SEEDLING BLIGHT OF RICE IN THE SOUTHERN UNITED STATES AND ITS MANAGEMENT

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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May 2021

Major Subject: Plant Pathology

ABSTRACT

Seedling blight of rice is one of the most important diseases in dry-seeded rice system in the southern U. S. Surveys were conducted in five southern rice-producing states (Arkansas, Louisiana, Mississippi, Missouri, and Texas) during the 2018 and 2019 cropping seasons to determine the distribution and importance of fungal pathogens associated with seedling blight in rice. A total of 349 fungal isolates were collected and identified as *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii*, and *Marasmius graminum*. *R. solani* was the most prevalent fungus in this survey study. There were two anastomosis groups of *R. solani* associated with rice seedlings, with *R. solani* AG-11 (n= 245) being the most predominant pathogen and *R. solani* AG-4 (n=7) being the most aggressive pathogen. *R. solani* AG-4 and *M. graminum* were identified as new pathogens causing seedling blight of rice and were more aggressive than *R. solani* AG-11, *Fusarium* spp., and *S. rolfsii*. A total of 202 rice breeding lines and commercial inbreed and hybrid cultivars were evaluated for resistance to *R. solani* AG-11 and AG-4 under greenhouse and field conditions. There were no cultivars or breeding lines showing a high level of resistance to either *R. solani* pathogen. However, the hybrid cultivar RT7521 FP and inbred cultivar CLL15 showed less than 50% stand loss against *R. solani* AG-11. To understand genetic structure of the populations of *R. solani* AG-11, the major seedling blight pathogen, we used whole-genome sequencing of the *R. solani* AG-11 populations from Arkansas (n= 31), Mississippi (n= 4), Missouri (n= 21), and Texas (n= 24). Demographic analysis suggested the Texas population is separated from

Arkansas, Mississippi, and Missouri populations. The results from the dendrogram and discriminate analysis of principal components grouped the Arkansas, Mississippi, and Missouri isolates in a single cluster, suggesting the possibility of genotype flow among these populations. The Texas population was further differentiated into two subpopulations with respect to geographical zone (east and northwest rice producing zones). This finding provides new insights into the evolution and structure of *R. solani* AG-11 populations in rice fields in the southern U.S. This research project provides the foundation for the future studies to develop effective management strategies for rice seedling blight, including proper diagnostics, cultivar resistance, and fungicide seed treatment.

DEDICATION

I dedicate this dissertation to my wife, Barsha Bastola, and my parents, Reshmi Raj Gaire and Krishna Kumari Gaire, who provided me with encouragement and support during my graduate study. Finally, I dedicate this dissertation to all the professors that mentored, supported, and believed in me.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Xin-Gen Zhou, my committee co-chair, Dr. Young-Ki Jo and my committee members, Drs, Daniel Ebbole, and Terry J. Gentry for their guidance and support throughout the course of this research.

Thanks also goes to my lab mates, friends and colleagues, the faculty and staff of Department of Plant pathology and Microbiology and Texas A&M AgriLife Research Center at Beaumont for making my time at Texas A&M University a great experience.

Thanks to the county extension agents and rice growers of Arkansas, Louisiana, Mississippi, Missouri, and Texas for their support in collecting rice samples in this disease survey study. Thanks also goes to Mr. Scott W. Winters at Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas for providing tester isolates of *Rhizoctonia solani* AG-11. The hybrid breeding lines used in this study were developed by Mr. Zongbu Yang and Dr. Ted L. Wilson, Professor, Center director at Texas A&M AgriLife Research Center at Beaumont, Texas.

Finally, thanks to my parents for their encouragement and to my wife, Barsha Bastola, for her patience and love through the most difficult periods.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professors Xin-Gen Zhou, Young-Ki Jo, and Daniel Ebbole of the Department of Plant Pathology and Microbiology, and Professor Terry J. Gentry of the Department of Soil and Crop Sciences at Texas A&M University.

