Molecular Analysis to Explore the Ecological Diversity of Arbuscular Mycorrhizal Fungi in the Marsh Grasses

Lathadevi Karuna Chintapenta[1], LaTaijah Crawford[1,2], Venu Kalavacharla[1,3], and Gulnihal Ozbay[1,3]

[1]College of Agriculture & Related Sciences, Delaware State University, Dover, Delaware 19901

[2]College of Mathematics, Natural Sciences & Technology[1], Delaware State University, Dover, Delaware 19901

[3]Center for Integrated Biological & Environmental Research (CIBER), Delaware State University, Dover, Delaware 19901

United States Department of Agriculture
National Institute of Food and Agriculture

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) are widely present in the roots of terrestrial plants through symbiotic associations. AMF fungi (Fig.1) are classified as special microbes that ensure efficient uptake of nutrients in its host plant even under stress conditions which is one of the key factors for sustainable agriculture. With global climate change, drought and salinity being the major reasons for worldwide crop loss, food production is becoming more challenging.

- The project outcome serves as the first study in this area and can provide baseline information to the resource managers to assist with restoring the native marsh (*Spartina alteniflora)*, in Blackbird Creek ecosystem.

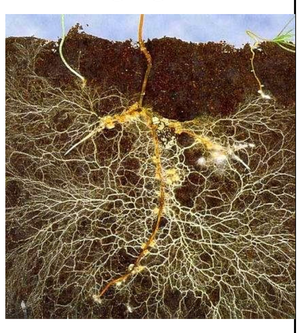


Fig: 1 Mycorrhizae within plant roots

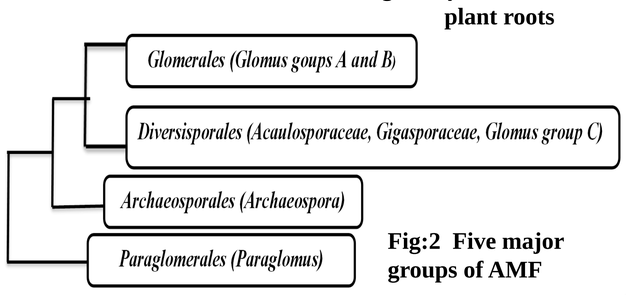


Fig:2 Five major groups of AMF

OBJECTIVES

- Investigate AMF species diversity from the soils and roots of the invasive and native marsh grasses from the Blackbird Creek marsh, Delaware.
- Determine if there are any changes in the types of AMF species and their abundance with respect to seasons.

SAMPLING SITE

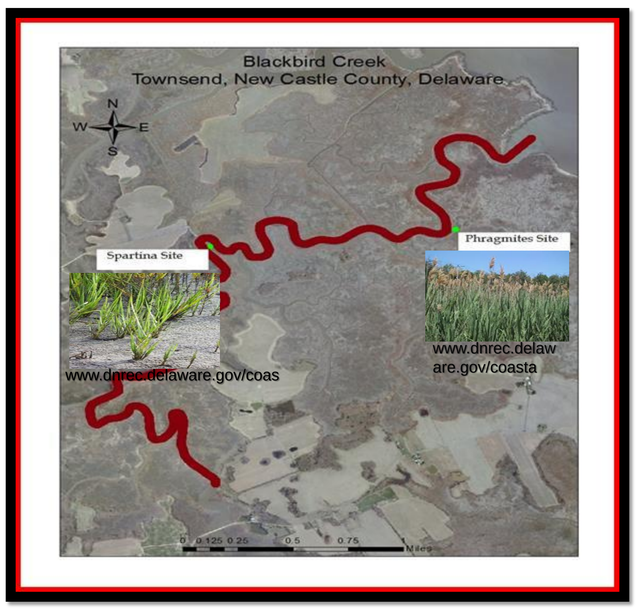


Fig 3: Sample collection sites at Blackbird Creek (Images by K. Ommanney)

- Blackbird Creek is located upstream from Taylor's Bridge, Townsend, Delaware.
- Blackbird Creek flows into the Delaware River just upstream from Delaware Bay.
- This marsh has areas dominated by the native marsh, *Spartina alterniflora* and the invasive marsh, *Phragmites australis*. Soil and root samples were collected from these marsh sites (Fig.3).

