

## **Supplementary material**

### **Field Inoculation of Bread Wheat with *Rhizophagus Irregularis* under Organic Farming: Variability in Growth Response and Nutritional Uptake of Eleven Old Genotypes and A Modern Variety**

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**Table S1.** List of arbuscular mycorrhizal fungal (AMF) sequences retrieved in the roots of Autonomia B, Frassineto and Bologna, the corresponding virtual taxon (VT) (MaarjAM database; <https://maarjam.botany.ut.ee>), code based on the clustering of the sequences in the Neighbor-Joining tree (see **Figures 6, S1 and S2**), accession number of the most similar VT and AMF taxon description.

Representative sequence	Other sequences	Virtual Taxon	Code	Accession number	Description
F10 66 22 alb M15R	F09 66 21 alb M15R, F08 66 20 alb M15R, F07 66 19 alb M15R, F06 66 18 alb M15R, F05 66 17 alb M15R, F04 66 16 alb M15R, F03 66 15 alb M15R, F02 66 14 alb M15R, F01 66 13 alb, M15R, G12 66 12 alb M15R, G11 66 11 alb M15R, G10 66 10 alb M15R, G09 66 9 alb M15R, G08 66 8 alb M15R, G07 66 7 alb M15R, G06 66 6 alb M15R, G05 66 5 alb M15R, G04 66 4 alb M15R, G03 66 3 alb M15R, G02 66 2 alb M15R, G01 66 1 alb M15R, E11 59 26 albM13R, E10 59 25 albM15R, E09 59 24 albM15R, E07 59 23 albM15R, E06 59 22 albM15R, E05 59 21 albM15R, D01 59 06 albM15R, D02 59 07 albM15R, D03 59 08 albM15R, D04 59 09 albM15R, D05 59 10 albM15R, E08 59 20 albM13R, E04 59 20 albM15R, E03 59 19 albM15R, E02 59 18 albM15R, E01 59 17 albM15R, D12 59 16 albM15R, D10 59 15 albM15R, D09 59 14 albM15R, D08 59 13 albM15R, D07 59 12 albM15R, D06 59 11 albM15R, G06 43 25 albM15R, G05 43 24 albM15R, G04 43 23 albM15R, G03 43 22 albM15R, G02 43 21 albM15R, G01 43 20 albM15R, F12 43 19 albM15R, F11 43 18 albM15R, F09 43 17 albM15R, F08 43 16 albM15R, F07 43 15 albM15R, E06 43 2 albM15R, F06 43 14 albM15R, F05 43 13 albM15R, F04 43 12 albM15R, F03 43 11 albM15R, F02 43 10 albM15R, F01 43 9 albM15R, E12 43 8 albM15R, E11 43 7 albM15R, E09 43 5 albM15R, E08 43 4 albM15R, E07 43 3 albM15R, E05 43 1 albM15R, C08 34 48 albM15R, B06 11 31 albM14R, B05 11 30 albM14R, B07 11 32 albM14R, B08 11 33 albM14R, B09 11 34 albM14R, B10 11 35 albM14R, B11 11 36 albM14R, B12 11 37 albM14R, C01 11 38 albM14R, C02 11 39 albM14R, C06 11 43 albM14R, C07 11 44 albM14R, C08 11 45 albM14R, C12 11 49 albM14R, D01 11 50 albM14R, D02 11 51 albM14R, D03 11 52 albM14R, D04 11 53 albM14R, D05 11 54 albM14R, D06 11 55 albM14R, D07 11 56 albM14R, D08 11 57 albM14R, F03 11 26 albM13R, E12 16 18 albM14R, F01 16 19 albM14R, F02 16 20 albM14R, F03 16 21 albM14R, F04 16 22 albM14, F05 16 23 albM14R, F06 16 24 albM14R, F07 16 25 albM14R, F08 16 26 albM14R, F09 16 27 albM14R, F10 16 28 albM14R, F11 16 29 albM14R, F12 16 30 albM14R, G01 16 31 albM14R, G02 16 32 albM14R, G03 16 33 albM14R, G04 16 34 albM14R, G05 16 35 albM14R, G05 16 36 albM14R, G07 16 37 albM14R, G08 16 38 albM14R, A06 34 22 albM15R, A07 34 23 albM15R, A08 34 24 albM15R, A09 34 25 albM15R, A10 34 26 albM15R, A11 34 27 albM15R, A12 34 28 albM15R, B01 34 29 albM15R, B02 34 30 albM15R, B03 34 31 albM15R, B04 34 32 albM15R, B05 34 33 albM15R, B06 34 34 albM15R, B07 34 35 albM15R, B08 34 36 albM15R, B09 34 37 albM15R, B10 34 38 albM15R, B11 34 39 albM15R, B12 34 40 albM15R, C01 34 41 albM15R, C02 34 42 albM15R, C03 34 43 albM15R, C04 34 44 albM15R, C05 34 45 albM15R, C07 34 47 albM15R, C06 34 46 albM15R	VTX00065	Fun	KJ952229	Glomeraceae <i>Glomus</i> sp. <sup>1</sup>

