Inoculation of Almond Rootstock with Symbiotic Arbuscular Mycorrhizal Fungi

Project No.: 07-HORT9-Hua

Project Leader: Sui Sheng (Sylvia) Hua USDA Western Regional Research Center 800 Buchanan Street Albany, CA 94710 (510) 559-5905 <u>ssth@pw.usda.gov</u>

Project Co-Investigators: Greg T. Browne USDA-ARS Department of Plant Pathology University of California One Shields Avenue Davis, CA 95616 (530) 754-9351 gtbrowne@ucdavis.edu

> Craig Ledbetter USDA-ARS San Joaquin Valley Agricultural Sciences Center 9611 S. Riverbend Avenue Parlier, CA 93648 (559) 596-2817 <u>cledbetter@fresno.ars.usda.gov</u>

Interpretive Summary:

Soil borne arbuscular mycorrhizal (AM) fungi form a symbiotic (mutualistic) relationship with most plants. The fungui colonize the root and grow out into the soil. The hyphae network, the part of the fungi that is in the soil acts as an extension of the root system. The AM symbiosis improves plant phosphorus, nitrogen and mineral nutrition. Evidence also suggests the symbiosis provides protection of the plant against pathogens and improves plant water relations. In addition to facilitating nutrient uptake, some mycorrhizae secrete a gluey substance, called glomalin, which helps develop soil structure and soil aggregation favorable for plant growth.

The purpose of this study is to determine if specific practices associated with planting almonds (e.g., pre-plant fumigation, inoculation with AM fungi, or other factors like choice of field grown vs. potted nursery stock), have an impact on AM fungal populations to the extent subsequent tree performance is affected.

In August 2007 root samples were collected from an existing fumigation plot (Firebaugh, CA) in which trees were planted January 2007. These samples were used to develop AM inoculum. This was done by using trap cultures of Sudan to multiply the residual AM fungi. A special nutrient medium was applied to irrigate Sudangrass for boosting AM inoculum production. AM spores were produced in January 2008 and used to inoculate almond rootstocks planted in February 2008.

Objectives:

- 1. Determine if there is value in adding AM fungi inoculum, particularly at planting of bare root (field grown) and the potted-plant nursery stock.
- 2. Determine if pre-plant fumigation impacts the extent and nature of mycorrhizal populations in the soil and is this of consequence?
- 3. Characterize the mycorrhizal fungi populations present on field grown nursery stock vs. potted plants at the time of planting and during the first season after planting as well as resulting tree performance.

Materials and Methods:

Sudangrass trap cultures and inoculum production

Five samples were collected from fumigated trees and the other from non-fumigated. Roots and sourounding soil were added with a potting mixture (sand: soil at 1: 1 ratio, autoclaved before use). Fifty Sudangrass seeds were planted in each 8 inch pot on September 7, 2007. The plants were maintained in greenhouse at Albany, CA. Quarter strength Hoagland solution with 1/8 strength phosphate was used to water the plants for the first four weeks and then phosphate was withdrawn from the nutrient until December 10, 2007 (David Douds, USDA-ARS-ERRC, Philadelphia, PA and Kendra Baumgartner, USDA-ARS, Dept. of Plant Pathology, Davis, CA, personal communication). Plants were left dry until harvest in January, 2008.

The tops of dried Sudan grass were cut off; roots and soil were collected in a pan. Roots were then cut into 1-cm pieces and mixed with the center portion of the soils containing the AM spores. Two hundred grams of soil-root mixtures were used as inoculum for each almond rootstock.

Almond rootstock field trial

As planned Nonpareil almond trees on Nemaguard rootstock were planted in spring 2008 at USDA Parlier. In essence there is a split plot design contrasting fumigation in fall (October) 2007 vs. no fumigation and this is being done under the supervision of Greg Browne. There are 5 fumigated and 5 control strips and within these treatments are:

Fumigated (chloropicrin 400 #/ac)

- 1. No inoculation with AM Field grown nursery stock
- 2. Inoculation with commercial AM inoculum Field grown nursery stock
- 3. Inoculation with trap culture AM inoculum developed by Sylvia Hua Field grown nursery stock
- 4. Inoculation with trap culture AM inoculum Potted nursery stock
- 5. No inoculation with AM Potted nursery stock

Not Fumigated

Same treatments as fumigated

The trial was planted on 7 February 2008 at the San Joaquin Valley Agricultural Sciences Center. Trees used in the study were either traditional bare root (1/2" caliper) Nonpareil/Nemaguard or 3/8" caliper 'potted' Nonpareil/Nemaguard trees. Three AM treatments were imposed on the bare root trees (control, field cultured AM and commercial cultured AM) and potted trees were utilized as either controls, or field cultured AM (five total tree treatments).

The trial site had been previously (September 2007) strip fumigated (chloropicrin) to provide ten single blocks (five fumigated, five non-fumigated), randomly arranged in two 5-block rows. Each block was of sufficient length to accommodate 12 trees planted at 12 ft intervals. Pairs of trees for each treatment were planted in a randomized order for each block, with a single Monterey/Nemaguard tree at the ends of each block. In addition to being guard or border trees, the Monterey/Nemaguard trees were planted to provide adequate pollination of the trial trees in future harvests. Table 1 shows the arrangement of trees in the plot.

As planned, tree performance data to be collected will include:

- a) Trunk circumference: initial and final yearly
- b) Annual pruning weights
- c) Nutrient status: Characterize nutritional deficiencies if and when symptoms arise
- d) At end of trial: Whole tree top weight, trunk diameter, etc.

AM fungal identification, cvolonization and molecular taxonomy

Spores were extracted from soils and roots by wet sieving. Sudangrass roots cut in 1cm segments were stained with trypan blue to detect AM colonization. Spores and root colonization were viewed under a Zeiss stero-microscope.