The disease survey work (Chapter 2) was supported by visiting scientists Dr. Jun Shi, Dr. Yong Zhou, and Mr. Kai Wang. The field experiment (Chapter 3) was supported by Dr. Guangjie Liu, research associate of Texas A&M AgriLife Research Center at Beaumont and Mr. Jason Samford, farm research services manager of Texas A&M AgriLife Research Wintermann Rice Research Station at Eagle Lake. The data analysis (Chapter 4) was supported by Dr. Shichen Wang at the Genomics and Bioinformatics Service, Texas A&M AgriLife. Portions of this research were conducted with the advanced computing resources and consultation provided by Texas A&M High Performance Research Computing. The portions of results depicted in Chapter 2 were published in the journal Plant Disease in 2020 and 2021.

Funding Sources

This study was supported by a graduate assistantship from Texas A&M AgriLife Research Center at Beaumont, TX, and USDA NIFA OREI grant (2015-51300-24286).

This work was also made possible in part by Texas Rice Research Foundation under Grant Number 2018-2020. Its contents are solely the responsibility of the authors

and do not necessarily represent the official views of the USDA NIFA OREI and Texas Rice Research Foundation.

NOMENCLATURE

AG	Anastomosis Group
ANOVA	Analysis of Variance
AR	Arkansas
BI	Bridging Isolate
BUSCO	Benchmarking Universal Single-Copy Ortholog
CA	California
CRD	Complete Randomized Design
DAPC	Discriminate analysis of principal components
DF	Degree of Freedom
DNA	Deoxyribonucleic Acid
ERS	Economic Research Service
GBS	Genotyping by Sequencing
HPRC	High Performance Research Computing
HSD	Honestly Significant Difference
ISSR	Inter-Simple Sequence Repeat
ITS	Internal Transcribed Spacer
LA	Louisiana
MI	Michigan
MO	Missouri
MS	Mississippi

NC	North Carolina
NCBI	National Center for Biotechnology Information
OR	Oregon
PDA	Potato Dextrose Agar
PT	Potato Type
RCBD	Randomized Complete Block Design
rDNA	Ribosomal Deoxyribonucleic Acid
RFLP	Restriction Fragment Length Polymorphism
SAS	Statistical Analysis Software
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TX	Texas
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
URRN	Uniform Regional Rice Nursery
US	United States
USA	United States of America
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops in the world and provides a main food source for more than half of the world's population. Asian countries dominate global rice production. India, Thailand, Vietnam, Pakistan, and USA are the top five rice exporting countries (USDA-ERS, 2020).

In the United States, rice is produced mainly in six states, Arkansas, California, Louisiana, Mississippi, Missouri, and Texas. Arkansas had the largest rice production acreage (469,840 ha) followed by California (180,085 ha), Louisiana (161,874 ha), Texas (99,674 ha), Missouri (68,392 ha), and Mississippi (46,538 ha) in 2019. The average rice productivity is lower in the southern four states (Arkansas, Mississippi, Missouri, Texas) as compared to California. Long-grain rice, accounting for around 68% of total U.S. rice production, is grown entirely in the South, whereas medium-grain, accounting for more than 30% of total U.S. rice production, is mainly grown in California (USDA-ERS 2020). Rice is mainly dry-seeded in the southern United States whereas rice is zero-grade water-seeded in California (Hill et al. 2006; Hardke 2015).

Cultivated rice belongs to two *Oryza* species: *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). *O. sativa* is grown worldwide, whereas *O. glaberrima* (African rice) is grown in parts of West Africa. Two subspecies *indica* and *japonica* of *O. sativa* have been widely recognized (Oka, 1988). *Indica* rice that domesticates in India is mainly cultivated in tropical and subtropical regions, whereas *japonica* rice that domesticates in southern China is predominately grown in temperate regions (Gross and Zhao, 2014). The *japonica* rice, consisting

of temperate and tropical cultivars, often is considered a *japonica* sub-group, designated as *temperate japonica* and *tropical japonica* (Cheng 1985). All medium-grain cultivars grown in the United States are classified as *temperate japonica*, and all long-grain cultivars are classified as *tropical japonica* (Mackill 1995).