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A12 46 24 alb M17R	E04 35 46 alb M18R, E02 35 44 alb M18R, E01 35 43 alb M18R, D12 35 42 alb M18R, B01 35 7 alb M18R, D05 35 35 alb M18R, D04 35 34 alb M18R, D03 35 33 alb M18R, D02 35 32 alb M18R, G07 23 5 alb M17R, A08 23 29 alb M18R, A06 23 28 alb M18R, A05 23 27 alb M18R, G06 4 28 alb M17R, G05 4 27 alb M17R, G04 4 26 alb M17R, G02 4 24 alb M17R, G01 4 23 alb M17R, F12 4 22 alb M17R		VTX00057	Claro1	FN869804	Claroideoglom us

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D08 35 38 alb M18R, C12 35 30 alb M18R, C09 35 27 alb M18R, C07 35 25 alb M18R, C03 35 21 alb M18R, B07 35 13 alb M18R, B03 35 09 alb M18R, C06 35 24 alb M18R, C05 35 23 alb M18R, C02 35 20 alb M18R, C01 35 19 alb M18R, B10 35 16 alb M18R, B09 35 15 alb M18R, B06 35 12 alb M18R, B05 35 11 alb M18R, B02 35 8 alb M18R, G04 23 4 alb M17R, H12 23 22 alb M17R, H09 23 19 alb M17R, H05 23 15 alb M17R, G05 23 5 alb M17R, G06 23 4 alb M17R, H10 23 20 alb M17R, H07 23 17 alb M17R, H06 23 16 alb M17R, H03 23 13 alb M17R, H02 23 12 alb M17R, G11 23 9 alb M17R, G10 23 8 alb M17R, F08 4 18 alb M17R, F07 4 17 alb M17R, F03 4 13 alb M17R, E10 4 8 alb M17R, E07 4 5 alb M17R, E05 4 3 alb M17R, F05 4 15 alb M17R, F04 4 14 alb M17R, F01 4 11 alb M17R, E12 4 10 alb M17R, E09 4 7 alb M17R, E08 4 6 alb M17R, E09 30 10 alb M16R, E10 30 11 alb M16R, E08 30 09 alb M16R, E11 30 12 alb M16R, D05 24 5 alb M16R, D04 24 4 alb M16R, D03 24 3 alb M16R, D02 24 2 alb M16R, E11 6 7 VTX00057 Claro2 FN869808 alb M13R, A07 6 34 alb M16R, E12 6 8 alb M13R, A06 6 33 alb M16R, F09 30 22 alb M16R, F08 30 21 alb M16R, F07 30 20 alb M16R, F06 30 19 alb M16R, F05 30 18 alb M16R, F04 30 17 alb M16R, F03 30 16 alb M16R, F02 30 15 alb M16R, D09 24 9 alb M16R, D08 24 8 alb M16R, D07 24 7 alb M16R, D06 24 6 alb M16R, G02 6 27 alb M13R, A09 6 36 alb M16R, G01 6 26 alb M13R, A08 6 35 alb M16R, D09 35 39 alb M18R, D06 35 36 alb M18R, C08 35 26 alb M18R, B11 35 17 alb M18R, C10 35 28 alb M18R, C04 35 22 alb M18R, B12 35 18 alb M18R, B08 35 14 alb M18R, B04 35 10 alb M18R, G03 23 3 alb M17R, H11 23 21 alb M17R, H01 23 11 alb M17R, G09 23 7 alb M17R, A03 23 25 alb M18R, H08 23 18 alb M17R, H04 23 14 alb M17R, G12 23 10 alb M17R, G08 23 6 alb M17R, F06 4 16 alb M17R, E04 4 2 alb M17R	<i>Claroideoglomus</i>
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**Table S2.** Effect of arbuscular mycorrhizal fungal inoculation (AMF inoc) and bread wheat genotype (Genotype) on richness, Shannon index ( $H'$ ) and Simpson index ( $\lambda$ ) of the AMF community in wheat roots.

