.1	46	2
AMF_Col_5	H	L5	Transcription factor	Solyc05g009790.1.1	48	
AMF_Col_5	M	L5	U3 small nucleolar RNA-associated protein 15 homolog	Solyc05g010480.2.1	117	
AMF_Col_6	N	L5	Erythrocyte membrane-associated giant protein antigen 332	Solyc06g075900.1.1	167	
AMF_Col_6	M	L5	Histone H4	Solyc06g075930.1.1	164	
AMF_Col_6	N	E9	X1 (fragment)	Solyc06g076200.1.1	137	
AMF_Col_6	L	L5	Genomic DNA chromosome 5 BAC clone F2O15	Solyc06g076250.2.1	132	
AMF_Col_6	M	L5	Phosphoserine phosphatase	Solyc06g076510.2.1	106	4
AMF_Col_6	VL	L5	Class I heat shock protein	Solyc06g076520.1.1	105	
AMF_Col_6	VL	L5	Class I heat shock protein	Solyc06g076540.1.1	103	
AMF_Col_6	Max	L5	**Laccase 1a**	Solyc06g076760.1.1	83	
AMF_Col_6	Max	L5	Cytochrome P450	Solyc06g076800.2.1	79	6
AMF_Col_6	H	L5	Pentatricopeptide repeat-containing protein	Solyc06g076900.2.1	69	3
AMF_Col_6	Max	L5	Receptor like kinase	Solyc06g076910.1.1	68	2
AMF_Col_6	VL	L5	Undecaprenyl pyrophosphate synthase	Solyc06g076920.2.1	67	
AMF_Col_6	Max	L5	SAGA-associated factor 11 homologue	Solyc06g082160.2.1	42	
AMF_Col_6	M	L5	Glutaredoxin family protein	Solyc06g082170.2.1	41	
AMF_Col_6	N	L5	Nodulin-like protein	Solyc06g082410.1.1	17	9
AMF_Col_6	M	L5	Ribosomal protein L10	Solyc06g082670.2.1	7	
AMF_Col_6	M	L5	Cytochrome b-c1 complex subunit 8	Solyc06g082680.2.1	8	
AMF_Col_6	M	L5	Protein phosphatase 2C	Solyc06g082700.1.1	10	4
AMF_Col_6	M	L5	60S ribosomal protein L5-1	Solyc06g082870.2.1	27	
AMF_Col_6	M	L5	Pentatricopeptide repeat-containing protein	Solyc06g082880.1.1	28	4, 3
AMF_Col_6	Max	L5	HAUS augmin-like complex subunit 3	Solyc06g083100.1.1	50	
AMF_Col_6	Max	L5	U-box domain-containing protein	Solyc06g083150.2.1	55	
AMF_Col_6	Max	L5	Plant-specific domain TIGR01615 family protein	Solyc06g083160.1.1	56	
AMF_Col_6	M	L5	Leucine-rich repeat family protein expressed	Solyc06g084060.2.1	146	
AMF_Col_6	Max	L5	Auxin responsive protein	Solyc06g084070.2.1	147	7
AMF_Col_6	H	L5	Guanylate-binding family protein	Solyc06g084080.2.1	148	
AMF_Col_6	Max	L5	High affinity sulphate transporter 1	Solyc06g084140.2.1	154	
AMF_Col_6	M	L5	Serine/threonine-protein kinase bud32 (EC 2.7.11.1)	Solyc06g084160.2.1	156	2
AMF_Col_6	L	L5	Universal stress protein	Solyc06g084540.2.1	194	
AMF_Col_8	M	L5	Ribonuclease 3-like protein 2	Solyc08g067210.2.1	87	
AMF_Col_8	N	L5	Floral homeotic protein FBP1	Solyc08g067220.1.1	86	
AMF_Col_8	L	L5	MADS box transcription factor	Solyc08g067230.2.1	85	
AMF_Col_8	M	L5	Cell division protein kinase 2	Solyc08g067450.1.1	63	
AMF_Col_8	M	L5	Pre-mRNA-splicing factor syf2	Solyc08g067460.1.1	62	
AMF_Col_8	M	L5	Monooxygenase FAD-binding	Solyc08g067470.2.1	61	
AMF_Col_8	M	E9	CW-type zinc finger family protein expressed	Solyc08g067490.1.1	59	2
AMF_Col_8	Max	L5	ATP-binding cassette transporter	Solyc08g067620.2.1	46	7
AMF_Col_8	N	L5	**MLO-like protein 3**	Solyc08g067760.2.1	34	
AMF_Col_8	L	L5	Globin	Solyc08g068070.2.1	2	
AMF_Col_8	M	L5	Globin	Solyc08g068090.2.1	0	
AMF_Col_8	Max	L5	DNA polymerase I family protein expressed	Solyc08g068120.1.1	0	
AMF_Col_8	Max	L5	BURP domain-containing protein (fragment)	Solyc08g068130.1.1	0	
AMF_Col_8	Max	L5	Ethylene receptor	Solyc08g068360.1.1	7	
AMF_Col_8	Max	L5	Class E vacuolar protein-sorting machinery protein HSE1	Solyc08g068370.2.1	8	
AMF_Col_8	M	L5	Lipoyl synthase	Solyc08g068440.2.1	15	
AMF_Col_8	N	L5	F-box family protein	Solyc08g068500.1.1	21	3
AMF_Col_8	H	L5	F-box/LRR-repeat protein At3g03360	Solyc08g068510.1.1	22	3
AMF_Col_8	VL	L5	F-box family protein	Solyc08g068520.2.1	23	3
AMF_Col_8	M	L5	Decarboxylase family protein	Solyc08g068640.2.1	34	
AMF_Col_8	Max	L5	*N*-Acetyltransferase	Solyc08g068730.1.1	42	
AMF_Col_8	Max	L5	***N*-Hydroxycinnamoyl-CoA:tyramine *N*-hydroxycinnamoyl transferase**	Solyc08g068780.1.1	47	