DNA was extracted from Spores and colonized roots by CTAB method (Doyle JJ, Doyle JL. *Phytochem Bull.* 1987. 19:11–15). Primers from different region of AM fungal ribosomal genes were designed for identification AM fungal species. A list of primers is summarized in Table 2.

Extracted DNA was used a template in conjuction with a pair of primers for PCR amplication using "HotStarTaq Plus Master Mix kit" (QIAGEN, Valencia, CA, USA). PCR was carried out in a Bio-Rad DNA Engine. Amplified PCR products were subjected to agarose gel eletrophoresis, stained with ethidium bromide, viewed and recorded using a Bio-Rad gel documentation system (Bio-Rad, Hercules, CA, USA). The GeneRuler DNA Ladder Mix was used as the molecular weight marker (Fermantas, Glen Burnie, MD, USA) as well as Bio-Rad EZ Load 100bp molecular ruler. The sizes of DNA fragments and banding profiles were analyzed by using the Bio-Rad software Quantity One 4.6.3.

Results and Discussion:

Forty days after planting, tree caliper was obtained approximately 30 cm above the graft union. Trees were just beginning to push vegetative buds at this date. Without exception, all trees were beginning to grow. Trunk caliper was measured again at 140 days after planting (DAP), with no tree deaths being noted at that time (mid-June). However, visual differences in tree growth were then quite evident as related to fumigation treatment.

Initial tree size differences between bare root and potted trees complicate growth rate analyses; however, large differences in tree growth (as measured by percentage increases in trunk caliper) for both fumigation and tree AM treatments were noted at 140 DAP. Averaged across both fumigation and AM treatments, percentage increases in tree caliper were 54.9% and 102.7% for bare root and potted trees, respectively. Bare root trees planted in fumigated blocks had the largest tree calipers at 140 DAP (22.2 mm averaged across AM treatments), but potted trees planted in fumigated blocks had the highest percentage growth increase between 40 and 140 DAP (139.5% averaged across AM treatments).

Performance evalution will be continued for at least two years. *Colonization by AM fungi causes a decrease of plant growth initially according to some published literature.*

Roots of Sudan grass from trap cultures were analyzed for AM fungal colonization in fumigated and non-fumigated soils. Sudan grass plants were grown under limiting phosphate nutrient conditions for boosting colonization and inoculum production. Five hundred and fifty root fragments (I cm long) were stained with trypan blue and scored for colonization. 60% of the roots from *non-fumigated* soil were colonized. About 40% of the roots from fumigated soil were colonized. The data indicate that fumigation did reduce residual soil AM population. Root samples from planted almond tress in Parlier will be collected for evaluating root colonization later this summer.

Preliminary results from PCR and gel electrophoresis analysis indicate that *Glomus Mosseae*, *Glomus 3*, *Gigasproa rosea*, *Glomus intraradices* were present in the soil and in colonized roots. More detailed analysis is in progress.

Recent Publications:

Hua, S.-S. T. Fungal media and methods for continuous propagation of vesicular arbuscular mycorrhizal (VAM) fungi in root organ culture. US patent No. 6,576,457 BI. 2003. (Patent)

Hua, S. S. T., Taurin, A. S., Pandey, S. N., Chang, L., and Chang, P. K. Characterization of *AFLAV*, a *Tf1/Sushi* Retrotransposon from *Aspergillus flavus*. Mycopathologia, 16: 97-104. 2007.

Hua, S. S. T. Environmental Adaptation of *Pichia anomala* WRL-076 as an effective biocontrol agent for pre-harvest application. IOBC Bulletin 30 (6) 241-244. 2007.

Hua, S. S. T., Brandl, M., and Eng, J. G. Fluorescent microscopic studies in the interactions of *Pichia anomala* and *Aspergillus flavus*. IOBC Bulletin 30 (6) 165-169. 2007.

Isakeit, T., Betran, F. J., Odvody, G., and **Hua, S. S. T**. Efficacy of *Pichia anomala* WRL-076 to control aflatoxin on corn in Texas, 2005. Plant Disease Management Reports 1: FC021. 2007.

Hua, S. S. T. Saprophytic Yeast, *Pichia anomala.* US patent, pending. 2006. Chang, P. K., and **Hua, S. S. T**. 2006. Nonaflatoxigenic *Aspergillus flavus* TX9-8 competitively prevents aflatoxin accumulation by *A. flavus* isolates of large and small sclerotial morphotypes. Int. J. Food Microbiol. 108: 172-177. 2007.

Hua, S. S. T., Parfitt, D. E., and Holtz, B. A. Evaluation of a biopesticide, *Pichia anomala* WRL-076 to control *Aspergillus flavus* in a commercial orchard. Proceedings of the California Conference of Biological Control V, pp. 152-155. 2006.

Hua, S. S. T., McAlpin, C. E., and Ly, S. B. Population of *Aspergillus flavus* on pistachio buds and flowers. In: A. Mendez-Vilas (ed.). Recent Advances in multidisciplinary Applied Microbiology, Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim, Germany, pp. 440-445. 2006. (Proceedings)

Hua, S. S. T. Progress in prevention of aflatoxin contamination in food by preharvest application of *Pichia anomala* WRL-076. In: A. Mendez-Vilas (ed.). Recent Advances in multidisciplinary Applied Microbiology, Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim, Germany, pp. 322-326. 2006.

Table 1. Plot randomization plan

South Row		North Row	
Guaru	1	Guaru	3
	1		3
	2		2
	2		2
	3		5
	3		5
	4		4
	5		4
	5		1
Guard		Guard	
Guard		Guard	
	5		4
	5		4 4
	1		2
	1		2
	3		5
	3		5
	2		3
	2		3 1
	4		1
Guard	•	Guard	·
Guard		Guard	
	2		5
	2		5
	4		3
	5		2
	5		2
	3		1
	3		1
	1		4
Cuard	1	Cuard	4
Guard		Guard	
Guara	2	Guara	3
	2		3
	5		4
	5		4
	3		2
	ა 1		∠ 1
	1		1
	4		5
	4		5
Guard		Guard	

Planted on 7 February 2008

1/2" Bare root Nonpareil/Nemaguard 1/2" Monterey/NG (guard trees) 1/4" - 3/8" potted NonP/NG

Trt 1 - Bare root control

Trt 2 - Bare root w/greenhouse inoc.