MATERIALS & METHODS

- Genomic DNA was isolated from the marsh soils (MoBio kit) and marsh grass roots (Qiagen plant DNeasy kit & CTAB method).
- *P. australis* root samples for the month of May were not collected as the plants were dead due to herbicide treatments in Blackbird Creek.
- Nested PCR was performed using 2 different primer sets
- First PCR- Universal eukaryotic primers (NS5/ ITS4) which amplifies most of the fungi.
- Second PCR- AMF specific primers (Table 1) which amplifies only specific AMF species.

Table 1: Primer sets used for Nested PCR

Primer Set	Region Amplified	AMF or Non AMF	Band Size (bp)
A. NS5/ ITS4i	SSU	AMF	1200
B. NS1/NS4	SSU	AMF	1100
SECOND PCR			
A. GLOM1310/ITS4i (A)	SSU/ITS	AMF	1,012
A. LETC1677/ITS4i (B)	SSU	AMF	676
A. ACAU1661/ITS4i (C)	SSU	AMF	645
A. ARCH1311/ITS4i (D)	SSU	AMF	1,052
A. GIGA 5.8R/ NS5 (E)	SSU	AMF	305
B. AML1/AML2	LSU	AMF	800
B. NS31/AM1	SSU	NON AMF	550

ITS- Internal Transcribed Spacer Region; SSU-Small Subunit Region; LSU- Large Subunit Region

RESULTS & DISCUSSION

- AMF species were observed in the roots of both *S. alterniflora* and *P. australis.*
- Seasonal transitions as well as environmental stresses regulate the presence and absence of AMF species.
- Both root samples from the invasive and native species have all 5 major groups (A-E) of AMF (Table 2).

Table 2: Presence and absence of the 5 major groups of AMF

SS-*Spartina* soil PS-*Phragmites* Soil SR-*Spartina* Root PR-*Phragmites* Root	PRIMERS	August 2015				February 2016				May 2016		
		SS	PS	SR	PR	SS	PS	SR	PR	SS	PS	SR
FIRST PCR	NS5/ ITS4	-	-	X	X	-	-	X	X	-	-	X
SECOND PCR	A-GLOM1310/ITS4	-	-	X	X	-	-	X	X	-	-	X
	B-LETC1677/ITS4	-	-	X	X	-	-	X	X	-	-	X
	C-ACAU1661/ITS4	-	-	X	X	-	-	X	X	-	-	X
	D-ARCH1311/ITS4	-	-	X	X	-	-	X	X	-	-	X
	E- NS5/ GIGA 5.8R	-	-	X	X	-	-	X	X	-	-	X

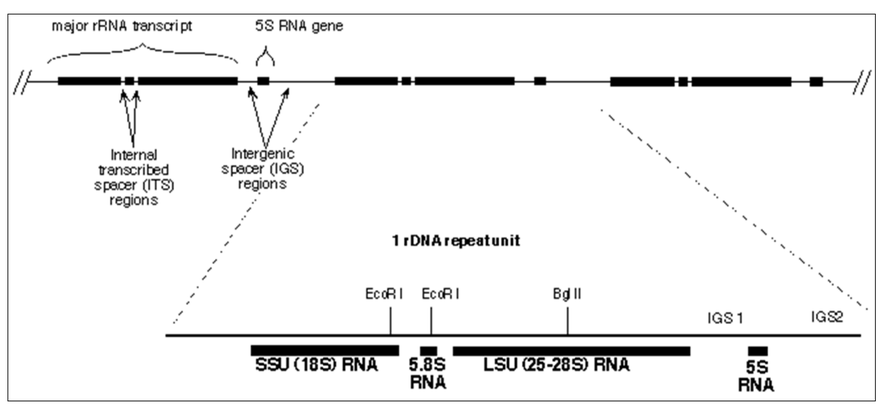
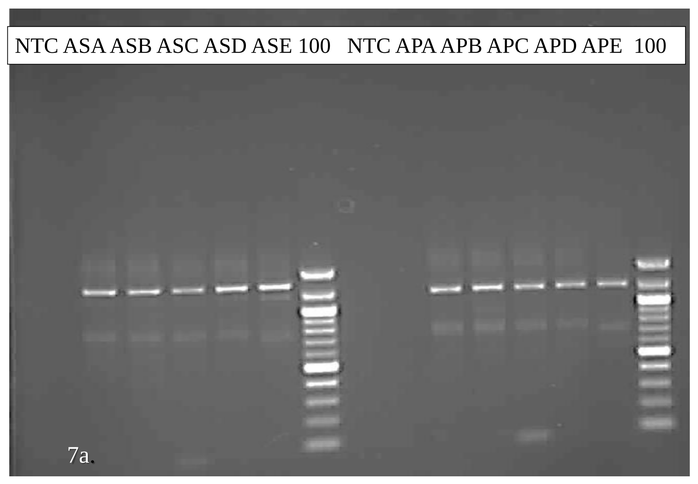