	Richness	$H'$	$\lambda$
Autonomia B -M	2.0 ± 0.0	0.58 ± 0.05 b	0.39 ± 0.05 b
Autonomi B +M	2.0 ± 0.0	0.49 ± 0.18 b	0.34 ± 0.01 b
Frassineto -M	3.0 ± 0.0	0.82 ± 0.05 b	0.48 ± 0.04 b
Frassineto +M	1.0 ± 0.0	0.00 ± 0.00 a	0.00 ± 0.00 a
Bologna -M	2.0 ± 0.0	0.64 ± 0.03 b	0.45 ± 0.03 b
Bologna +M	1.0 ± 0.0	0.00 ± 0.00 a	0.00 ± 0.00 a

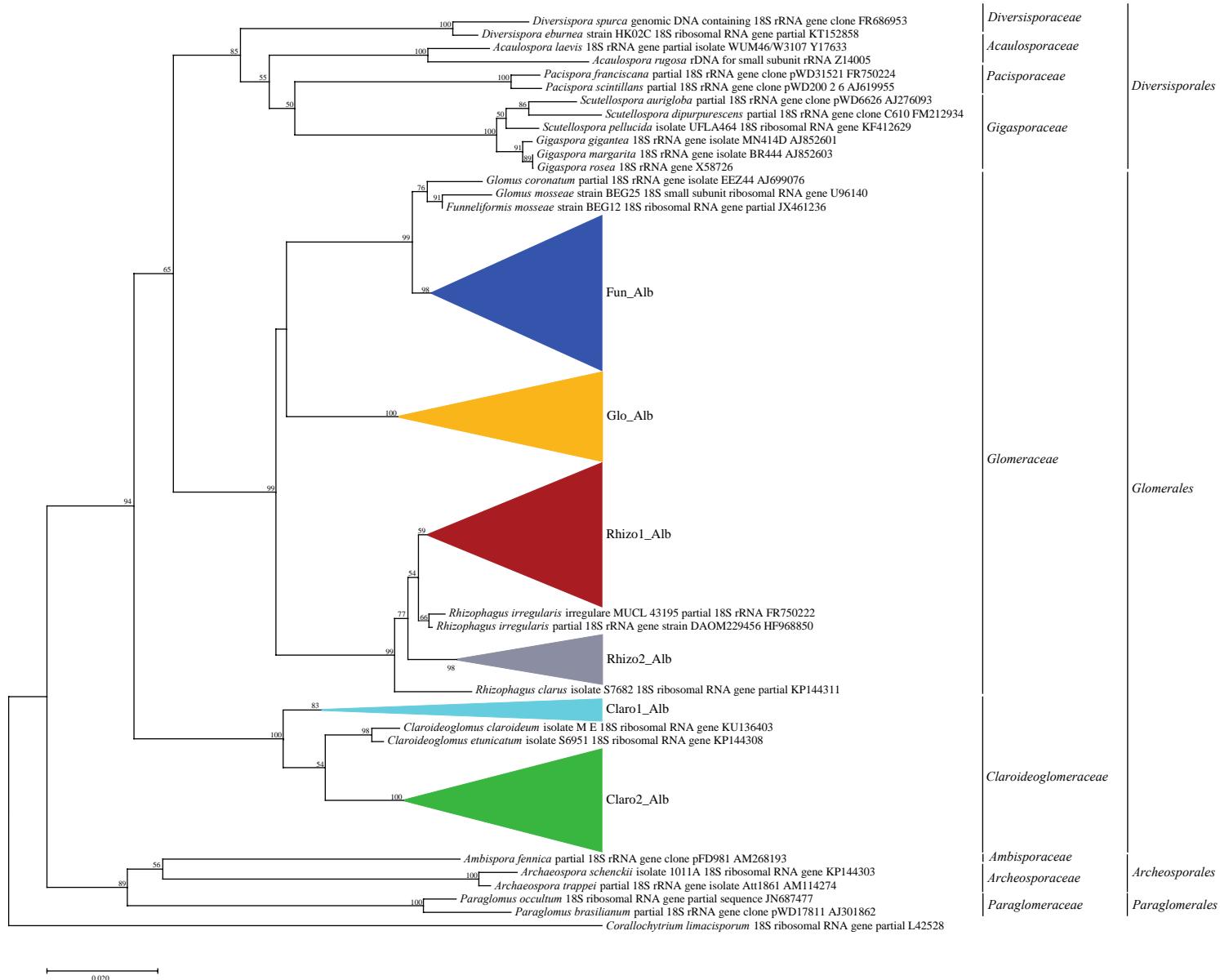
<sup>a</sup>Values are means ± SE of three replicate plots for each treatment. Values in the same column followed by different letters are statistically different, according to Tukey's-b test ( $P \leq 0.05$ ). The values that did not show any statistical difference were reported only with SE.

**Supplementary Table 3.** PERMANOVA on the effect of arbuscular mycorrhizal fungal inoculation (AMF inoc) and bread wheat genotype (Genotype) on the AMF community structure in wheat roots.

Treatment	Total df	Pseudo-F	P (perm)
AMF inoc <sup>†</sup>	1	149.12	<b>0.001<sup>‡</sup></b>
Genotype	2	172.73	<b>0.001</b>
AMF inoc x Genotype	2	177.89	<b>0.001</b>
<i>PERMDISP</i>			
AMF inoc <sup>†</sup>	-	-	0.542
Genotype	-	-	<b>0.002</b>