Trt 3 - Bare root w/commercial inoc.

Trt 4 - Potted

control Trt 5 - Potted w/ greenhouse inoc.

Guard	Guard				
	4		5		
	4		5		
	3		3		
	3		3		
	1		1		
	1		1		
	2		4		
	2		4		
	5		2		
	5		2		
Guard		Guard			

•

Table 2. List of primers for Arbuscular Mycorrhizal fungal identification and map of ribosomal structural genes

Mycorrhizae Primers



Forward Primers Reverse Primers

Forward	Forward Sequence	Reverse	Reverse Sequence	PCR/Seq	Species or Region
ITS1*	TCCGTAGGTGAACCTGCGG	ITS4	TCCTCCGCTTATTGATATGC	PCR2	Internal transcribed spacer (ITS) Region
ITS1F*	CTTGGTCATTTAGAGGAAGTAA	GIGA5.8R	ACTGACCCTCAAGCAKGTG	PCR2	Gigasporaceae (ITS and 5.8S Region)
ITS1F*	CTTGGTCATTTAGAGGAAGTAA	GLOM5.8R	TCCGTTGTTGAAAGTGATC	PCR2	Glomus mosseae/intraradices group
					(ITS and 5.8S)
ACAU1660*	TGAGACTCTCGGATCGGG	ITS4	TCCTCCGCTTATTGATATGC	PCR2	Acaulosporaceae sensu stricto
					(part of 18S(SSU), ITS, 5.8S)
ARCH1311*	TGCTAAATAGCTAGGCTGY	ITS4	TCCTCCGCTTATTGATATGC	PCR2	A. gerdemannii/A. trappei group/
					G. occultum/G. brasilianum group
					(part of 18S(SSU), ITS, 5.8S)
LETC1670*	GATCGGCGATCGGTGAGT	ITS4	TCCTCCGCTTATTGATATGC	PCR2	Glomus etunicatum/claroideum group
					(part of 18S(SSU), ITS, 5.8S)
GLOM1310*	AGCTAGGCTTAACATTGTTA	ITS4	TCCTCCGCTTATTGATATGC	PCR2	Glomus mosseae/intraradices group
				T	(part of 18S(SSU), ITS, 5.8S)
G. Mosseae***	AAAGCCTTCGGATTCGCGG	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Glomus Mosseae(LSU)
Glomus 2***	CATGAGGAGGAAACCCTCG	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Glomus 2 (LSU)
Glomus 3***	GAGCGTGAGGAGTTAAACGC	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Glomus 3 (LSU)
Glomus 4***	TCCTTATTTGCAAATTTGTATTC	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Glomus 4 (LSU)
Glomus 5***	GCCTTCGTTGCTTGCGTTA	FLR4	ТАСӨТСААСАТССТТААСӨАА	PCR2	Glomus 5 (LSU)
A.spor. 1***	CAACATGAGGGTTCGCTTTC	FLR4	ТАСӨТСААСАТССТТААСӨАА	PCR2	Acaulosporaceae 1
					(LSU)
A.spor. 2***	TGTTCCCCCGGGAGCGATCT	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Acaulospora 2 (LSU)
A.spor. 3***	TTCGCTCGCGTACTTTCCGG	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Acaulospora 3 (LSU)
S.spor. 1***	GAACCTAACCTTGAAGTGCAC	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Scutellospora 1 (LSU)
S.spor. 2***	AGGGGAAACTCTGAGTGCA	FLR4	TACGTCAACATCCTTAACGAA	PCR2	Scutellospora 2 (LSU)
NS5	AACTTAAAGGAATTGAGGGAAG	ITS4	TCCTCCGCTTATTGATATGC	PCR1	Universal Primers
					(part of 18S (SSU), ITS, 5.8S)