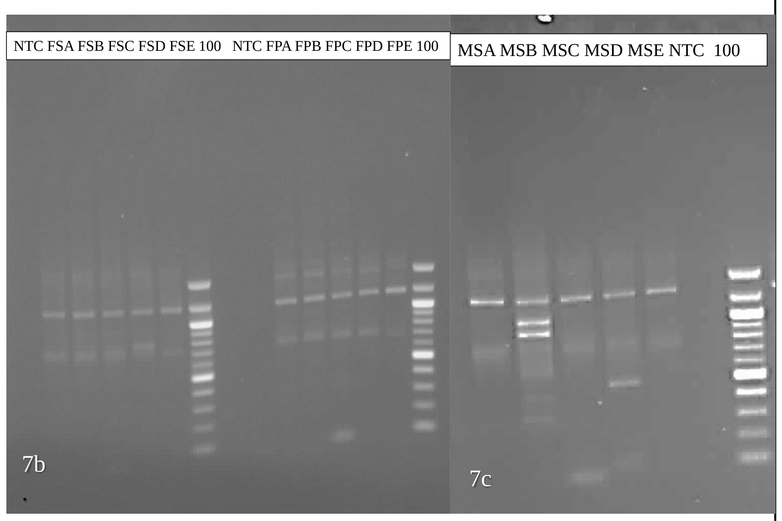
Fig. 6 Ribosomal RNA regions targeted for designing the primers of AMF

- The results from this study predicts that *P. australis* species are well adapted in the foreign environment due to AMF associations.

VALIDATION OF THE FIVE MAJOR GROUPS (A-E) OF AMF



- The intensity of the amplified PCR product for AMF is low in the soil samples of both *Spartina* and *Phragmites.*
- May samples had non-specific bands for 3 sets of AMF specific primers.



Figs.7a,b,c: Nested PCR using 5 primer set (A-E) on both *Spartina* (S) and *Phragmites* (P) root samples from August (A), February (F), and May (M).

CONCLUSION & FUTURE GOALS

- VAM Fungi have been confirmed by molecular methods in marsh roots of *S. alterniflora* and *P. australis*.
- VAM fungi are present in low numbers in the soils than the root samples.
- Studies including Real Time PCR and Cloning are being performed to study the abundance of VAM fungi and identify the species by Sanger Sequencing.
- Testing for potential non AMF species using NS31/AM1 primers will be done in soil samples.
- Green house experiments will be performed to understand plant and microbe interactions under abiotic stress conditions as a sustainable agricultural approach.

LITERATURE CITED

1. Redecker D (2000). Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80.
2. Shepherd, M., Nguyen, L., Jones, M.E. et al. A method for assessing arbuscular mycorrhizal fungi group distribution in tree roots by intergenic transcribed sequence variation. Plant Soil (2007). 290: 259. doi:10.1007/s11104-006-9157-5.

ACKNOWLEDGEMENTS

- We acknowledge the financial support NSF- EPSCoR program (EPS-1301765) and USDA-NIFA CBG Grant Program.
- We would also like to extend gratitude to the Aquatic Sciences Lab Members and PMGG Lab Members.