Continued

TABLE 2. *Continued*

QTL	Exp.	M.	Annotated gene	mRNA	Order	Reference
AMF_Col_8	M	L5	Tyramine hydroxycinnamoyl transferase	Solyc08g068790.1.1	48	
AMF_Col_8	M	L5	**Glutathione peroxidase**	Solyc08g068800.2.1	49	5
AMF_Col_8	M	L5	Mitochondrial import inner membrane translocase subunit	Solyc08g069210.1.1	88	
AMF_Col_8	M	L5	DDB1- and CUL4-associated factor homologue 1	Solyc08g074370.2.1	105	
AMF_Col_8	M	L5	DDB1- and CUL4-associated factor homologue 1	Solyc08g074380.1.1	106	
AMF_Col_9	N	L5	MYB transcription factor	Solyc09g007580.1.1	135	2
AMF_Col_9	L	L5	Histone H4	Solyc09g007590.1.1	134	
AMF_Col_9	N	L5	Acetyl xylan esterase A	Solyc09g008540.1.1	39	
AMF_Col_9	Max	L5	NCS1 family transporter cytosine/purines/uracil/thiamine/allantoin	Solyc09g008550.2.1	38	
AMF_Col_9	Max		1-aminocyclopropane-1-carboxylate oxidase	Solyc09g008560.2.1	37	9
AMF_Col_9	M	L5	Repressor of silencing 1	Solyc09g009080.2.1	9	
AMF_Col_9	N	L5	F-box protein family-like	Solyc09g009320.1.1	33	
AMF_Col_9	N	L5	Fatty acyl CoA reductase	Solyc09g009570.1.1	58	
AMF_Col_9	VL	L5	Z-box binding factor 2 protein	Solyc09g009760.1.1	77	
AMF_Col_9	Max	L5	50S ribosomal protein L12-2	Solyc09g010030.1.1	104	
AMF_Col_9	M	L5	1-Aminocyclopropane-1-carboxylate oxidase-like protein	Solyc09g010040.1.1	105	9
AMF_Col_9	M	L5	Cullin 1B	Solyc09g010050.1.1	106	
AMF_Col_10	N	L5	Helicase-like protein	Solyc10g054180.1.1	15	
AMF_Col_10	N	L5	Helitron helicase-like protein	Solyc10g054190.1.1	16	
AMF_Col_10	N	L5	Helicase-like protein	Solyc10g054210.1.1	18	
AMF_Col_10	N	L5	Helicase-like protein	Solyc10g054220.1.1	19	
AMF_Col_10	N	L5	Nucleic acid-binding OB-fold	Solyc10g054240.1.1	21	
AMF_Col_10	N	L5	**LRR resistance protein fragment**	Solyc10g054360.1.1	33	
AMF_Col_10	N	L5	**CC-NBS-LRR resistance protein**	Solyc10g054370.1.1	34	
AMF_Col_10	N	L5	Fructose-bisphosphate aldolase	Solyc10g054380.1.1	35	
AMF_Col_10	N	L5	Fructose-bisphosphate aldolase	Solyc10g054390.1.1	36	
AMF_Col_10	M	L5	General transcription factor IIF subunit 2	Solyc10g054400.1.1	37	
AMF_Col_10	M	L5	FAD-binding domain-containing protein	Solyc10g054480.1.1	45	
AMF_Col_10	N	L5	FAD-binding domain-containing protein	Solyc10g054490.1.1	46	
AMF_Col_10	M	L5	V-type proton ATPase 16 kDa proteolipid subunit	Solyc10g054590.1.1	56	
AMF_Col_10	N	L5	Ulp1 protease family	Solyc10g054630.1.1	60	
AMF_Col_10	N	L5	Mutator-like transposase	Solyc10g054640.1.1	61	