Almond Board of California

ITS1 TCOGRAGETGACCTGOGG NDL22 TGETCOGTETTTCAAGACG PCR1 Universal Primers - First amplification (roots) (TS, 5.8S and LSU) LR1*** GCATATCAATAAGOGGAGGA NDL22 TGGTCOGTUTTCAAGACG PCR20 2.01 ampl. for Coning to sequence (LSU) LR1 GCATATCAATAAGOGGAGGA FLR2 GTGCTUTTCAAGACG PCR20 2.01 ampl. for Coning to sequence (LSU) LR1*** GCATATCAATAAGOGGAAGCA FLR2 GTGCTUTTCAAGACCATTCCTAACOT PCR10 Universal Primers - First amplification (roots) (re end of LSU) 523*** GTAGGUTTAATAGOGGAAGCA FLR2 GTGCTUTTAAAGCCATTACGT PCR20 2.01 ampl. for Coning to sequence (LSU) 523*** GTAGGUTTAATAGOGGAAGCA FLR2 GTGCTUTTAAAGCCATACGT PCR2 C. mosseee (D2 & D3 of LSU) 523*** GTAGGUTTAATAGOGGAAGCA 23.46 GCGUTTCAAACCACTACTC PCR2 G. interedices (D1 D2D3 of LSU) 1TS3 CCAATCGATGAAGAACCCAGC NDL22 TGGTCCGUTTCAAGACG PCR2 G. interedices (D1 D2D3 of LSU) 1TS4 CAATGGATGAAGAACCAGCAGC NDL22 TGGTCCGUTTCAAGACG PCR2 G. interedices (D1 D2D3 of LSU) 1TS4 CAATGGATGAAGAACCAGCAGC NDL22 TGGTCCGUTTCAAGACG PCR2 G. interedices (D1 D2D3 of LSU) 1TS4 CAATGGATGAGAAGCAGCAGC NDL22 TGGTCCGUTTCAAGACG PCR2	M13F	GTAAAACGACGGCCAG	M13R	CAGGAAACAGCTATGAC		After TOPO Cloning for Sequencing
IRI** GCATATCAATAAGGGGAGGA NDL22 TGGTCGTTTTAAGGCG PCR2/CI Znd ampl. for cloning to sequence (LSU) LR1 GCATATCAATAGCGGAGGA FLR2 GTCGTTTAAGCCATAGCTC PCR1 Universal Primers - First amplification (roots) (5' end of LSU) FLR3*** TTGAAGGGAAACGATTGAGT FLR4 TACGTCAACATCGTTAACGAC PCR2/CI 2nd ampl. for cloning to sequence (LSU) FLR3*** TTGAAAGGGAAACGATTGAGGT FLR4 TACGTCAACATCGTCAACGAC PCR2/CI 2nd ampl. for cloning/seq. for primer design (FLR3) between DI and D2 of LSU. FLR4 is in the D2 domain) 523*** GTICGGTTGATCAACATCGGG FLR2 GTCGTTTAAAGCCATTACGTC PCR2 G. mossaee (D2 & D3 of LSU) 623*** GTCGGTTGATCAAGACGCGGGA 23.46 GTCGTTTAAAGCCATTACGTC PCR2 G. mossaee (D2 & D3 of LSU) 1R3*** GCATATCAATAAGCGGAGGA 23.49 GTCGTTTAAAGCCATTACGTC PCR2 G. mossaee (D1 oc LSU) 1F4*** TAATCACCTGGTTGCAAGACGCAGGC NDL2 TGGTCGTTTTAAGCCATTACGTC PCR2 A. pauLinas (D1 of LSU) 1F4*** TAATCACATGAGGGAGGA 822 TGGTCGTTGTCGAAGCG PCR2 G. Intracatalcase (367bp) 1F4*** <td>ITS1</td> <td>TCCGTAGGTGAACCTGCGG</td> <td>NDL22</td> <td>TGGTCCGTGTTTCAAGACG</td> <td>PCR1</td> <td>Universal Primers - First amplification (roots) (ITS, 5.8S and LSU)</td>	ITS1	TCCGTAGGTGAACCTGCGG	NDL22	TGGTCCGTGTTTCAAGACG	PCR1	Universal Primers - First amplification (roots) (ITS, 5.8S and LSU)
LR1 GCATATCAATAAGCGGAGGA FLR2 GTGGTTAAAGCCATTACGTC PCR1 Universal Primers - First amplification (roots) (5' end of LSU) Used as tat & 2nd ampl. FLR3*** TTGGAAGGGAAACGATTGAAGT FLR4 TACGTCAACTCCTTAAGGA PCR2/Cl 2nd ampl. for cloning/seq. for primer design (FLR3 is in the D2 domain) 5.23*** GTACGGTTAGTCAACATCG FLR2 GTGGTTTAAAGCCATACGTC PCR2/Cl 2nd ampl. for cloning/seq. for primer design (FLR3 is between D1 and D2 of LSU, FLR4 is in the D2 domain) 5.23*** GTACGGTTAGTCAAGACGAGC FLR2 GTGGTTTAAAGCCATACGTC PCR2 G. mosseee (D2 & D3 of LSU) 8 23*** GTACGGTTAGTCAGAGGAGA 23.46 GTATCCCTAAGACCAATACGT PCR2 G. mosseee (D1 & D2 of LSU) 1TS3 GCATTCAATAAGCGAAGCC NDL22 TGGTCGTGTTCAAGACG PCR2 A. paulinae (D1 of LSU) 1f4**** TAAATCCCGATGCTGGTC TAAATCCCCAAGCT PCR2 G. morseee (267bp) (D1 and part D2 of LSU) 1LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCAGGCTCCACGCT PCR2 Gigagora rosea (630bp) (D1 and part D2 of LSU) 1LR1*** GCATATCAATAGCGGAGGA 8.22 GATCCACAGTCAGCT PCR2 Gigagora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAGCGGAGGA 8.22 GATCCACGTCACGCT PCR2 Gigagora rosea (630bp) (D1 and part D2 of LSU) LR1**** </td <td>LR1**</td> <td>GCATATCAATAAGCGGAGGA</td> <td>NDL22</td> <td>TGGTCCGTGTTTCAAGACG</td> <td>PCR2/CI</td> <td>2nd ampl. for cloning to sequence (LSU)</td>	LR1**	GCATATCAATAAGCGGAGGA	NDL22	TGGTCCGTGTTTCAAGACG	PCR2/CI	2nd ampl. for cloning to sequence (LSU)
Image: State	LR1	GCATATCAATAAGCGGAGGA	FLR2	GTCGTTTAAAGCCATTACGTC	PCR1	Universal Primers - First amplification (roots)
FLR3*** Used as fat & 2nd ampl. FLR3*** TTGAAAGGGAAACGATTGAAGT FLR4 TACGTCAACATCCTTAACGAA PCR2/C 2nd ampl. for cloning/seq, for primer design (FLR3 is between D1 and D2 of LSU, FLR4 is in the D2 domain) 5.23*** GTGCGGTTGATCAGAACTCCG FLR2 GTGCTTTAAGCACATTAGTC PCR2 G. mosseea (D2 & D3 of LSU) 8.23*** GTTCGGTTGATCAGAACGCAGC FLR2 GTGCTTTAAGCCATTAGTC PCR2 G. mosseea (D2 & D3 of LSU) LR1*** GCATTCAATAAGCGAGGA 23.46 GCTATCCGTTAACGAATCGT PCR2 Gig. Rosea (D1 & D2 of LSU) ITS3 GCATCGATGAAGAACGCAGC NDL22 TGGTCGTGTTTCAAGACG PCR2 A. paulinae (D1 of 1SU) f4*** TAAATCTCCGAGOTTTCCTGCC r1 TOATCTTTCCCCACAGATCGC PCR2 Glomus osp. (D1 and part D2 of 1SU) f4*** GCATTCAATAAGCGGAGGA 8.22 AACTCCTCAACGCTCACGAGA PCR2 G. Intraadloss (455bp) (D1 and part D2 of 1SU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCCAATCACGAAGCACAGCA PCR2 Gigasporta cosea (63Dp) (D1 and part D2 of 1SU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCCAATCACGCAAGCATTACGT PCR2 Gigasporta cosea (63Dp) (D1 and part D2 of 1SU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCCAATACCCAATCACT PCR2 Giomus mosseae (Af5Dp) (D1 and part D2 of 1SU) <						(5' end of LSU)