Diseases occur in all rice growing regions of the world. In the United States, disease pressure is higher in the southern rice growing regions than in the arid California production areas. The United States has limited number of nematode and bacterial diseases and does not have any of the devastating viral diseases. Unfortunately, there are several fungal diseases that significantly reduced crop yields and increase production costs. Sheath blight, blast, kernel smut, narrow-brown leaf spot, and seedling diseases are the top five yield limiting diseases in the southern rice-producing states (Allen et al. 2020).

Seedling diseases in rice

Seedling diseases can affect rice production in water-seeded rice, direct-seeded rice, and in seedbeds rice grown for transplanting. The seed-rot, water-mold complex, and seedling blight are among the common seedling diseases in rice (Rush 1992). These diseases cause stand reduction and stand irregularities, resulting in yield loss and potentially replanting that causes more significant monetary loss.

Seed-rot and seedling damping-off, caused by *Achlya klebsiana*, and *Pythium* species, are major problems in the water-seeded rice of California (Webster et al. 1970). *Achlya* spp. normally attack the endosperm of germinating seeds, whereas *Pythium* spp. directly attack the developing embryo. *Pythium arrhenomanes* and *P. irregulare* were found strongly pathogenic to rice seedlings (Cother and Gilbert 1993; Eberle et al. 2007). Seeds attacked immediately after sowing by the pathogens. The endosperm can be destroyed, and the seeds do not germinate. To

control these diseases, planting at warm-enough time is an option. Fungicide seed treatment is another effective option to get uniform plant stands and improved yields.

Seedling blight is an important seedling disease in dry-seeded rice throughout the world. In the southern rice growing states of the United States, seedling blight is one of the most important disease complexes of rice that can result in significant stand loss. The disease is caused by several seed- and soil-borne pathogens. These pathogens included *Alternaria padwickii, Cochliobolus miyabeanus*, *Curvularia lunata, Fusarium roseum*, *F. moniliforme, Pythium arrhenomanes*, *P. irregulare, P. dissotocum, P. spinosum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Sarocladium oryzae* (Eberle et al. 2007; Groth et al. 1991; Oh 1985; Rush 1992).

Multiple fungal and oomycetes species have been reported to cause rice seedling blight in different countries. *Fusarium oxysporum*, *F. moniliforme*, *F. verticillioides*, *F. tricinctum*, *F. redolens*, *F. equiseti*, *F. solani*, *Rhizoctonia solani*, *Alternaria alternata*, *Curvularia coatesiae*, and *Pythium aristosporum* were reported in China (Gao et al. 2001; Liu et al. 2019), *Helminthosporium oryzae*, *Pyricularia oryzae*, *S. rolfsii*, and *Fusarium verticilliodes* in India (Amin 1976), *Pythium graminicola* in Korea (Sung et al. 1983) and Japan (Kato et al. 1985), and *Pythium arrhenomanes* in Philippines (Buyten and Hofte 2013).

Fungal pathogens isolated from rice seeds of the United States include *Curvularia* spp., *Fusarium solani*, *F. oxysporum*, *Phoma* spp., *Penicillium* spp., *Helminthosporium* spp., and *Aspergilus flavus* (Dossu and Silue 2018). Imolehin (1983) isolated pathogenic fungi, *Helminthosporium oryzae*, *Fusarium moniloforme*, *Penicillium* spp., *Curvularia lunata*, *Aspergillus* spp., *Rhizopus arrhizus*, *Geotrichum* spp., and *Alternaria* spp. from rice seed in Nigeria.

Symptoms of seedling blight disease includes pre- and post-emergence damping-off of rice seedlings, resulting in significant stand loss. Pathogens attack the embryo area of seed, causing pre-emergence damping-off. Dark brown necrotic lesions are visible on the coleoptile, mesocotyl or even in leaves in post-emergence damping-off (Rush 1992). The seedlings subsequently become blighted and die. The seedling blight of rice is greatly influenced by environmental conditions. The disease is more severe when temperature is cool, the condition unfavorable for the growth of rice seedlings (Groth et al. 1991).

