CHAPTER 7[1]

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION AND BIOCHAR AMENDMENT ON GROWTH AND YIELD OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

7.1 ABSTRACT

A field study was conducted to investigate the interactive effects of inoculation of arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* and soil amendment with biochar on AMF root colonisation, plant growth, fruit yield and nutrient uptake of tomato (*Solanum lycopersicum* L.). A 2 × 2 factorial experiment arranged in a randomised complete block design included two *G. mosseae* treatments (inoculated at sowing or uninoculated) and two biochar levels (5 t ha^{-1} or unamended) with six replications were used. At mid-season, 12 weeks after transplanting, biochar addition did not increase the percentage of AMF root colonisation on tomato plants. Inoculation with *G. mosseae* increased dry shoot mass and total plant biomass by 11% and 9%, respectively, whereas biochar amendment decreased dry root mass by 13%. Similarly, biochar amendment lowered shoot K content by 9% when compared to unamended plants. Generally, inoculation with *G. mosseae* and biochar did not affect shoot Ca, B, Cu, Mn, Na or Zn but lowered shoot P by 26% when compared to uninoculated plants. Inoculation with AMF and

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biochar combined did not affect tomato growth variables, yield or yield components. Microbial community assessment revealed that AMF-treated plants shared specific bacterial species, which they did not share with untreated-AMF plants. Interestingly, when AMF-treated plants were transplanted with biochar, fungal diversity was different to treatments without biochar. Nursery inoculation with AMF had the highest dominant bacteria in the rhizosphere. Tentative identification of Denaturing Gradient Gel Electrophoresis (DGGE) suggested that *Alternaria* spp. were only found in untreated plots, whereas *Penicillium pinophilum* was only restricted to the AMF-treated sample without biochar. In conclusion, combined application of AMF and biochar had no effect on AMF root colonisation and performance of tomato plants, but altered the composition of microbes in the rhizosphere of tomato.

Keywords: Arbuscular mycorrhizal fungi, biochar, DGGE, inoculation, microbial community, tomato

7.2 INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligatory symbiotic soil fungi which colonise roots of most plants (Douds & Millner, 1999). These fungi form mutualistic relationships with more than 80% of terrestrial plants (Ulrich *et al*., 2002) and provide the host with mineral nutrients in exchange for carbohydrates (Tahat *et al*., 2008). Generally, plants inoculated with AMF are more efficient in nutrient and water acquisition, thus resulting in an improved plant growth (Oseni *et al*., 2010). Colonisation of roots by AMF enhanced crop productivity by enhancing tolerance to various biotic and abiotic stress factors (Al-Garni, 2006; Khaosaad *et al*., 2007; Javaid & Riaz,

2008). In tomato, AMF are widely used to improve plant growth and health (Oseni *et al*., 2010). However, even with AMF nursery inoculation or field application, tomato plants exhibit low root mycorrhizal colonisation. Low AMF colonisation in field-grown plants has been variously attributed to (i) use of unsuitable strains, (ii) relatively high available soil P (iii) cultural practices and (iv) microbial competition in the rhizosphere (Strzemska, 1975; Jasper *et al*., 1989).

Soil amendments, which increase AMF abundance and/or functionality, could be beneficial to plant hosts (Rillig & Mummey, 2006; Warnock *et al*., 2010). Biochar (biomass-derived black carbon) can serve as refuge for AMF hyphae and protect them from fungal grazers (Warnock *et al*., 2007), thus enhancing plant host-fungus symbiosis. Ishii and Kadoya (1994) argued that additions of biochar altered soil physico-chemical characteristics, leading to increased soil nutrient availability and enhanced mycorrhizal root colonisation. Similarly, Saito (1990) observed an increase of more than 300% in mycorrhizal root colonisation in field-grown soybean. According to Lehmann *et al*. (2003), biochar addition can improve plant productivity directly as a result of its nutrient content and release characteristics or indirectly, through improved nutrient retention. Although numerous studies indicated that soil biochar amendments can increase AMF percent root colonisation (Ezawa *et al*., 2002; Yamato *et al*., 2006; Warnock *et al*., 2010), little is known about the resultant effects on the soil microbial community (Glaser, 2007; Steinbeiss *et al*., 2009).

Molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) of ribosomal DNA (rDNA) fragments amplified from total community DNA have been widely used to evaluate the composition of bacterial and fungal communities (Muyzer & Uitterlinden, 1993).

Most commonly, 16S rRNA genes are used to give an overall indication of the species composition of a sample since they can easily be compared on gene databases (Reynolds & Surridge, 2009).

Since AMF might alter the microbial community in the rhizosphere, while biochar could affect percentage mycorrhizal root colonisation and that both could improve crop performance, there is an increasing interest in understanding their potential synergisms in crop production. The objective of this study was two-fold: (i) to investigate the effects of AMF-inoculated transplants and biochar-amended soil on mycorrhizal root colonisation, nutrient content, plant growth and yield of field-grown tomato and (ii) to assess their resultant effects on microbial community in the rhizosphere.

7.3 MATERIALS AND METHODS

7.3.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production

Site description

The experiment was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria. Details of the study location and duration are presented in Chapter 5, with the exception that this study was conducted in 2010 growing season.