The mRNA code, its relative root expression (Exp.) in the Heinz cultivar (Max, maximum; H, high; M, medium; VL, very low; L, low; and N, no data) and the number of genes counted from the QTL peak (Ord.) are shown.

As indicated by the reference number, members of gene families of some candidates were previously associated with AMF colonization (1, 2, 3, 4, 6, 7, 8 and 9) and/or the secretome plant defence response against pathogens (5).

Annotated genes related to plant defence are given in bold.

(1) Amiour *et al*., 2007; (2) Hohnejc *et al*., 2015; (3) Gaude *et al.*, 2012; (4) Labbè *et al.*, 2011; (5) Yeom *et al.*, 2011; (6) Ruyter-Spira *et al*., 2013; (7) Mohanta and Bae, 2015; (8) Buendia *et al.*, 2016; (9) Siciliano *et al.*, 2007.

processes were also over-represented when the gene analysis was performed within each AMF_Col QTL separately (Supplementary Data Fig. S3). Among over-represented biological processes using just polymorphic candidates from Table 2, are N utilization, response to stimulus, lipid metabolic process, DNA replication and mitochondrial transport (Fig. 5). As indicated by the reference number, members of gene families of the mutational candidates included in Table 2 were previously associated with mycorrhization and/or the plant defence response against pathogens.

DISCUSSION

It is well known from the literature that mycorrhizal fungi improve soil functionality (Bitterlich *et al.*, 2018), plant nutrients and water use efficiency under abiotic-stressed environments (Bowles *et al.*, 2016; Chitarra *et al.*, 2016; Ruiz-Lozano *et al*., 2016) and increase their tolerance/resistance against pests and diseases (Pozo and Azcón-Aguilar, 2007), thus representing key organisms in agricultural systems, including organic farming (Hohmann and Messmer, 2017; Tsvelkov *et al*., 2018). However, it is not clear yet whether those benefits come just from the association or by the way the plant manages the association for its own benefit because several studies (reviewed by Hohmann and Messer, 2017) found no correlation between the response of the AMF-colonized plant and its percentage of AMF-colonized root. In general, these studies fail to detect genetic factors (or QTLs) underlying the variation for AMF colonization (frequently estimated as F%) that could explain the lack of correlation as the absence of common genes controlling both traits. Therefore, as a first step, the genetic basis of AMF association has to be studied to test further if those QTLs, only some of them or none of them, are involved in the beneficial effects of the AMF regarding the plant's tolerance to biotic and abiotic stresses. Genetic variation for the AMF root colonization has not been exploited yet in tomato. The present study shows that the three measures of AMF association (F%, M% and A%), although apparently very similar to one another (Fig. 1), differ in heritability in the population of RILs, being the highest for M% (0.24) and the lowest for F% (0.19). Larger heritability estimates of AMF association were reported in wheat (0.54; Lehnert *et al.*, 2017) and poplar (0.38; Labbé