Population genetics study of *Rhizoctonia solani*

Understanding genetic structure of the populations of plant pathogen is important to develop effective management strategies (Wolfe and Caten 1987). The pathogen populations can be affected by the application of fungicides, use of resistant varieties, changes in cropping patterns etc. We should consider the population rather than a single isolate as a representative of the population in disease control strategies. The major processes contributing to pathogen evolution within the populations are mutation, gene flow, migration, natural selection, and genetic drift (McDonald 1997). Population genetics analysis can be used to infer genetic patterns, degree of recombination, and processes of pathogen emergence (Milgroom et al. 2014).

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk.) is a highly heterogenous fungal species with respect to colony morphology, host range, biochemical and molecular characters, and pathogenicity. To date, the members of this species are classified into 14 anastomosis groups (AG-1 to 13 and AG-BI) based on hyphal anastomosis reactions (Carling 1996). The hyphae of similar AGs can fuse with each other while different AGs fail to achieve hyphal fusion (Anderson 1982). Presently, 7 AGs (AG 1, 2, 3, 4, 6, 8, and 9) have been further divided into subgroups based on host-range specificity, nutrient utilization, and genetic

differences (Vilgalys and Cubeta 1994). Field isolates of *R. solani* are multinucleated, mostly heterokaryon and lack clamp connection. The heterokaryon formation is possibly due to somatic fusion of genetically different homokaryons. Heterokaryosis, recombination, and mutation can cause genetic variation in *R. solani* (Flentje and Stretton 1964).

Population genetic structure of *R. solani* has been intensively studied in two anastomosis groups, AG-1 -IA and AG-3 PT. *R. solani* AG-1-IA, the causal agent of sheath blight of rice is an important subgroup within the *R. solani* complex. Various molecular tools like restriction fragment length polymorphism (RFLP) marker, random amplified polymorphic DNA (RAPD) markers, microsatellite marker, inter-simple sequence repeat (ISSR) marker, sequencing the ITS region, single nucleotide polymorphism (SNP) data obtained by genotyping by sequencing (GBS) have been used to study genetic diversity, population structure and reproductive biology of *R. solani* AG-1-IA. Rosewich et al. (1999) provided evidence that the population of *R. solani* AG-1-IA from six major rice growing counties in Texas displays high genetic diversity, lack of population structure with high level of gene flow, and mixed reproductive biology. Similar findings except restricted long-distance migration were reported by several researchers on *R. solani* AG-1-IA population from rice in China (Bernardes-de-Assis et al. 2008; Shu et al. 2014; Wang et al. 2013). Cumagun et al. (2020) revealed that rice-infecting populations of *R. solani* sampled from China, Japan and the Philippines are characterized by high levels of genetic diversity maintained by a mixed reproductive system. No subdivision was found among populations within Japan or within the Philippines, but subdivision was detected among populations within China. Padasht-Dehkaei et al. (2012) also reported mixed reproductive mode and low differentiation among rice infecting *R. solani* populations in Iran.

However, the *R. solani* AG-1-IA populations from rice and soybean in Louisiana (Bernardes-de-Assis et al. 2008), and from rice and maize in Latin America (Gonzalez-Vera et al. 2010) have been significantly differentiated with respect to host species and characterized by both sexual and asexual reproduction. Ciampi et al. (2008) reported the populations of *R. solani* AG-1-IA from soybean in Brazil had low genetic diversity, high degree of population subdivision, evidence of sexual recombination but predominantly asexual reproduction, short-distance dispersal of vegetative propagules and limited long-distance dispersal.