Experimental design and treatments

The four treatment combinations (2 AMF × 2 biochar), M_1B_1 (AMF-inoculated seedlings with biochar-amended soil), M_1B_0 (AMF-inoculated seedlings without biochar), M_0B_1 (uninoculated seedlings with biochar) and M_0B_0 (untreated/control), were arranged in a randomised complete block design with six replicates.

Tomato cv. Nemo-Netta seedlings either pre-inoculated with commercial inoculum Biocult© containing spores of *Glomus mosseae* or uninoculated, were supplied by Hishtill nursery, Mooketsi, South Africa. Pre-inoculated AMF seedlings had less than 15% mycorrhizal root colonisation, whereas uninoculated seedlings had no colonisation. Where applicable, biochar was added to the transplanting hole (30 cm depth) at planting at a rate of 500 g/hole corresponding to 5 t ha^{-1}(Hossain *et al*., 2010).

Cultural methods are presented in Chapter 6.

Biochar production

Biochar was produced at the Natuurboerdery Research Center in Mooketsi, South Africa from *Eucalyptus globolus* trees. The trees were cut down, chipped and pyrolysed in a fixed bed reactor. The pyrolysis temperature was maintained at 450ºC for 1 h. Physical and chemical characteristics of biochar are shown in Table 7.1.

Table 7.1 Chemical and physical characteristics of biochar produced from *Eucalyptus globulus*

Parameters	Biochar	Unit
Total Carbon	338	g kg^{-1}
Total Nitrogen	3.7	g kg^{-1}
pH (H_20)	7.6	
Moisture content	3.5	%
Ash content	3.3	%
Phosphorus-Bray 2	84.7	mg kg^{-1}
Total Sulfur	43	mg kg^{-1}
Total Magnesium	0.7	g kg^{-1}
Total Boron	8.45	mg kg^{-1}
Cation exchangeable capacity	9.3	$mmol_c$ kg^{-1}
Bulk density	560	kg m^{-3}

Data collection

The procedures for root colonisation and shoot chemical analysis are presented in Chapter 4. Harvesting was done as described in Chapter 5. Details of yield and marketable yield determination are presented in Chapter 3, while dry matter determination is presented in Chapter 6.

Data analysis

The analysis of data has been previously described (Chapter 3). Relevant ANOVA tables can be found in the Appendix.

7.3.2 Effect of AMF and biochar amendment on fungal and bacterial populations

Site description and soil sampling

Soil samples were collected from tomato roots in the rhizosphere at the end of the growing season. Plants were pulled out and soils gently removed. Soil samples were kept into a cooler box and sent to the laboratory (Soil microbiology laboratory, Department of Plant Production and Soil Science, University of Pretoria, South Africa) where they were maintained at 4ºC until DNA extraction.

***Microbial community structure: denaturing gradient gel electrophoresis* (DGGE)**

Total DNA was extracted from 0.25 g soil using the Zymo Fast spin soil DNA extraction kit (Inqaba Biotec, Pretoria, South Africa). The DNA concentration was determined by agarose gel electrophoresis. A segment of 16S bacterial rDNA was amplified by means of PCR using primers K (Siciliano *et al*., 2003) and M (Fjellbirkeland *et al*., 2001). Complimentary screening of eukaryotic diversity was carried out on a portion of the internal transcribed spacer (ITS) gene sequence of the DNA by means of PCR using the primer set ITS3 and ITS4 (White *et al*., 1990). The PCR product was subjected to DGGE (Muyzer *et al*., 1993), whereas image analysis was performed using the Gel2K (Norland, 2004). Dominant bands were compared and analysed for population diversity determination.

Band reamplification and sequencing were conducted by Ingaba Biotec (Pretoria, South Africa) for DGGE sequencing. Each sequence was subjected to BLAST analysis on the GenBank database and matching hits were selected for alignment using Clustal X (Thompson *et al*., 1994). Phylogenetic analysis was based on parsimony using PAUP 4.0b8 (Phylogenetic Analysis Using

Parsimony) (Swofford, 2000). Heuristic searches were done with random addition of sequences (1000 replicates), tree bisection-reconnection (TBR), branch swapping, MULPAR-effective and MaxTrees set to auto-increase. Phylogenetic signal in the data sets was assessed by evaluating tree length distributions over randomly generated phylogenetic trees. The consistency (CI) and retention indices (RI) were determined for all data sets. Phylogenetic trees of sequences were rooted with *E. coli* as outgroup to the remaining taxa. Bootstrap analyses were conducted, retaining groups with 70% consistency, to determine confidence in branching points (1000 replicates) for the most parsimonious trees generated.

Data analysis

Bacterial community fingerprints were recorded and digital images were analysed using software based on the Shannon-Weaver index. Numbers of dominant bacterial species per sample were plotted. Dendrograms depicting similarities and differences between communities were generated using Jaccard statistics and a group average across the different types of samples.

7.4 RESULTS

7.4.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production

Growth parameters and mycorrhizal root colonisation

There was a significant main effect of AMF inoculation on dry shoot mass and total plant biomass (Table 7.2). The main effect of biochar was only significant for dry root mass. The interaction of AMF inoculation × biochar amendment was not significant for any parameter.

Regardless of biochar amendment, AMF inoculation increased the shoot dry mass and total plant biomass by 11% and 9%, respectively. Biochar amendment decreased the root dry mass by 13%. Tomato shoot length and root length were not affected by any treatment. Root colonisation of AMF was 15%, with or without biochar addition, whereas, uninoculated seedlings roots had no mycorrhizal colonisation (Table 7.3).