FLR3*** TIGAAAGGGAAACGATTGAAGT FLR4 TAGGTCAACATCCTTAACGAA PCR2/CI 2nd ampl. for cloning/sec, for primer design (FLR3 is between D1 and D2 of LSU). 5.23*** GTACGGTTGAACACTCG FLR2 GTCCGTTAAACGCATACGTC PCR2 G. mossee (D2 & D3 of LSU) 8.23*** GTTCGGTTGAACACATCG FLR2 GTCGGTTAAACGCATACGTC PCR2 G. mossee (D2 & D3 of LSU) 8.23*** GTTCGGTTGAACACATCGC FLR2 GTGCGTTAAACGCATACGTC PCR2 G. mossee (D2 & D3 of LSU) 8.23*** GTTCGGTTGAACACATCGCT FLR2 GTGCGTTTAAAGCCATACGTC PCR2 G. mossee (D2 & D3 of LSU) 8.23*** GCATATCAATAAGCGAAGGA 23.46 CGTTCGTCTATCCAATACGTC PCR2 G. mossee (D1 & D2 Of LSU) ITS3 GCATATCAATAGGAGAGCC NDL22 TGGTCGTGTTTCAAGAGC PCR2 Glomus sp. (D1 and part D2 of LSU) I4**** TAATCTACATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACAGA PCR2 G. intraradices (458pp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 GAATCCAAGTCAGCATCGT PCR2 Gigrappora rossea (G30p) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 GAATCCACAGTCAGCATCGT PCR2 Gigrappora rossea (G30p) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 GATCCACAGTCAGCATCGT PCR						Used as 1st & 2nd ampl.
5.23*** GTACGGTTAGTCAACATCG FLR2 GTCGTTTAAAGCCATACGTC PCR2 G. mosseae (D2 & D3 of LSU) 8.23*** GTTCGGTTGATCAGATCGGCT FLR2 GTGCGTTAAAGCCATACGTC PCR2 G. mosseae (D2 & D3 of LSU) 8.23*** GTTCGGTTGATCAGATCAGGTC FLR2 GTGCTTTAAAGCCATACGTC PCR2 G. intradices (D1 & D2 & D3 of LSU) 8.23*** GCATCGATGAGAACGCAGC NDL22 TGGTCGTGTTCCAGAGCG PCR2 G. intradices (D1 & D2 & D3 of LSU) ITS3 GCATCGATGAGAGACGCAGCC NDL22 TGGTCGTGTTTCAAGAGG PCR2 A. paulinae (D1 of LSU) f4*** TAAATCTCCCGAGGTCTCGTGC r1 TCATCTTTCCCTCACGGTCAGCAGCA PCR2 Glomus sp. (D1 and part D2 of LSU) f4*** GCATTCAATAAGCGGAGGA NDL22 TGGTCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) (D2 and part D2 of LSU) f2*** GCATATCAATAAGCGGAGGA 8.22 ACCTCTCACGCTCACCAA PCR2 G. intradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTGAGCACTTCGT PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Gigaspora rosea (630bp) (D1 and part D	FLR3***	TTGAAAGGGAAACGATTGAAGT	FLR4	TACGTCAACATCCTTAACGAA	PCR2/CI	2nd ampl. for cloning/seq. for primer design
5.23****0 GTGGGTTGATACACATGG FLR2 GTGGTTTAAAGCCATTACGT PCR2 G. mosseae (D2 & D3 of LSU) 8.23***0 GTGGGTTGATACAATAGCGGATGA 23.46 GCATATCAATAGCG AGAGA 23.46 GCATACCATACGAT PCR2 G. Intrandices (D1 D2 D3 of LSU) LR1***0 GCATCGATGAAGAACGCAGC NDL22 TGGTCCGTGTTTCAAGACG PCR2 Gig. Rosea (D1 & D2 of LSU) f6*** TAATCTCACGAGGTTTCCTTOCC F1 TCATCTTCCCTCAGGGTACTG PCR2 Glomus age. f4*** TAATCTCACGAGGTTCCTTOCC F1 TCATCTTCCCCCAGGTACTG PCR2 Glomus age. f4**** TAATCTACTGGTTCCCAGGTC F2 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) f1 TCATCTTCCCCAGGTCCCAGGA NDL22 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) f01 and part D2 of LSU) CTTTTGAGCTCGGGGAGGA 8.22 AACTCCCACAGCAGCAGCA PCR2 Glomus mosseae (367bp) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCCACAGCACCCAGGA PCR2 Glomus mosseae (367bp) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCCACAGCACCCAGGA PCR2 Glomus mosseae (363bp) LR1*** GCATATCAATAAGC						(FLR3 is between D1 and D2 of
5.23*** GTACGGTTAGTCAACATCG FLR2 GTGGTTTAAAGCCATACGTC PCR2 G. mossee (D2 & D3 of LSU) 8.23*** GGTGGTTGACAGTCCGCT FLR2 GTGGTTTAAAGCCATACGTC PCR2 G. mossee (D1 8D2 of LSU) 8.23*** GGTGGTTGACAGTCCGCT FLR2 GTGGTTAAAGCCATACGTC PCR2 G. mossee (D1 8D2 of LSU) ITTS3 GCATCGATGAAGACGCAGC NDL22 TGGTCCGTGTTTCAAGACG PCR1 Universal Primer (5.85 to LSU) 15*** TAAATCTACCTGGGTTCCTAGGT r1 TCATCTTTCCCTAAGACG PCR2 A. paulinae (D1 of LSU) 5.21*** CCTTTTGAGCTCGGGTCCGTGT NDL22 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACAGA PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACAGA PCR2 Glomus mosseae (367bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCACATA PCR2 Gigagora rosea (43bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Glomus mosseae (part of LSU) LR1***						LSU, FLR4 is in the D2 domain)
8.23*** GTGGGTTGATCAGATCGACTCAGTC FLR2 GTGGTTTAAAGCCGATAGCTC PCR2 G. Intradices (01.02.03 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.46 GCATATCCATAACC PCR2 Gig. Rosea (D1&& D2.D3 of LSU) ITS3 GCATCGATGAAGAACGCAGC NDL22 TGGTCGTTTCAAGACG PCR2 Gig. Rosea (D1&& D2.D3 of LSU) f4*** TAAATCTCCGATGATCCAGGTTTCCTTGGC r1 TCATCTTTCCCCAGGTACTTG PCR2 A. paulinae (D1 of LSU) f4*** TAAATCTCCGGTTCCCAGGTC r2 TGATCCGTGGTTTCCAAGACG PCR2 Glomus sp. f4*** TAAATCTCCGGTCCGGTCTCGTG NDL22 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) (D2 of LSU) f1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCCACGA PCR2 Gig. Intradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCCAGGA PCR2 Gig. Social castanea (615bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCAGTCACTT PCR2 Glomus mosseae (part of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCAGTTACGT PCR2 Glomus mosseae (part of LSU) LCha2-rtF	5.23***	GTACGGTTAGTCAACATCG	FLR2	GTCGTTTAAAGCCATTACGTC	PCR2	G. mosseae (D2 & D3 of LSU)