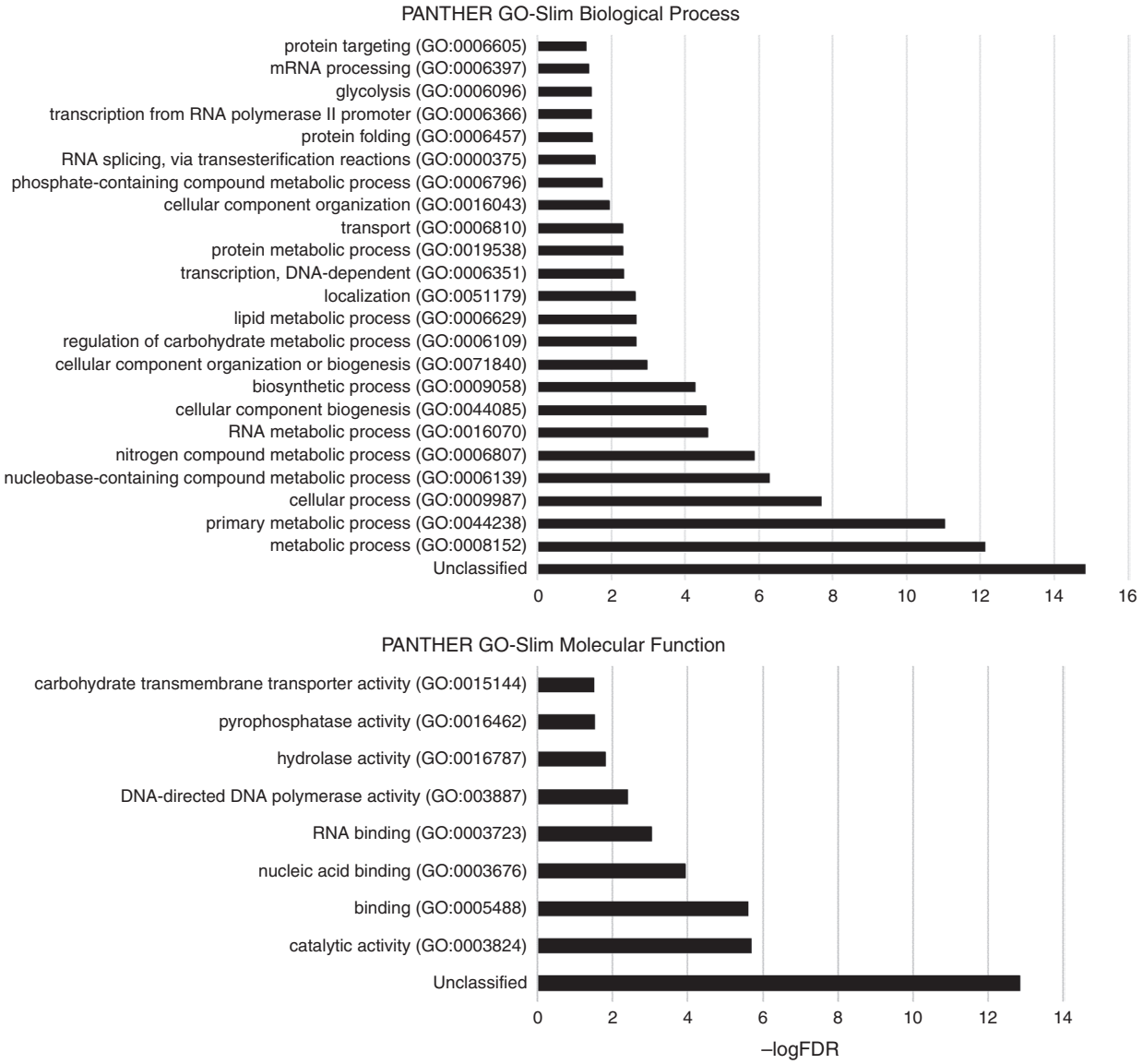

FIG. 4. Over-represented biological processes and molecular functions of all genes within AMF colonization QTLs (Supplementary data Table S2) by means of the PANTHER Classification System (http://www.pantherdb.org/) (Mi *et al*., 2013, 2016) using Fisher's exact test with FDR multiple test correction.