Table 7.2 Growth variables of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Response variable	Shoot length (cm)	Root length (cm)	Dry shoot mass (g plant $^{-1}$)	Dry root mass (g plant $^{-1}$)	Plant biomass (g plant^{-1})
AMF					
M_0	149.88	59.18	10.60b	2.05	12.65b
M_1	148.60	61.19	11.87a	2.00	13.87a
Biochar					
B_0	150.11	58.37	10.85	2.15a	13.00
B_1	148.37	61.99	11.62	1.90b	13.52
ANOVA					
M	ns	ns	*	ns	*
B	ns	ns	ns	*	ns
M×B	ns	ns	ns	ns	ns

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

ns, * are levels of significance (not significant, $P \leq 0.05$, respectively according to LSD test)

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

Yield and yield components

The yield and yield components of tomato were not affected significantly by the main effects or AMF inoculation × biochar amendment interaction (Appendix A, Table 7.2). However, the AMF inoculation with (M_1B_1) or without biochar (M_1B_0) increased the total yield by 8% (Table 7.3). Uninoculated seedlings combined with biochar (M_0B_1) decreased both early and total yields of tomato by 9%.

Table 7.3 Percentage of mycorrhiza root colonisation, mean yield and yield components of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Response variable	Marketable fruit* (plant^{-1})	Early yield* (kg plant^{-1})	Total yield* (kg plant^{-1})	Marketable yield* (kg plant^{-1})	Mycorrhiza (%)
M_0B_0	89.91	1.73	7.16	6.10	-
M_0B_1	83.04	1.59	7.04	6.13	-
M_1B_0	92.85	1.82	7.69	6.57	15
M_1B_1	94.78	1.72	7.47	6.45	15

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

Shoot chemical analysis

There was no significant effect of either AMF inoculation or biochar amendment on shoot N, Ca, Na, B, Cu, Mn or Zn contents of tomato plants (Table 7.4). Regardless of the seedlings status, amending soil with biochar (B_1) resulted in 9% decrease in shoot K content of tomato as compared to the control (B_0).

Table 7.4 Shoot nutrients content of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Response variable	K (%)	Ca (%)	N (%)	Na (ppm)	B (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
AMF inoculation								
M_0	2.73	1.93	4.05	2981.3	28.67	37.42	191.25	127.17
M_1	2.70	2.03	4.10	2966.5	30.08	34.00	255.75	150.67
Biochar addition								
B_0	2.83a	2.04	4.05	2929.9	29.33	37.33	217.50	136.33
B_1	2.60b	1.92	4.10	3017.8	29.42	34.08	229.50	141.50
ANOVA								
M	ns	ns	ns	ns	ns	ns	ns	ns
B	*	ns	ns	ns	ns	ns	ns	ns
M×B	ns	ns	ns	ns	ns	ns	ns	ns

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

ns, *, are levels of significance (not significant, $P \leq 0.05$, respectively according to LSD test)

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

Growing AMF-inoculated seedlings with (M_1B_1) or without biochar (M_1B_0) resulted in 26% and 29% decreases in shoot P content, respectively (Table 7.5). Similarly, uninoculated seedlings with biochar added (M_0B_1) also showed a decrease of about 32% in shoot P content as compared to the uninoculated seedlings grown without biochar amendment (M_0B_0).

Table 7.5 Phosphorus shoot content of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Parameter	P (mg kg^{-1})			
	Biochar amendment			
AMF inoculation	B_0	R-E (%)	B_1	R-E (%)
M_0	0.45a		0.31b	-32
M_1	0.32b	-29	0.33b	-26

Means followed by the same letter within column and row were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

7.4.2 Effect of AMF and biochar amendment on fungal and bacterial populations

DNA extraction and PCR

DNA was successfully extracted from all samples collected. No evidence of RNA or protein contamination was visible either below the lanes or in the wells of the gel, respectively (Figure 7.1).

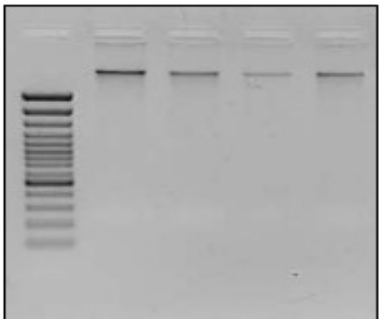

Figure 7.1 Tris-acetate–EDTA (TAE) agarose gel (1.5%) showing high-quality, clean genomic DNA extracted from soil samples

PCR of prokaryotes was successful yielding a ca. 510bp PCR product on a 1.5% TAE agarose gel. The negative control lane (first in row) shows that there was no contamination of the

reaction and that PCR product is thus a true indication of the microbial population being targeted (Figure 7.2).

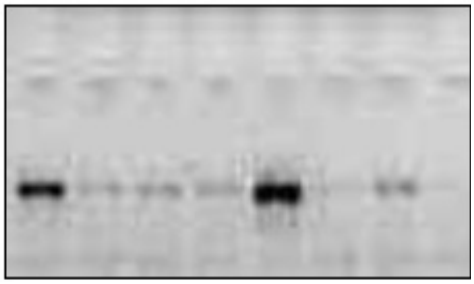

Figure 7.2 Tris-acetate –EDTA (TAE) agarose gel (1.5%) showing 5µl of PCR product from each of the 16S bacterial gene amplifications

DGGE

DGGE yielded gels showing clear multiple banding, forming a fingerprint in each lane (Figure 7. 3). These gel images were loaded into Gel2K (Norland, 2004) and a graphical image of the gels was produced (Figure 7.4) for further species diversity bioinformatics analysis. Dominant species per lane are indicated as dark prominent bands across the lane.