LR1*** GCATATCAATAGECGAGGA 23.46 CCTATCCGTAATCCAATACTG PCR2 Gig. Rosea (D1&D2 of LSU) ITS3 CCATCGATGAAGAACGCAGC NDL22 TGGTCCGTGTTTCAAGACG PCR1 Universal Primer (5.85 to LSU) f6*** TAAATCTCCGAGGTTTCCTTGCC r1 TCATCTTTCCCAAGGTC PCR2 A. paulinae (D1 of LSU) f4*** TAAATCTACCTGGTTCCCAGGTC r2 TGGTCCGTGTTTCCAAGGCG PCR2 Glomus sp. (D1 and part D2 of LSU) 5.21*** CCTTTTGAGCTCGGTCCTGTG NDL22 TGGTCCGTGTTTCCAAGGCG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 ACTCCTCACGCTCCACGGA PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 ACTCCTCACGCTCCACGGA PCR2 Glomus mosseae (367bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 ACTCCTCACGCTCCACGGT PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Gigaspora rosea (610bp) (D1 and part D2 of LSU) S25Forward *** ATCAACCTTTGAGCTCG FLR2 GTCGTTTAAGCCAACTTCGT PCR2 Glomus mosseae (part of LSU) </td <td>8.23***</td> <td>GTTCGGTTGATCAGATCCGCT</td> <td>FLR2</td> <td>GTCGTTTAAAGCCATTACGTC</td> <td>PCR2</td> <td>G. intraradices (D1,D2,D3 of LSU)</td>	8.23***	GTTCGGTTGATCAGATCCGCT	FLR2	GTCGTTTAAAGCCATTACGTC	PCR2	G. intraradices (D1,D2,D3 of LSU)
ITS3 GCATCGATGAAGAACGCAGC NDL22 TGGTCCGTGTTTCAAGACG PCR1 Universal Primer (5.88 to LSU) f6**** TAAATCTCCGAGGGTTTCCTTGGC r1 TCATCTTTCCCCAGGTACTG PCR2 A paulinae (D1 of LSU) f4*** TAAATCTACCGGAGGTTCCCTGGT r2 TGGACCCAAAACCGACGAACTG PCR2 Glomus sp. (D1 and part D2 of LSU) 5.21*** CCTTTTGAGCTCGGTCCGTG NDL22 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATACAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACGA PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCCACAGTCAGCACGTCA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCGAACTTCGT PCR2 Scuttellospora castanea (615bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA FLR2 GTGGTTTAAAGCGAATTACGT PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) 5.25Forward *** ATCAACCTTTGAGCTCG FLR2 GTGGTTTAAAGCCAATTACGT PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LeChs2-rtF TTCGGTTAAGCGTTTGTGAGCTG FLR2 GTGGAGCACTTGTTGTTGTGAGCATTACGT P	LR1***	GCATATCAATAAGCGGAGGA	23.46	GCTATCCGTAATCCAATACTG	PCR2	Gig. Rosea (D1&D2 of LSU)
f6***0 TAAATCTCCGAGGTTTCCTTGCC r1 TCATCTTTCCCTCAGGTACTTCC PCR2 A. pullinae (D1 of LSU) f4***0 TAAATCTCCGAGGTTTCCTTGCC r2 TGAACCCAAAACCCACAAACCC PCR2 Glomus sp. 5.21*** CCTTTTGAGCTCGGTCTCGTG NDL22 TGGTCCGTGTTTCCAAGACG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCAGGCTCCACGA PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCAGGTCACGCA PCR2 Glomus mosseae (367bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCAGGCTCGCACGAA PCR2 Glomus mosseae (367bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Glomus mosseae (615bp) (D1 and part D2 of LSU) LEChs2-rtF TCCGTTAAGCGGCTCATGA Lechs2-rtR CTCGAGCACCTTGTTGTCTC PCR2 Glomus mosseae (part of LSU) Mtchit1-rtF GGTGATGGTTGTCCAGTCAATG Mtchit1- rtR CTGTCGTCACGGTGCTTGAS RT Medicago truncarula R108 (Chitinase 1 (GenBank # : Y10373	ITS3	GCATCGATGAAGAACGCAGC	NDL22	TGGTCCGTGTTTCAAGACG	PCR1	Universal Primer
f6*** TARATCTCCGAGGTTTCCTTGGC r1 TCATCTTTCCCTCAGGTACTTG PCR2 A. paulinae (DI of LSU) f4*** TARATCTACCTGGTTCCCAGGT r2 TGAACCCAAAACCG PCR2 Glomus sp. (DI and part D2 of LSU) 5.21*** CCTTTGAGCTGGGTCTCGTG NDL22 TGGTCGTGTTTCAAGAGC PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 ACTCCTCACGCTCACGAA PCR2 G. intraradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCAGCATGCTA PCR2 Gigaspra rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Gigaspra rosea (615bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Glomus mosseae (part of LSU) 5.25Forward *** ATCACCTTTTGAGCTCG FLR2 GTCGTTTAAGCCACTTGTTTTTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG rtR Medicago truncarula R108 (Di tinase 1 GenBank # : Y10373						(5.8S to LSU)
f4*** TARATCTACCTGGTTCCCAGGTC r2 TGAACCCAAAACCCACCAAACTG PCR2 Glomus sp. (D1 and part D2 of LSU) 5.21*** CCTTTTGAGCTCGGTCCTGTG NDL22 TGGTCCGTGTTTCAAGAGCG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGGCTCCACAGA PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGGCTCCACAGA PCR2 Glagspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCTCGTCG PCR2 Glagspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Glamus mosseae (part of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCGTTTAAAGCCAATTAGCTC PCR2 Glomus mosseae (part of LSU) LCh2>-rtF TTCGGTTAAGCGGCTCATGA Lechs2-rtR CTCGAGCACCCTTGTTGTTCTC PCR2 Glomus mosseae (part of LSU) Lechs2-rtF GGTGATGGTTGTCGAGTCAATG Lechs2-rtR CTCGAGCACCCTTGTTGTTCTC RT Lycopersion esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGG	f6***	TAAATCTCCGAGGTTTCCTTGGC	r1	TCATCTTTCCCTCACGGTACTTG	PCR2	A. paulinae (D1 of LSU)
5.21*** CCTTTTGAGCTCGGTCTCGTG NDL22 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGGTCCACAGA PCR2 G. intraradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCATGCTA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCAAGTCAGCATTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Glomus mosseae (part of LSU) LR1*** GCATATCAATAAGCGGAGGA LeChs2-rtR GTCGAGCCCTTGTTGTCTC PCR2 Glomus mosseae (part of LSU) LC1*** TTCGGTTAAGCGGCTCATGA LeChs2-rtR CTCGAGCACCCTTGTGTTGTCTC PCR2 Glomus mosseae (part of LSU) LeChs2-rtF TTCGGTTAAGCGGCCCATGA LeChs2-rtR CTCGAGCACCCTTGTTGTCTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG rtR Medicago truncarula R108 Chitinase 1 GenBank # : Y10373	f4***	TAAATCTACCTGGTTCCCAGGTC	r2	TGAACCCAAAACCCACCAAACTG	PCR2	Glomus sp.