R. solani AG-3 PT is an important potato pathogen, causing significant yield loss. A population genetic study of *R. solani* AG-3PT populations from North Carolina demonstrates genetic diversity deviated from panmixia, both recombination and clonality, and no population subdivision suggesting that long-distance gene flow was occurring (Ceresini et al 2002; Ciampi et al. 2008). However, Muzhinji et al. (2016) found genetically distinct geographical populations of *R. solani* AG-3PT from potato in South Africa, and among distant populations in Colombian Andes (Ferrucho et al. 2013).

Genetic structure of the *R. solani* AG-2-2IIIB soybean populations from Illinois, Ohio, and Ontario revealed high genotypic diversity in the Ontario population. Percentage clonality was low for the Ontario and Ohio populations but high for the Illinois population (Ajayi-Oyetunde et al. 2019). *R. solani* AG-4 HG-I populations from Iran provided evidence of high genetic diversity, high to moderate levels of gene flow, and mixed reproduction (Haratian et al. 2013).

The populations of *R. solani* have a predominantly mixed reproductive system. Evidence based on population genetic structure analyses of *R. solani* AG-1 IA in China, India, Japan, Philippines, United States, Brazil, Iran, and Latin America, *R. solani* AG-3 PT in Unites States,

South Africa and Colombia, *R. solani* AG-2-2IIIB from North America and *R. solani* AG-4 from Iran indicates that, despite an important clonal component, sexual recombination is common. High genetic diversity and gene flow have been detected in most of the *R. solani* populations, which might be a major evolutionary force in determining population structure, as it affects the level and distribution of genetic variation of the pathogen.

Seedling diseases are commonly present throughout the rice-growing areas in the southern United States. However, it becomes more severe in early plantings when soil temperature is relatively low, which is unfavorable for the growth of rice seedlings. Seedling blight causes stand of rice to be spotty, irregular, and thin, leading to replanting under severe conditions. Identification of pathogens associated with stand loss is the first step to develop effective management strategies.

The overall goal of this research is to manage seedling blight disease of rice by identifying fungal pathogens associated with this disease in the southern United States.

Specific objectives

Objectives 1: Conducting surveys in the rice growing belts of the southern United States to identify fungal pathogens associated with seedling blight in rice.

Objectives 2: Greenhouse and field evaluation of the inbred and hybrid cultivars and breeding lines resistance to seedling blight caused by *R. solani* AG-11 and AG-4.

Objectives 3: Using whole-genome sequence to study genetic structure of populations of *R. solani* AG-11 in the southern United States.

Hypothesis

Hypothesis 1: Soil-borne fungi along with some new fungal pathogens may cause seedling stand loss in the southern United States.

Hypothesis 2: The US rice genotypes may have certain degree of resistance to seedling blight caused by *R. solani* AG-11 and *R. solani* AG-4.

Hypothesis 3: The populations of *R. solani* AG-11 in the southern United States are differentiated with respect to geographical locations (AR, MS, MO, and TX).

CHAPTER II

FUNGAL PATHOGENS ASSOCIATED WITH RICE SEEDLING BLIGHT IN THE SOUTHERN UNITED STATES

Synopsis

Surveys were conducted in five southern rice-producing states (Arkansas, Louisiana, Mississippi, Missouri, and Texas) in the United States during the 2018 and 2019 cropping seasons to determine the distribution and importance of fungal pathogens associated with seedling blight in rice. A total of 384 fungal isolates were collected and identified as belonging to four genera: *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii*, and *Marasmius graminum* based on morphological characteristics, molecular analysis, and koch's postulates. *R. solani* was the most prevalent fungus isolated from diseased samples. Of the 252 pathogenic *R. solani* isolates, 245 were further classified as anastomosis group 11 (AG-11) and 7 as AG-4 based on cultural morphology and sequencing of the rDNA-ITS region. *R. solani* AG-4 and *M. graminum* were most aggressive toward rice, followed by *R. solani* AG-11, *Fusarium* spp., and *S. rolfsii*. *R. solani* and *Fusarium* spp. were predominant in all the five states surveyed. *R. solani* AG-4 and *M. graminum* that has been not recognized as causing any diseases in rice were also the casual agents of seedling blight. The results of this first survey in the southern United States will help develop effective fungicide seed treatment strategies for control of stand loss caused by seedling blight, one of major factors limiting rice production.