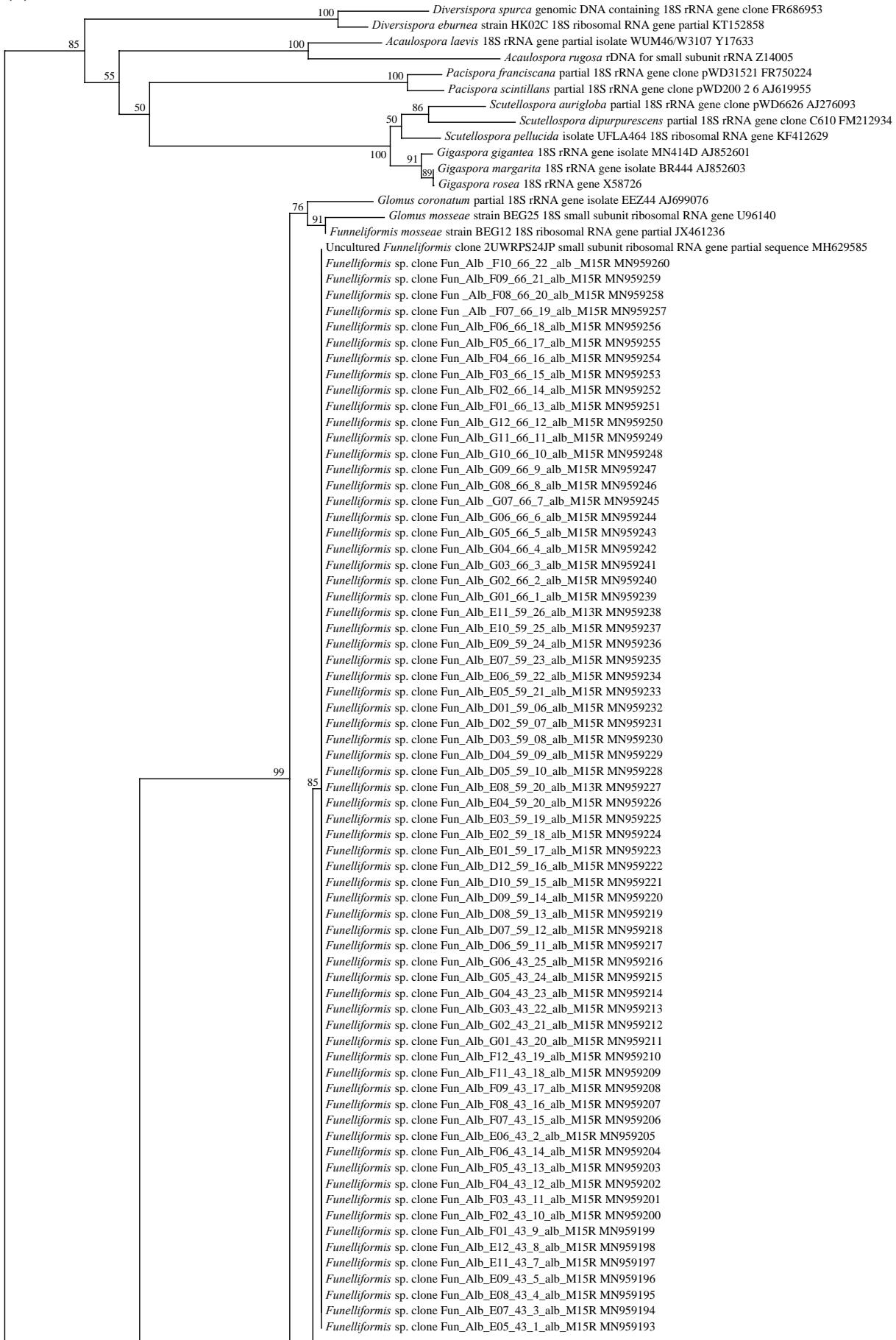
<sup>†</sup>PERMANOVA was performed following a split-plot design with AMF inoc as main-plot factor and genotype as subplot factor and with three replicate plots per treatment: AMF inoc (inoculated and mock inoculated) and genotype (Autonomia B, Frassineto and Bologna).

<sup>‡</sup>In bold statistically significant values in the PERMANOVAs ( $P \leq 0.05$ ).



**Figure S1.** Collapsed Neighbour-Joining (NJ) tree of 504 arbuscular mycorrhizal fungal (AMF) sequences retrieved in the roots of inoculated and not inoculated the three bread wheat genotypes (Autonomia B, Frassineto and Bologna) and 31 representative AMF sequences. NJ tree is based on the sequences obtained from the amplification of the partial 16 SSU rRNA gene. The AMF taxa were assigned to Molecular Operational Taxonomic Unit (MOTU) by BLAST against the NCBI database and by clustering the sequences  $\geq 97\%$  similarity threshold. The name of each MOTU is composed by the name of the AMF genus and the abbreviation of the location where the samples were collected (Alberese, Grosseto, Italy).