5.21*** CCTTTTGAGCTCGGTG NDL22 TGGTCCGTGTTTCAAGACG PCR2 Glomus mosseae (367bp) (D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACAGA PCR2 G. intraradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCAGCATGCTA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCAACTTCGT PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Gigaspora rosea (615bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Glomus mosseae (part of LSU) Lchs2-rtF ATCAACCTTTTGAGCTCG FLR2 GTCGTTTAAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) Lechs2-rtF TTCGGTTAACGCGCTCATGA Lechs2-rtR CTCGAGCACCTTGTTGTTCTC RT Lycopersicon esculentum (chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						(D1 and part D2 of LSU)
LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACAGA PCR2 G. intraradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCATGCTA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) 5.25Forward *** ATCAACCTITTGAGCTCG FLR2 GTCGTTTAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) 5.25Forward *** ATCAACCTITTGAGCTCG FLR2 GTCGTTTAAGCCATTGCTC PCR2 Glomus mosseae (part of LSU) Lechs2-rtF TTCGGTTAAGCGGCTCATGA Lechs2-rtR CTCGAGCACCCTTGTTGTTCTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGGTCGTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373	5.21***	CCTTTTGAGCTCGGTCTCGTG	NDL22	TGGTCCGTGTTTCAAGACG	PCR2	Glomus mosseae (367bp)
LR1*** GCATATCAATAAGCGGAGGA 8.22 AACTCCTCACGCTCCACAGA PCR2 G. intraradices (455bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCATGCTA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) 5.25Forward *** ATCAACCTTITGAGCTCG FLR2 GTCGTTTAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) 5.25Forward *** ATCAACCTTITGAGCTCG FLR2 GTCGTTTAAGCCCATTGCT PCR2 Glomus mosseae (part of LSU) 1eChs2-rtF TTCGGTTAAGCGGCTCATGA LeChs2-rtR CTCGAGCACCCTTGTTGTTCT RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						(D2 of LSU)
LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCATGCTA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) 5.25Forward *** ATCAACCTITTGAGCTCG FLR2 GTCGTTTAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) LeChs2-rtF TTCGGTTAAGCGGCTCATGA Lechs2-rtR CTCGAGCACCCTGTGTTGTTCTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG TtR CTGTCGTCGCGGTCCTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373	LR1***	GCATATCAATAAGCGGAGGA	8.22	AACTCCTCACGCTCCACAGA	PCR2	G. intraradices (455bp)
LR1*** GCATATCAATAAGCGGAGGA 23.22 GAATCACAGTCAGCATGCTA PCR2 Gigaspora rosea (630bp) (D1 and part D2 of LSU) LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) 5.25Forward *** ATCAACCTITIGAGCTCG FLR2 GTCGTTTAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) LeChs2-rtF TTCGGTTAAGCGGCTCATGA LeChs2-rtR CTCGAGCACCCTGTGTGTCTCC RT Lycopersion esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						(D1 and part D2 of LSU)
Index Index <th< td=""><td>LR1***</td><td>GCATATCAATAAGCGGAGGA</td><td>23.22</td><td>GAATCACAGTCAGCATGCTA</td><td>PCR2</td><td>Gigaspora rosea (630bp)</td></th<>	LR1***	GCATATCAATAAGCGGAGGA	23.22	GAATCACAGTCAGCATGCTA	PCR2	Gigaspora rosea (630bp)
LR1*** GCATATCAATAAGCGGAGGA 4.24 TGTCCATAACCCAACTTCGT PCR2 Scutellospora castanea (615bp) (D1 and part D2 of LSU) 5.25Forward *** ATCAACCTTITGAGCTCG FLR2 GTCGTTTAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) LeChs2-rtF TTCGGTTAAGCGGCTCATGA LeChs2-rtR CTCGAGCACCCTTGTTGTTCTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						(D1 and part D2 of LSU)
Image: Substraint state Image: Substraint state <td>LR1***</td> <td>GCATATCAATAAGCGGAGGA</td> <td>4.24</td> <td>TGTCCATAACCCAACTTCGT</td> <td>PCR2</td> <td>Scutellospora castanea (615bp)</td>	LR1***	GCATATCAATAAGCGGAGGA	4.24	TGTCCATAACCCAACTTCGT	PCR2	Scutellospora castanea (615bp)
5.25Forward *** ATCAACCTTTTGAGCTCG FLR2 GTCGTTTAAAGCCATTACGTC PCR2 Glomus mosseae (part of LSU) LeChs2-rtF TTCGGTTAAGCGGCTCATGA LeChs2-rtR CTCGAGCACCTTGTTGTCTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373					1	(D1 and part D2 of LSU)
LeChs2-rtF TTCGGTTAAGCGGCTCATGA LeChs2-rtR CTCGAGCACCCTTGTTGTTCTC RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195 Mtchit1-rtF GGTGATGGTTGTCGAGTCAATG Mtchit1- rtR CTGTCGTCACGGTGCTTGAG RT Lycopersicon esculentum cv.Micro-Tom Chalcone synthase 2 GenBank # : X55195	5.25Forward ***	ATCAACCTTTTGAGCTCG	FLR2	GTCGTTTAAAGCCATTACGTC	PCR2	Glomus mosseae (part of LSU)
Mtchitl-rtF GGTGATGGTTGTCGAGTCAATG Mtchitl- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373	LeChs2-rtF	TTCGGTTAAGCGGCTCATGA	LeChs2-rtR	CTCGAGCACCCTTGTTGTTCTC	RT	Lycopersicon esculentum
Mtchitl-rtF GGTGATGGTTGTCGAGTCAATG Mtchitl- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						cv.Micro-Tom
Mtchitl-rtF GGTGATGGTTGTCGAGTCAATG Mtchitl- rtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						Chalcone synthase 2
Mtchitl-rtF GGTGATGGTTGTCGAGTCAATG PtR CTGTCGTCACGGTGCTTGAG RT Medicago truncarula R108 Chitinase 1 GenBank # : Y10373						GenBank # : X55195
Chitinase 1 GenBank # : Y10373	Mtchit1-rtF	GGTGATGGTTGTCGAGTCAATG	Mtchit1- rtR	CTGTCGTCACGGTGCTTGAG	RT	Medicago truncarula R108
GenBank # : Y10373						Chitinase 1
						GenBank # : Y10373