et al., 2011), and similar values were reported in alfalfa, maize and sorghum (Lackie *et al.*, 1988; Kaeppler *et al.*, 2000; Leiser *et al.*, 2016). In spite of heritability differences, the number of QTLs detected here for M% or A% in the tomato RIL population was similar to that reported by Lehnert *et al.* (2017) for the percentage of root length colonized in wheat. Differences in heritability estimates among species could be due to differences in the trait definition (F% instead of M%), in the method to assess colonization (Trouvelot, 1986 vs. McGonnigle *et al.*, 1990), in population structure and/or in statistical methods used (Asins, 2002). Initially, only four QTLs were detected by IM in chromosomes 3, 5, 9 and 10 (QTLs in bold in Table 1); but, allowing for multiple QTLs (MQM procedure), eight significant QTLs were obtained, although three of them (AMF_Col_4, _6 and _8) were not supported by KW. Therefore, no major, but eight minor QTLs were detected (Table 1; Supplementary data Fig. S1), each contributing 4.1–8.7 % to the total variance of AMF. The fact that the increasing alleles come from both parents (*S. lycopersicum* or *S. pimpinellifolium* depending on the QTL) would explain the lack of significant differences between them. Interestingly, deviations from additivity were observed at both the intralocus (underdominance) and interlocus (epistatic) levels. Thus, the AMF colonization ability of heterozygotes (h) at QTLs AMF_Col_3 and AMF_Col_9 was found to be worse than that of any homozygote (a and b) (Fig. 3). Therefore, exploiting heterosis in tomato breeding programmes for improving AMF colonization does not seem advisable, contrary to what Labbé *et al.* (2011) suggested regarding ectomycorrhization in poplar. On the other hand, some epistasis (particularly involving AMF_Col_3, AMF_Col_8 and AMF_Col_6) was found to be relevant to increase AMF colonization (Fig. 2).