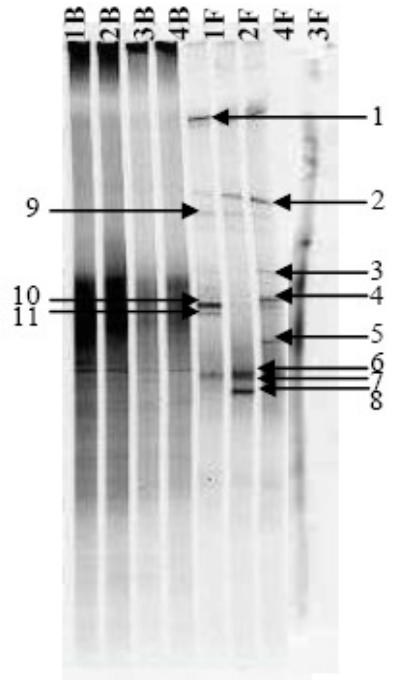

Figure 7.3 Denaturing gradient gel electrophoresis (DGGE) gel showing species diversity of bacteria (B) and fungi (F) from soil samples, run at 40-60% denaturants. PCR product is separated according to base-pair sequence differences to determine community richness and diversity of microorganisms based on these fingerprints

Arrows (1-11) point to bands that were excised for sequencing and tentative fungal identification
Sample 1 = no AMF + Biochar added (M_0B_1); Sample 2 = no AMF + no Biochar added (M_0B_0);
Sample 3 = AMF + Biochar added (M_1B_1); Sample 4 = no AMF + no Biochar added (M_0B_0)
B: Bacterial population; F: Fungal population

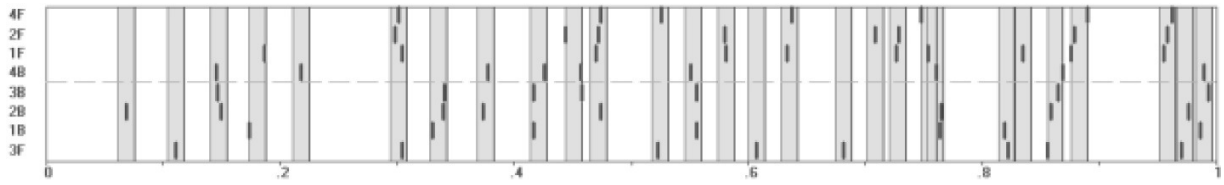

Figure 7.4 Graphic representation of the denaturing gradient gel electrophoresis (DGGE) gel in Figure 3 depicting the band pattern, indicating species diversity within bacterial (B) and fungal (F) populations, produced by each of the samples

Results suggested that soil amended with biochar had the highest dominant fungal species when compared with AMF, AMF and biochar or the untreated plots, whereas AMF alone had the highest number of bacterial species (Figure 7.5).

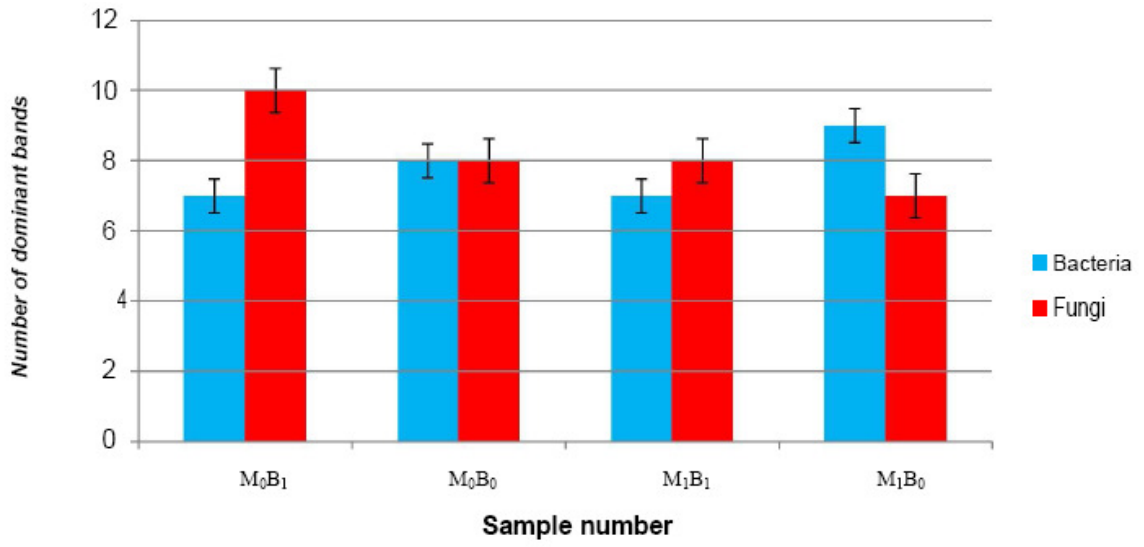

Figure 7.5 Number of dominant bacterial or fungal species per sample visible from denaturing gradient gel electrophoresis (DGGE) band