GiAM-rtF	GCTCTGGTGCCGAAAGCTT	GiAM-rtR	TAACCCGTTCTAACCTATTGACCAT	RT	Glomus intraradices
					28S rDNA subunit
					GenBank # : AF396797
ITS1	TCCGTAGGTGAACCTGCGG	NL4	GGTCCGTGTTTCAAGACGG	PCR1	ITS and 26S rRNA
NL1	GCATATCAATAAGCGGAGGAAAAG	NL4	GGTCCGTGTTTCAAGACGG	PCR1/seq	D1/D2 region of LSU
LSU0061	AGCATATCAATAAGCGGAGGA			PCR1/Seq	Glomus sp. LSU rDNA , roots
LSU3f	AGTTGTTTGGGATTGCAGC			Seq.	Glomus sp. LSU rDNA , roots
LSU4f	GGGAGGTAAATTTCTCCTAAGGC			PCR2	Glomus sp. LSU rDNA , roots
LSU6f	AAATTGTTGAAAGGGAAACG			Seq.	Glomus sp. LSU rDNA , roots
LSU9f	ATTCGTTAAGGATGTTGACG			Seq.	Glomus sp. LSU rDNA , roots
LSU5r	CCCTTTCAACAATTTCACG			Seq.	Glomus sp. LSU rDNA , roots
LSU7r	ATCGAAGCTACATTCCTCC			PCR2	Glomus sp. LSU rDNA , roots
LSU8r	GGGTATCCGTTGCAATCCTC			Seq.	Glomus sp. LSU rDNA , roots
LSU0599	TGGTCCGTGTTTCAAGACG			PCR1	Glomus sp. LSU rDNA , roots
LSU0805	CATAGTTCACCATCTTTCGG			PCR1/seq	Glomus sp. LSU rDNA , roots

*Nested Primers : Use NS5 and ITS4 first PCR

**Nested primers : Use ITS1 and NDL22 first PCR