Introduction

In the United States, rice (*Oryzae sativa* L.) is produced in four regions, Arkansas Non-Delta, Mississippi River Delta (parts of Arkansas, Mississippi, Missouri, and Louisiana), Gulf Coast (Texas and southwest Louisiana) and Sacramento Valley of California. Among the major rice-producing states, Arkansas had the largest rice production acreage (467,817 ha) followed by California (201,533 ha), Louisiana (171,991 ha), Missouri (75,676 ha), Texas (63,536 ha), and Mississippi (47,348 ha) in 2019 (USDA, ERS, 2020). Most rice in the southern United States (Arkansas, Louisiana, Mississippi, Missouri, and Texas) is dry-seeded, whereas almost all rice in California is water-seeded.

Seedling blight is an important seedling disease in dry-seeded rice, causing significant stand loss annually. Seedling blight is a disease complex caused by several seedborne and soilborne pathogens. In the United States, the soil-borne fungi *Pythium arrhenomanes*, *P. irregulare, P. dissotocum, P. spinosum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and seed-borne fungi *Alternaria padwickii*, *Cochliobolus miyabeanus*, *Curvularia lunata*, *Fusarium roseum*, *F. moniliforme,* and *Sarocladium oryzae* have been frequently isolated from blighted seedlings in dry-seeded rice (Eberle et al. 2007; Groth et al. 1991; Oh 1985; Rush 1992). Multiple species of *Pythium, Phytophthora, Fusarium, Rhizoctonia,* and *Rhizopus* were reported causing rice seedling blight in China, Japan, and Korea (Gao et al. 2001; Kobori et al. 2004; Liu et al. 2019; Rush 1992).

Symptoms of rice seedling blight include pre- or post-emergence damping-off, resulting in irregular or thin plant stands. Even if blighted seedlings survive, they lose vigor, become stunted, and are weakened for growth. Seed germination and growth of seedlings are often poor when rice is planted during March to early April in the southern rice-growing belts in the United

States. Low soil temperatures that inhibit the rapid germination and growth of rice favor the development of seedling blight (Groth et al. 1991). Due to lack of resistant cultivars against seedling diseases (Okubara et al. 2014), growers rely only on fungicide seed treatment for control of seedling diseases. Fungicides registered for seed treatment in dry-seeded rice in the United States include Dynasty® (azoxystrobin), Apron® XL (mefenoxam), Maxim® 4 FS (fludioxonil), CruiserMaxx® Rice (azoxystrobin, fludioxonil, mefenoxam, and thiamethoxam (insecticide)), NipsIt® SuiteRice (fludioxonil, metalaxyl and clothianidin (insecticide)), Vitavax® 200 (carboxin + thiram), Dithane® (mancozeb), Vibrance® (sedaxane), Allegiance® FL (metalaxyl), Trilex® 2000 (trifloxystrobin and metalaxyl), and EverGol Energy® (prothioconazole, penflufen and metalaxyl). These fungicides are mainly targeted for control of a range of soil- and seed-borne pathogens, including *Pythium* and *Rhizoctonia*. However, these seed treatments are not always effective due to the complex pathogens involved in seedling diseases.

Although various seedborne and soilborne pathogens can cause seedling blight, there is no data available in literature on which species are dominant in the current drill-seeded rice production systems in the southern United States. Disease surveys are needed to better understand the distribution, frequency, and importance of fungal pathogens associated with rice seedling diseases in the region, which will serve as the foundation to develop and implement effective management options for control of seedling diseases in rice. Therefore, surveys were carried out in the southern United States with the objectives of identifying fungal species associated with rice seedling blight and determining the prevalence and importance of these fungal pathogens in rice in the southern United States.