(a)



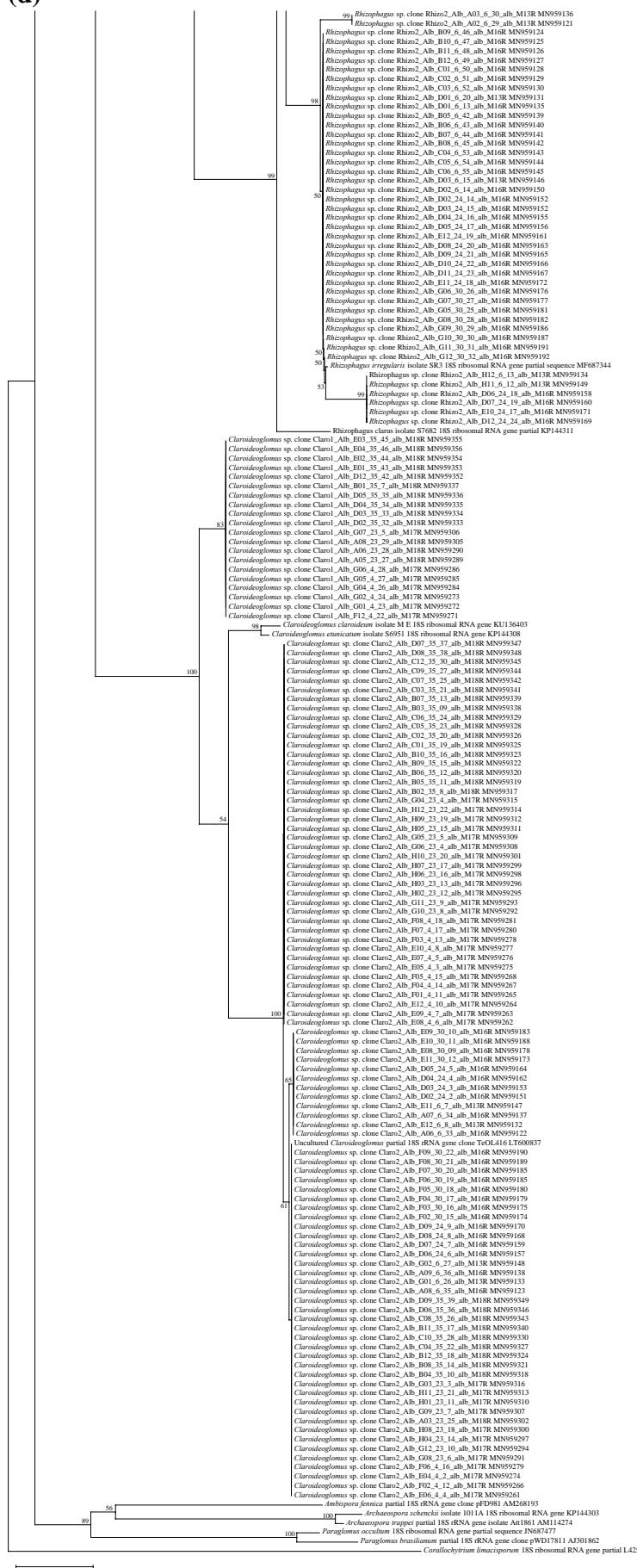
(b)