Patterns (error bars were calculated using standard error across the respective sampling)

M_0B_1 (Sample 1) = no AMF + Biochar added; M_0B_0 (Sample 2) = no AMF + no Biochar added; M_1B_1 (Sample 3) = AMF + Biochar added; M_0B_0 (Sample 4) = no AMF + no Biochar added

The average number of dominant bands found between the pro- and eukaryotes screened for diversity shows higher diversity within the fungi, although this was not significant. Similarities between samples within the profile are indicated by branch lengths (Figure 7.6). The dendrogram forms two distinct clades/groupings. Clade I contains only fungal samples, whereas clade II only contains bacterial samples, with the exception of combined AMF and biochar (Sample 3, M_1B_1). Focusing on clade I, AMF-treated sample (sample 1, M_0B_1) and untreated-AMF sample with biochar added (sample 4, M_1B_0) grouped together, whereas on clade II, AMF-treated samples (sample 3, M_1B_1 and sample 4, M_1B_0) grouped together. Interestingly, combined AMF and biochar sample (M_1B_1) did not share common fungal species with any other treatments.

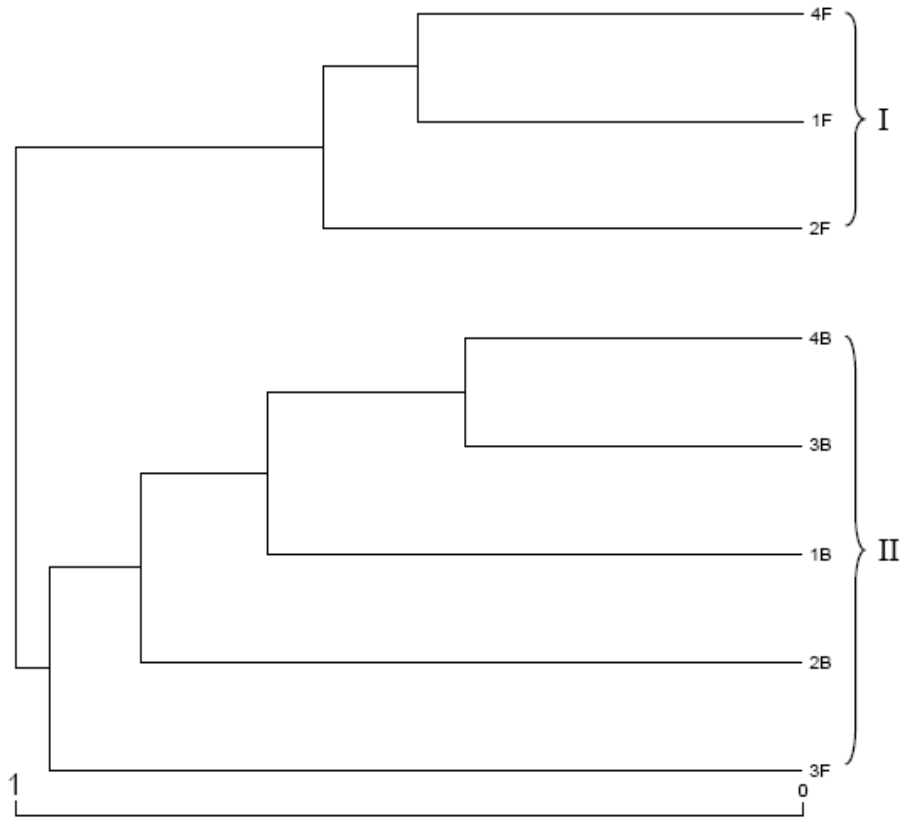

Figure 7.6 Cluster analysis of the banding pattern in Fig. 4, using a jaccard matching, group average setting to separate bacterial (B) and fungal (F) populations on the basis of community differences

Sample 1 = no AMF + Biochar added (M_0B_1); Sample 2 = no AMF + no Biochar added (M_0B_0);
Sample 3 = AMF + Biochar added (M_1B_1); Sample 4 = no AMF + no Biochar added (M_0B_0)

Tentative identification of the bands cut from the DGGE gel as indicated by arrows above (Figure 7.3) and confirmed in the phylogenetic tree (Figure 7.7) are presented in Table 7.6. There was 94% DGGE confidence AMF-treated (M_1B_0) soil sample contained *Penicillium pinophilum*, which was not found in any other samples.

Table 7.6 Tentative identification of denaturing gradient gel electrophoresis (DGGE) bands sequenced according to BLAST results from the NCBI GenBank database

Seq. no.	Species	Accession no.	Similarity (%)	Associated literatures	Samples
1	Ascomycete sp.	DQ683976	96	Conley *et al.* (2006)	M_0B_1
2	*Mortierella elongata*	GU446646	98	Bukovska (2009)	M_1B_0
3	*Penicillium pinophilum*	HQ589152	94	Iskandar *et al.*(2009)	M_1B_0
4	Uncultured Chlorophyta	HQ219393	81	Monchy *et al.*(2007)	M_1B_0
5	*Leptosphaeria* sp.	AM921719	90	Marquez *et al.* (2008)	M_1B_0
6	*Alternaria* sp.	EF432296	98	Mwangi *et al.*(2009)	M_0B_0
7				No match	M_0B_0
8				No match	M_0B_0
9				No match	M_0B_1
10	*Sporormiella septenaria*	GQ203790	90	Kruys & Wedin (2009)	M_0B_1
11				No match	M_0B_1