Materials and Methods

Field surveys and fungal isolation

Surveys were conducted during the 2018 and 2019 crop seasons in 70 commercial rice fields located across five southern rice-producing states of the United States (Arkansas, Louisiana, Mississippi, Missouri, and Texas) (Figure 1). Most of the fields surveyed were planted with fungicides treated seeds. Typically, 10 to 15 seedlings showing damping-off symptoms were uprooted and collected from each field at 2 to 3 weeks after emergence. These samples were brought to the laboratory for fungal isolation.

Symptomatic tissues (0.5 to 1 cm) from diseased seedlings were surface sterilized with 1% sodium hypochlorite, double rinsed in sterilized distilled water, blotted dry on sterilized filter paper, and plated on two agar media. Potato dextrose agar (PDA) amended with streptomycin sulfate (50 mg/liter) was used to isolate general fungi, and corn meal agar amended with streptomycin sulfate (50 mg/liter) and benomyl (100 mg/liter) was used to isolate oomycetes. The plates were incubated at 24°C with a 12-h photoperiod in a growth chamber. After 96 h, hyphal tips of all fungal colonies were transferred separately into PDA to obtain pure cultures, and the pure cultures were stored in 4°C refrigerator. For long-term storage, two PDA plugs (4 mm in diameter) from actively growing 5-days-old cultures of each isolate were placed in a1.5-ml microcentrifuge tube containing 750 µl of 15% glycerol and maintained at -80°C.

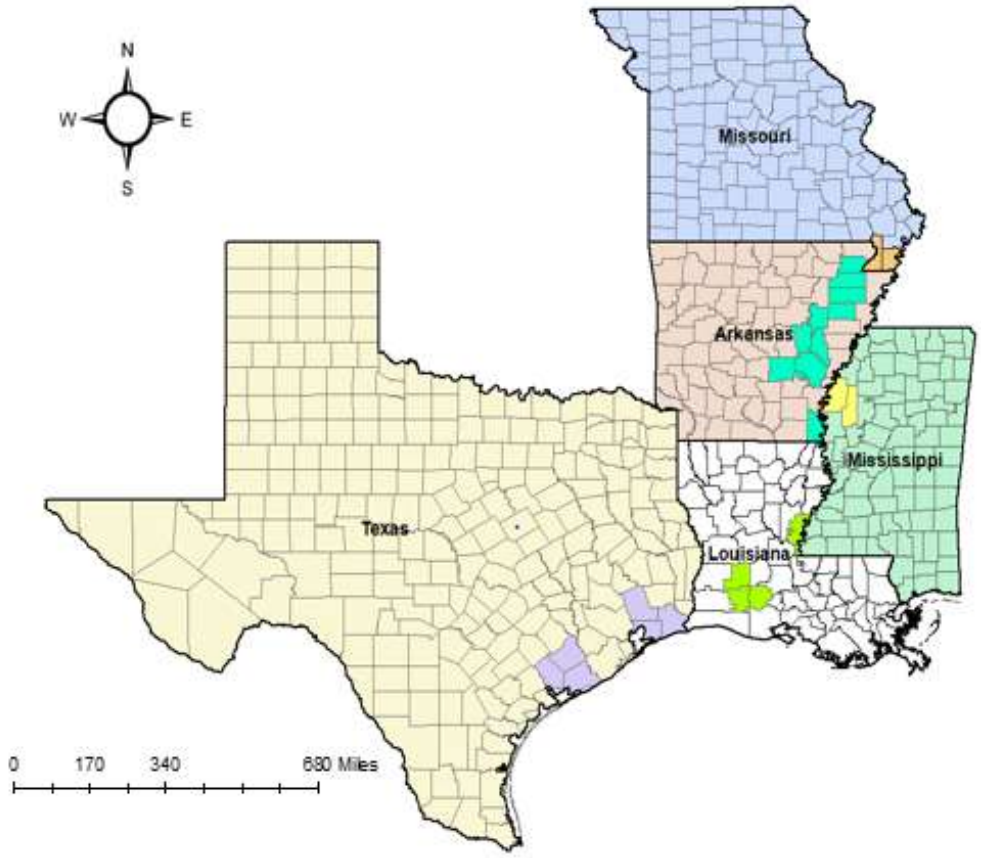

Figure 1. Sampling sites of diseased rice seedlings in the southern United States in 2018 and 2019. Symptomatic seedlings were collected from 10 counties in Arkansas, four parishes in Louisiana, two counties in Mississippi, two counties in Missouri, and six counties in Texas.