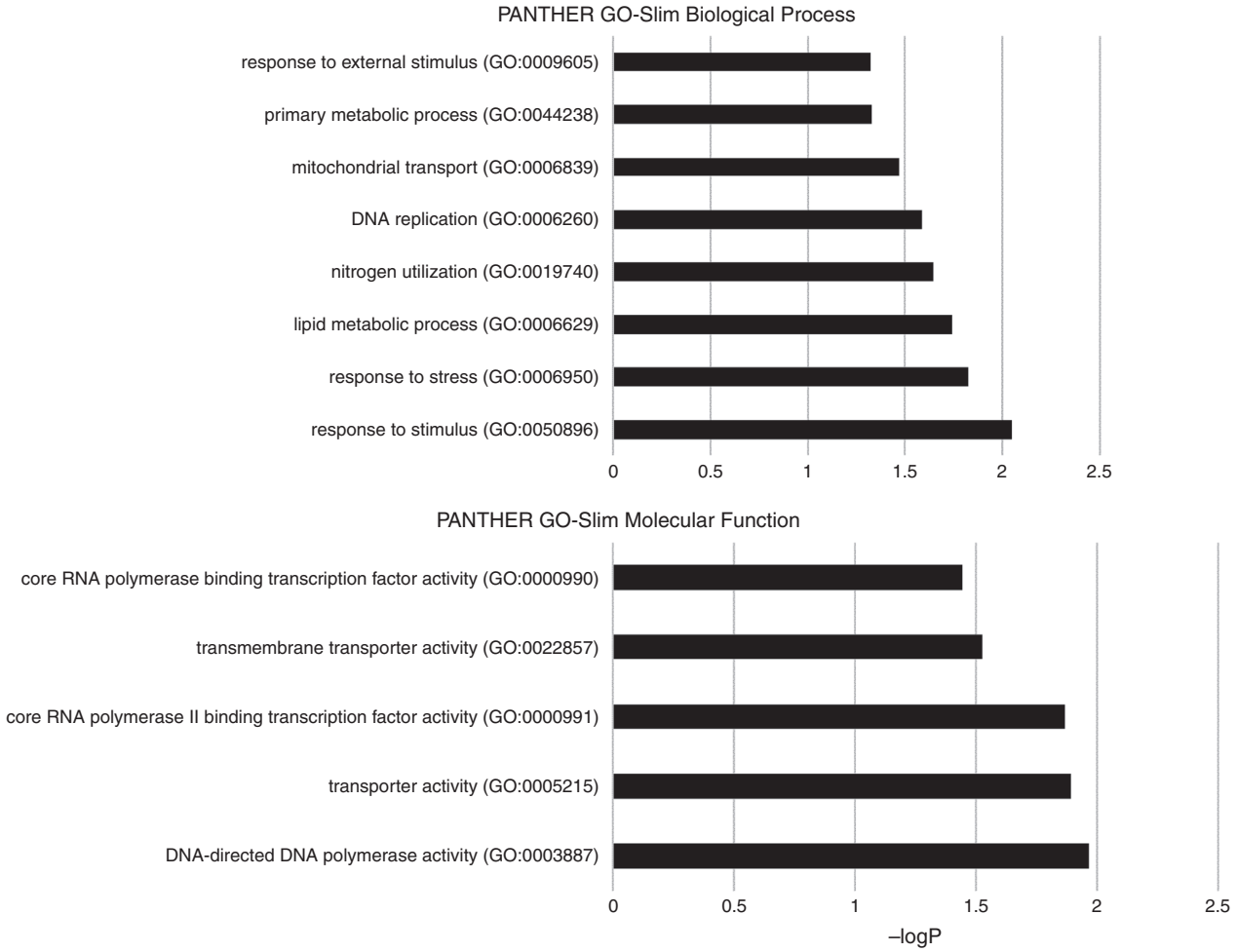

FIG. 5. Over-represented biological processes and molecular functions of segregating genes within AMF colonization QTLs (Table 2) by means of the PANTHER Classification System (http://www.pantherdb.org/) (Mi *et al*., 2013, 2016) using the Binomial test.

Genes involved in AMF root colonization have been previously identified by the reverse genetic strategy through expression studies in mutants and transgenic genotypes (Siciliano *et al.*, 2007; Kretzschmar *et al*., 2012; Dermatsev *et al.*, 2010; Hohnejc *et al*., 2015; Mohanta and Bae, 2015; Buendia *et al.*, 2016). Some of these genes could be represented among the genes found within the tomato AMF_Col QTLs (Table 2). Specifically, SlCCaMK, coding for a calcium- and calmodulin-dependent protein kinase that is required for penetration of *Rhizophagus irregularis* into the roots of tomato plants (Buendia *et al.*, 2016), and SlLYK13, coding for a lysin motif receptor-like kinase of the phylogenetic group LYKI (Buendia *et al*., 2018) are within AMF_Col_1. Members of the LYKI group have dual roles in endosymbiosis and defence, and they might be co-receptors rather than ligand-binding proteins (Buendia *et al*., 2018).

Another effort to identify genes involved in the AMF–plant symbiosis consisted of comparing the transcriptomic profile of arbuscular-containing and non-colonized cortical cells. Thus, Gaude *et al.* (2012) reported some differentially expressed genes similar to some included in our list of segregating candidates (Table 2): genes coding for mitochondrial carrier, ammonium transporter, ABC transporter, lipases, pentatricopeptide repeat-containing proteins, cysteine-type peptidase, F-box proteins and ankyrin-repeat proteins, and genes involved in signalling such as leucine-rich receptor (LRR) proteins and rapid alkalinization factor.

The forward genetics approach to identify candidate genes controlling agronomic traits has scarcely been used. In a previous QTL analysis of ectomycorrhizal colonization, Labbè *et al.* (2011) tried to reduce the list of candidate genes within the QTL intervals by comparing transcript levels in ectomycorrhizal root tips of the parents of the poplar population. They found differential expression in 41 genes that located in three out of the four QTLs detected. Among mutational candidates in Table 2, genes encoding pentatricopeptide repeat-containing proteins, MATE proteins and phosphatases from AMF_Col_6, AMF_Col _5 and AMF_Col _3 are similar to those reported by Labbé *et al.* (2011) in poplar.

The origin of a QTL could be segregation at one or more genes whose products are involved in the trait under study, and/or at one or more genes (or elements) involved in the pattern of expression of genes whose products are involved in the trait under study. Thus, a strategy to narrow the list of candidate genes underlying QTLs for AMF colonization, similar to that reported by Asins *et al.* (2017), could be based on the