98	<i>Funneliformis</i> sp. clone Fun_Alb_C08_34_48_alb_M15R MN959000
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	<i>Funneliformis</i> sp. clone Fun_Alb_B05_11_30_alb_M14R MN958910
	<i>Funneliformis</i> sp. clone Fun_Alb_B07_11_32_alb_M14R MN958911
	<i>Funneliformis</i> sp. clone Fun_Alb_B08_11_33_alb_M14R MN958911
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	<i>Funneliformis</i> sp. clone Fun_Alb_B10_11_35_alb_M14R MN958911
	<i>Funneliformis</i> sp. clone Fun_Alb_B11_11_36_alb_M14R MN958911
	<i>Funneliformis</i> sp. clone Fun_Alb_B12_11_37_alb_M14R MN958911
	<i>Funneliformis</i> sp. clone Fun_Alb_C01_11_38_alb_M14R MN958911
	<i>Funneliformis</i> sp. clone Fun_Alb_C02_11_39_alb_M14R MN958911
	<i>Funneliformis</i> sp. clone Fun_Alb_C06_11_43_alb_M14R MN958920
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	<i>Funneliformis</i> sp. clone Fun_Alb_F01_16_19_alb_M14R MN958949
	<i>Funneliformis</i> sp. clone Fun_Alb_F02_16_20_alb_M14R MN958950
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	<i>Funneliformis</i> sp. clone Fun_Alb_G05_16_35_alb_M14R MN958965
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	<i>Funneliformis</i> sp. clone Fun_Alb_G07_16_37_alb_M14R MN958967
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	<i>Funneliformis</i> sp. clone Fun_Alb_A07_34_23_alb_M15R MN958971
	<i>Funneliformis</i> sp. clone Fun_Alb_A08_34_24_alb_M15R MN958971
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	<i>Funneliformis</i> sp. clone Fun_Alb_B05_34_33_alb_M15R MN958985
	<i>Funneliformis</i> sp. clone Fun_Alb_B06_34_34_alb_M15R MN958986
	<i>Funneliformis</i> sp. clone Fun_Alb_B07_34_35_alb_M15R MN958987
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	<i>Funneliformis</i> sp. clone Fun_Alb_B09_34_37_alb_M15R MN958989
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	<i>Funneliformis</i> sp. clone Fun_Alb_B11_34_39_alb_M15R MN958990
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	<i>Funneliformis</i> sp. clone Fun_Alb_C02_34_42_alb_M15R MN958999
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	<i>Funneliformis</i> sp. clone Fun_Alb_C05_34_45_alb_M15R MN958999
	<i>Funneliformis</i> sp. clone Fun_Alb_C07_34_47_alb_M15R MN958999
	<i>Funneliformis</i> sp. clone Fun_Alb_C08_34_46_alb_M15R MN958999
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	<i>Globose</i> sp. clone Glo_Alb_C07_70_31_alb_M16R MN959103
	<i>Globose</i> sp. clone Glo_Alb_C06_70_30_alb_M16R MN959102
	<i>Globose</i> sp. clone Glo_Alb_C05_70_29_alb_M16R MN959101
	<i>Globose</i> sp. clone Glo_Alb_C04_70_28_alb_M16R MN959100
	<i>Globose</i> sp. clone Glo_Alb_C03_70_27_alb_M16R MN959099
	<i>Globose</i> sp. clone Glo_Alb_C12_50_36_alb_M15R MN959066
	<i>Globose</i> sp. clone Glo_Alb_C11_50_35_alb_M15R MN959065
	<i>Globose</i> sp. clone Glo_Alb_C10_50_34_alb_M15R MN959064
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	<i>Globose</i> sp. clone Glo_Alb_C08_50_32_alb_M15R MN959062
	<i>Globose</i> sp. clone Glo_Alb_C07_50_31_alb_M15R MN959061
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(c)

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	77	Rhizophagus irregularis irregular MUCL_43195 partial 18S rRNA gene strain DAOM229456 HF968850

(d)



**Figure S2.** Neighbour-Joining (NJ) tree of 504 arbuscular mycorrhizal fungal (AMF) sequences retrieved in the roots of inoculated and not inoculated the three bread wheat genotypes (Autonomia B, Frassineto and Bologna) and 31 representative AMF sequences (a,b,c,d). NJ tree is based on the sequences obtained from the amplification of the partial 16 SSU rRNA gene. The AMF taxa were assigned to Molecular Operational Taxonomic Unit (MOTU) by BLAST against the NCBI database and by clustering the sequences  $\geq 97\%$  similarity threshold. The name of each MOTU is composed by the name of the AMF genus and the abbreviation of the location where the samples were collected (Alberese, Grosseto, Italy).