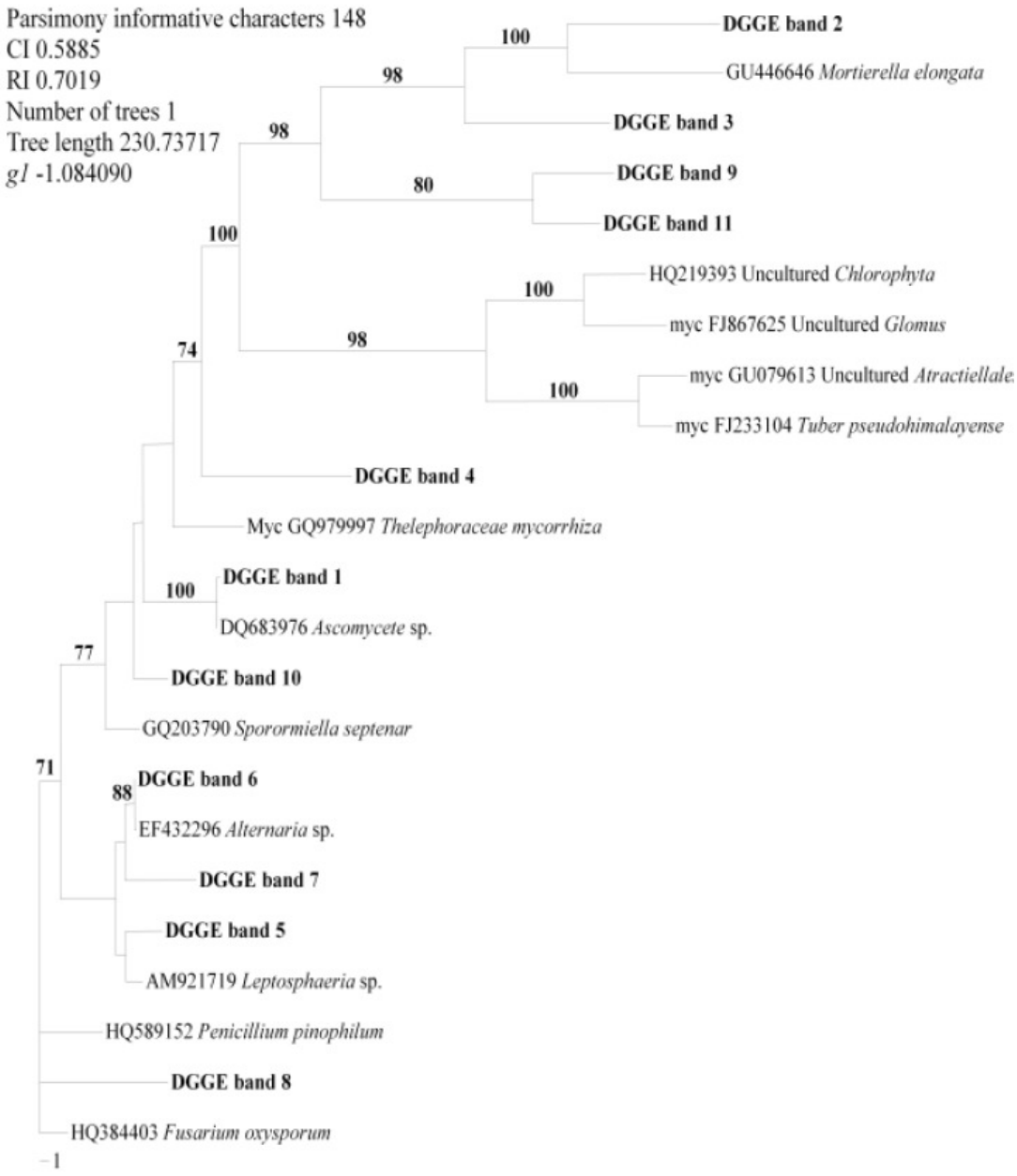

Figure 7.7 Phylogram of the denaturing gradient gel electrophoresis (DGGE) bands sequenced for tentative identification of fungi found in Mittal soil samples

7.5 DISCUSSION

7.5.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production

The addition of biochar to the planting hole of AMF-inoculated tomato seedlings did not increase the percentage of root colonisation, growth, yield or yield components of tomato plants. However, this combination influenced shoot P content. Effects of biochar addition to soil on root colonisation by AMF have been contradictory. Ishii and Kadoya (1994) observed increased percentage of root colonised by AMF on citrus. Wallstedt *et al*. (2002) argued that biochar could reduce root mycorrhizal colonisation by decreasing nutrient availability or creating unfavourable nutrient ratios in soils. In this study, biochar had no effect on mycorrhizal colonisation rate probably due to four reasons: (i) low seedling mycorrhizal colonisation (< 11%) before transplanting (ii) soil disturbance during production, (iii) use of synthetic fertilisers, especially P and (iv) application of pesticides, more especially copper-based products, which were used for the control of bacterial diseases. All these factors have been correlated with low mycorrhizal root colonisation in field production (Martin, 2007).