hypothesis that those genes segregating for a frameshift InDel mutation in the population and which are expected to be predominantly expressed in root (taking into account expression data from the Heinz cultivar under control conditions) would be more likely to be responsible for the QTL detected. Discarding genes coding for proteins of unknown function in this list (71) and considering proximity to the QTL peak marker, the best candidates from Table 2 would be: cyclin-dependent protein kinase for AMF_Col_1, Kunitz-type trypsin inhibitor α chain and receptor-like kinase for AMF_Col_3, mitochondrial carrier for AMF_Col_4, choline transporter for AMF_Col_5, SAGA-associated factor 11 and HAUS augmin-like complex subunit 3 for AMF_Col_6, BURP domain-containing protein and ethylene receptor for AMF_Col_8, and NCS1 transporter for AMF_Col_9. However, candidates SlCCaMK and SlLYK13 within AMF_Col_1 are not segregating for a frameshift mutation, and all candidates within AMF_Col_10 (the QTL contributing most to AMF symbiosis here) show low relative expression in root of the reference cultivar Heinz. Perhaps differences in the patterns of expression of parental alleles (E9 from *S. lycopersicum* and L5 from *S. pimpinellifolium*), including the effect of P starvation, are the origin of these AMF colonization QTLs in chromosomes 1 and 10. Two fructose-biphosphate aldolases, involved in glycolysis, could underlie AMF_Col_10 by increasing the glucose pool available for the AMF. This enzyme is regulated by gibberellin in rice roots and physically associates with vacuolar H-ATPase (Konishi *et al.*, 2004). It is noteworthy that this genomic region overlaps that including GA3_N_10, a QTL governing gibberellin concentration in the xylem sap under N deficit in the same population of RILs (Asins *et al.*, 2017), and a V-type proton ATPase 16 kDa proteolipid subunit-encoding gene is a few genes further on (Table 2). It is also important to point out that AMF_Col_10 contains genes relevant for the biosynthesis of alditol (Solyc10g054280), spermidine (Solyc10g054440) and ergosterol (Solyc10g054130), close to numerous fragments of transposable elements (Supplementary data Table S2). Alditol is a sugar alcohol whose probable functions are storage of reduced carbon and reducing power, and osmoregulation (Loescher, 1987). Regarding spermidine, polyamines play a critical role in stress tolerance by modulating the homeostasis of reactive oxygen species (ROS), regulating antioxidant systems or suppressing ROS production (Liu *et al.*, 2015). Thus, soil drench application of polyamines positively influenced mycorrhizal inoculation in *Freesia hybrida* (Rezvanypour *et al.*, 2015). Perhaps frameship mutations in the transposable elements of this region (Table 2) have provoked changes in the pattern of root expression of those genes within AMF_Col_10, favouring mycorrhization of homozygotes for the *pimpinellifolium* allele.

A likely candidate for AMF_Col_5 encodes a choline transporter-like protein. In arabidopsis, a similar gene is required for sieve plate development, phloem conductivity (Dettmer *et al.*, 2014) and plasmodesmata maturation (Kraner *et al.*, 2016) mediating long- and short-range cell–cell communication. In addition, extracellular choline might be important for plant–microbe interaction (Chen *et al.*, 2013). Other candidate genes from Table 2 related to plant–microbe interaction are: two aldo/keto reductases (Sengupta *et al.*, 2015) within AMF_Col_1, and a lipoyl synthase (Allary *et al.*, 2007) within AMF_Col_8.

Multiple factors such as auxin and phosphate levels affect the strigolactone levels in plants (Ruyter-Spira *et al.*, 2013). Thus, a likely candidate for AMF_Col_9 encodes a nucleobase cation symporter (NCS1) which plays, in general, an important role in the salvage of nucleosides and nucleobases as an alternative to the *de novo* synthesis of nucleotides preserving phosphate and N for plant development (Witz *et al.*, 2014). Nucleobase biochemistry highly depends on extensive intra- and intercellular transport; therefore, a mutation in this gene decreasing the nucleotide salvage pathway for plant growth could benefit AMF development. Another possibility is that the mutation could affect just substrate affinity, making the heterozygote the most efficient genotype in nucleotide salvage that could explain the underdominance effect detected at this QTL (Fig. 3). Alternatively, more than one gene could underlie this QTL, resulting in pseudo-underdominance.

Since root-secreted proteins play a crucial role in the numerous complex defence responses that are provoked by the presence of pathogens (Yeom *et al.*, 2011), it is not surprising that AMF also have the ability to induce plant defence mechanisms (Hohmann and Messmer, 2017). It is noteworthy that many genes within AMF_Col QTLs are similar to those encoding proteins found in the root secretome that are involved in the plant response against pathogens such as peroxidases, chitinases and pathogenesis-related proteins (Yeom *et al.*, 2011). The abundance of genes related to the plant response against pathogens within AMF colonization QTLs (in bold in Table 2) suggests that AMF colonization might involve genes that also participate in the plant defence response against pathogens, particularly fungi. Hohmann and Messmer (2017) reviewed evidence that AMF initially trigger the plant defence mechanisms similarly to a biotrophic pathogen but then modulate plant responses for successful colonization (Paszkowski, 2006). Our data could be interpreted as the other way around: changes (perhaps failures) in some genes involved in plant defence might facilitate the AMF–plant association. Therefore, it is possible that not all beneficial alleles at the AMF_Col QTLs reported here are also beneficial for enhancing the AMF–plant defence against pathogens. However, the situation is more complex than testing one or the other hypothesis because AMF association intensity (under P deficit) and concentration of jasmonic acid (JA) in the xylem sap under N deficit (data from Asins *et al.*, 2017) are significantly correlated ($P \leq 0.0075$, $r = 0.23$). In fact, AMF_Col_4 and AMF_Col_9 are linked to QTLs controlling JA concentration in the xylem sap under N deficit (Asins *et al.*, 2017). Since this phytohormone is frequently found in microbe-induced resistance (Pozo *et al.*, 2007; Van der Ent *et al.*, 2009) and apparently in AMF-mediated resistance and priming (reviewed in Jung *et al.*, 2012), at least a positional connection between plant resistance and AMF colonization exists in tomato. Supporting this connection, based on genetic linkage, several biological processes related to plant defence, such as phenylpropanoid and cinnamic acid biosynthetic processes, negative regulation of endopeptidase activity and oxylipin biosynthetic process, are over-represented in AMF_Col_9, AMF_Col_3 and AMF_Col_1, respectively (Supplementary data Fig. S3). This finding echoes the results reported by Rivero *et al.* (2018) on the reorganization of metabolic profiles of tomato roots that occurred as a consequence of establishment of mycorrhiza in the absence of stress.

On the other hand, AMF symbiosis is characterized by the exchange of photosynthetic products (mainly glucose) produced by the plants and nutrients (mainly P and N) by the fungus (Mohanta and Bae, 2015); therefore, it is not surprising that carbohydrate transmembrane transporter activity, regulation of the carbohydrate metabolic process, the N compound metabolic process and the phosphate-containing compound metabolic process are accessions over-represented among AMF colonization candidate genes (Fig. 4). Thus, a connection between AMF colonization and carbohydrate regulation and transport is supported by the over-representation of these biological processes in AMF_Col_3 (Supplementary data Fig. S3), and N compound and phosphate-containing compound metabolic processes are over-represented in AMF_Col_1, AMF_Col_3 and AMF_Col_5 (Supplementary data Fig. S3). AMF depend on their plant host for palmitic acid synthesis, this lipid being its major form of carbon storage (Trépanier *et al.*, 2005), and a genetic connection between AMF colonization and lipid metabolism and transport exists through the over-representation of these biological processes in AMF_Col_1 and AMF_Col_8 (Supplementary data Fig. S3). On the other hand, AMF symbiosis alleviates drought stress in plants, and Ruiz-Lozano *et al.* (2016) have shown that drought induces strigolactone biosynthesis only in mycorrhized plants, while the synthesis of this hormone was reduced in non-AMF-colonized plants. It is noteworthy that regulation of stomatal closure is over-represented in AMF_Col_1 (Supplementary data Fig. S3). Therefore, gene linkage within AMF colonization QTLs might be important to explain the functioning of the AMF–plant mutualism and the coevolution of both organisms as a genotype × genotype interaction for fitness (Kiers *et al.*, 2011). Then, a new question arises: is the activation (or regulation) of genes within AMF colonization QTLs what finally benefit the plant regarding water and nutrient use efficiency, and disease resistance? Future experiments on mycorrhizal responsiveness to salinity, drought and pathogens using this RIL population could test this hypothesis and integrate the AMF–plant association and its response to ecosystem changes in comparison with non-AMF-colonized plants.

CONCLUSION

Intensity (M%), instead of frequency (F%), as defined by Trouvelot *et al.* (1986), is genetically the best morphological measure of root AMF colonization. Wild alleles from *S. pimpinellifolium* can improve AMF colonization in tomato, and the gene contents of AMF colonization QTLs might be important for explaining the establishment and functioning of the AMF–plant symbiosis.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: LOD profiles of QTLs for AMF colonization traits. Table S2: list of downloaded genes at peaks (2–2.6 Mbp) of AMF colonization QTLs indicating the presence of frameshift Indels in parental genomes, E9 and L5, and relative root expression in the cultivar Heinz using the tomato eFP Browser. Figure S3: significant over-represented biological processes within each AMF colonization QTL by means of the PANTHER Classification System using Fisher's exact test with FDR multiple test correction.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Spanish Government (to M.J.A.) (AGL2014-56675-R, AGL2017-82452-C2-2-R) and the European Union (FP7-KBBE-2011–5), contract # 289365 (ROOTOPOWER).

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