In this study, biochar had no positive effect on yield or yield components with or without AMF inoculation. Similarly, Graber *et al*. (2010) did not find any effect of biochar on the number of flowers or fruit yield of tomato grown in a soil-less medium. However, Steiner *et al*. (2007) observed increased yield in rice and sorghum with an application of 11 t ha^{-1} biochar over two years in an oxisol in Brazil. Similar results were observed for maize following three repeated applications of 7 t ha^{-1} of biochar over two growing seasons in Kenyan soils cropped to maize for up to 100 years (Kimetu *et al*., 2008). Even with 20 t ha^{-1} biochar applied, Major *et al*. (2010)

only found a significant yield response in maize in subsequent cropping years. Despite the clear evidence that increased yield is usually observed in subsequent years, some authors found positive results in the first year. For instance, in cherry tomato, Hossain *et al*. (2010) reported a 20% yield increase with combined biochar and fertiliser. In their studies, Hossain *et al*. (2010) used a low pH chomosol with 10 t ha^{-1} of biochar applied. The absence of a clear yield increase in our study could partly be attributed to the soil used (acid), application rate (5 t ha^{-1}), one growing season and application frequency.

Generally, K and Na are affected by salinity, nematodes and AMF (Graham & Sylvesten 1989; Mashela & Nthangeni, 2002). In this study, AMF inoculation did not affect shoot K content, probably due to low mycorrhizal root colonisation. The lower shoot K content in biochar-amended transplants was likely due to enhanced N and P by biochar resulting in an imbalance ratio of N/K and P/K in the rhizosphere, which then reduced K uptake. Shoot P content was the only mineral nutrient whose uptake was decreased by both AMF inoculation and biochar application probably due to the use of P fertilisers and non-stressed growing conditions during this study.

7.5.2 Effect of AMF and biochar amendment on fungal and bacterial populations

Results of this study showed that AMF-treated plants with or without biochar addition, shared specific bacterial species with each other, but which they did not share with other treatments, suggesting that AMF might influence bacterial community development in the rhizosphere. Generally, plant growth-promoting rhizobacteria (PGPR), which are important contributors to

overall plant growth and nutrition, are often associated with mycorrhizal hyphae (Garbaye, 1994; Vestergard *et al*., 2008; Rooney *et al*., 2009). In addition, some bacterial communities specifically attach to dead hyphae, whereas others use exudates from living hyphae as a growth substrate (Rooney *et al*., 2009). In this study, the AMF-treated sample had the highest dominant bacterial band. Albertsen *et al*. (2006) observed increased bacterial and saprophytic fungal biomass in the presence of AMF *G. intraradices*, whereas Andrade *et al*. (1997) found higher numbers of bacteria in AMF-untreated plant roots. According to Garbaye (1991), AMF might alter root exudation in the rhizosphere and therefore, indirectly affect bacterial growth.

Cluster analysis showed that fungal diversity of AMF-untreated (M_0B_1) and biochar-amended (M_1B_0) treatments were closer, when compared to other samples. Surprisingly, combination of AMF and biochar (M_1B_1) did not share common fungal species with M_0B_1 or M_1B_0, suggesting that biochar might modify the mycorrhizosphere community. Biochar contains organic pyrolytic byproducts, including phenolic and polyphenolic compounds, which might inhibit soil organisms including AMF (Warnock *et al*., 2010).

Tentative identification of DGGE band suggested that *Alternaria* sp. was found in the untreated control only. Physical field scouting supported this finding, as untreated plots had the highest disease incidence of early blight (*Alternaria solani*), *Fusarium* and *Verticillium* wilts when compared to other treatments. In this study, *Penicillium pinophilum* was found in AMF-treated sample (M_1B_0) only. Rando *et al*. (1997) classified *P. pinophilum* as a minor pathogen due to growth retardation observed in tomato. Fan *et al*. (2008) observed AMF symbiosis in strawberry roots when inoculated with *P. pinophilum*. However, Hempel (2009) questioned the finding that

P. pinophilum was capable of forming AMF symbiosis and called for further investigations with other plants. Synergistic effects between AMF and *Penicillium* spp. have been reported on wheat and maize (Babana & Antoun, 2006; Chandanie *et al*., 2006; Zaidi & Khan, 2007).

7.5.3 Conclusions

In conclusion, the addition of biochar in the planting hole during transplanting of AMF-inoculated seedlings had no effect on root colonisation, yield or yield components, or most of the shoot nutrients measured. However, the treatment reduced shoot P content. Findings in this study also suggest that biochar amendment might modify the rhizosphere, resulting in the altered development of microorganisms. Consequently, biochar should first be researched in detail before attempting any combination with AMF.

CHAPTER 8

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The aim of this study was to investigate the potential use of *T. harzianum* and AMF mixture as pre-sowing treatment in improving tomato seedlings quality, yield and fruit quality of tomato. To attain this goal, growth chamber, greenhouse, nethouse and field experiments were conducted. Major findings are presented below, followed by recommendations for future studies.

Trichoderma harzianum and AMF mixture was compared with SWE as well as Si in improving seedling growth and development, fruit yield and *Verticillium* wilt incidence on tomato plants. Because SWE contains growth hormones, susceptible to inhibit seedling germination and development, a preliminary study comparing two types of SWE derived from *Ecklonia maxima* and *Ascophyllum nodosum* at different concentrations, found 10% dilution with *E. maxima* as ideal SWE pre-sowing treatment. Combining *T. harzianum* and AMF mixture with *E. maxima* extract and or Si had no effect on seedling growth and development of tomato. However, *Ecklonia maxima* inhibition of AMF root colonisation of tomato seedlings suggested that fungal mixture should not be combined with SWE as pre-sowing treatment. Investigations on the influence on *T. harzianum* and AMF mixture, *E. maxima* and Si, each applied alone on the incidence of *Verticillium* wilt, confirmed the potential of the fungal mixture in reducing the deleterious effect of the disease during the early season. However, *T. harzianum* and AMF mixture could not improve tomato yield when compared with control plants. Evidences are that *T. harzianum* and AMF interact on each other and the nature of interactions, which are synergistic, antagonistic or neutral, depends on strains, inoculation time and crops. In this study,

combination of *T. harzianum* with four AMF species as single inoculation might have reduce the efficacy of each fungus in improving seedling quality and increasing tomato yield. Further study looked at *T. harzianum* and AMF (*G. mosseae*), each alone or in combination.

Trichoderma harzianum and AMF (*G. mosseae*) were inoculated alone or in combination before sowing or two weeks after sowing, and their effects on seedling growth and development were evaluated. Interestingly, interactions between *T. harzianum* and AMF (*G. mosseae*) on root colonisation were neutral. In this study, high *T. harzianum* root colonisation and low AMF root colonisation observed are simply indications of each fungus colonisation capacity rather antagonism between the two fungi. Findings of this study suggested that *T. harzianum* and AMF (*G. mosseae*) could simultaneously be applied to improve seedling growth and development, except when both fungi are applied two weeks after sowing. Another major finding in this study is the capacity of each fungus to induce seedling growth and development, confirming the potential use of each as biofertiliser and pre-sowing treatment on tomato.

Investigations were carried out to find out whether those benefits could be translated into increased yield and fruit quality under greenhouse conditions. There was no evidence of increasing yield or yield components of tomato plant following *T. harzianum* and AMF inoculation. However, increased percentage of extra-large fruit by *G. mosseae* confirmed previous studies that AMF could increase crop fruit size. The lowering of fruit K content in late AMF inoculation supported suggestions that early AMF inoculation was preferable than late inoculation. Although Vitamin C and fruit lycopene varied among treatments, there was no clear evidence of influence of *T. harzianum* and/or AMF on fruit phytochemical contents. Findings of

this study suggested that *T. harzianum* and AMF have negligible effect on yield of tomato under greenhouse conditions.

In South Africa, the large majority of fresh produce tomato originates from open field production. Experiments were conducted to investigate the influence of inoculation with *T. harzianum* and AMF on field-grown tomato. Growth promotion following microbial inoculation observed in previous studies with seedlings or greenhouse production was confirmed under field conditions. However, as previously observed, increased dry matter production was not translated into increased yield or yield components of tomato. Interestingly, when observing yield of first four weeks harvest, pre-inoculated seedlings increased early yield of tomato, which suggest that *T. harzianum* and AMF have the potential to influence yield of tomato. In this study, the role of AMF in increasing the percentage of extra-large fruit was confirmed. Inoculation with AMF also increased Vitamin C, while AMF alone or in combination with *T. harzianum* increased fruit TSS. However, the non-response of the fruit qualities during subsequent years confirmed suggestions that variations in fruit quality are not restricted to the impact of fungal inoculation. Although, AMF performed better than *T. harzianum* or combined *T. harzianum* and AMF, there was no indication of any antagonistic effect between the two fungi. The major setback in this study was the inability of obtaining high root mycorrhizal colonisation, despite inoculation in the nursery.

Although no actual evidence exist to support the premise that high mycorrhizal root colonisation could increase crop yield, strategies to improve mycorrhizal root colonisation should not be overlooked. Indications are that biochar could serve as refuge for AMF against soil predators and increase mycorrhizal root colonisation of crop. In this study, when AMF-inoculated seedlings

were transplanted with biochar, no effects on root colonisation yield or yield components of tomato were observed. However, assessment of the microbial communities in the rhizosphere showed that AMF-inoculated plants shared specific bacterial species with each other suggesting that AMF might influence bacterial community such as PGPR, which are associated with improved plant growth , nutrient uptake and disease control in the rhizosphere. Results of this study also showed that when AMF was applied simultaneously with biochar, the fungal community differed with the rest of the treatments, suggesting that biochar might modify the mycorrhizosphere.

Finally, for commercial fresh produce tomato farmers, nursery inoculation with *T. harzianum* and AMF during sowing could be considered as an effective integrated nutrient and disease management strategy. However, the persistence of low AMF root colonisation in this and numerous previous findings present opportunities for further studies into strategies to improve the situation through using crop specific AMF species, and by investigating the effect of AMF species alone or in combination. Investigations should not be restricted *in vitro* or to seedlings but expanded to field conditions as well. The fact that biochar had an effect on the mycorrhizosphere also opened new avenues on understanding the interactions between AMF and biochar. Arbuscular mycorrhizal fungi are well-documented for stress alleviation, such as salinity and drought, so are *Trichoderma spp*., particularly in disease control. Future studies should investigate the combined effect of *Trichoderma* and AMF on stress (salinity, drought and disease) alleviation and the resultant effect on growth promotion, yield and fruit quality. Similar studies should also be conducted under nutrient stressed conditions, especially where P is limited.