Fungal identification

The fungal isolates recovered from the symptomatic rice seedlings were identified based on colony characteristics, microscopic observations of hyphal and spore morphology, and sequencing of nuclear ribosomal internal transcribed spacer (ITS) region of the representative isolates using primers ITS1 and ITS4 (White et al. 1990). Specifically, *R. solani* strains were identified using morphological characteristics of hyphae, sclerotia, and cellular nuclei number in young vegetative hyphae (Andersen 1996; Parmeter et al. 1967, 1970; Sneh et al. 1991). *S. rolfsii*

strains were identified based on colony characteristics and sclerotia formation (Punja and Damiani 1996). Morphological and molecular characteristics were used to identify sterile white basidiomycetes fungi (Bell and Sumner 1984; Howard et al. 1977; Vinnere et al. 2005). The *Fusarium* isolates were identified at the genus level based on their conidia, colony appearance and pigmentation (Leslie and Summerella 2006).

Anastomosis grouping of R. solani

R. solani stains were further classified into anastomosis group (AG) by sequencing of nuclear ribosomal ITS region (Kuninaga et al. 1997; Sharon et al. 2008). *R. solani* isolates were first grouped based on cultural morphology by growing the isolates on PDA at 25°C for 5 days (Figure 3) and then sequencing of rDNA-ITS region of the representative isolates from each group (Table 1).

Table 1 Representative isolates of *Rhizoctonia solani* AG-11, *R. solani* AG-4, and *Marasmius graminum* used for sequencing and molecular identification in this study

Fungal species	**Isolate**	**Location (County/Parish, state)**	**GenBank accession number of ITS sequence**
R. solani AG-11	SG_R15	Jefferson Co., TX	MW522910
	SG_S2	Chamber Co., TX	MW522911
R. solani AG-4	SG_732	Jefferson Co., TX	MN053033
	SG_2T	Wharton Co., TX	MN053034
M. graminum	SG_388	Jefferson-Davis Parish., LA	MT524457

ITS = Internal transcribed spacer

DNA extraction, PCR amplification, and sequencing

Representative isolates of *R. solani* and sterile white basidiomycetes fungi were selected for molecular identification (Table 1). For genomic DNA extraction, each isolate was grown on PDA at 25°C. Fungal mycelia were harvested from 5-day-old cultures and placed in 1.5-ml microcentrifuge tube. DNA extraction was carried out using the Zymo Research Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research Corp.) following the manufacturer's recommendations. Genomic DNA was amplified using primer ITS1 and ITS4 (White et al. 1990). PCR products were separated on 2% agarose gels, stained with ethidium bromide, and viewed using UC transilluminator. The PCR products were purified using Zymoclean Gel DNA Recovery Kit (Zymo Research Corp.) following manufacturer's recommendations and sent for Sanger sequencing at Eton Bioscience, Inc. San Diego, CA. Each isolate was sequenced in both directions, allowing for sequence analysis of both the ITS1 and ITS4 regions and sequences were deposited at NCBI database (Table 1).

Pathogenicity tests

The pathogenicity of all recovered fungal isolates was determined in a growth chamber based on the method described by Carling and Leiner (1990). Briefly, pots (6.5 cm in diameter x 7.5 cm in height) were filled with 100 g of sterilized sand that had been sterilized twice at 121°C for 30 min on each of two consecutive days and added water to its field capacity. Five PDA plugs (4 mm in diameter) from 5-days-old growing cultures were placed on the sand surface of each pot. Pots inoculated with PDA plugs without any fungus served as the control. Five seeds of the rice cultivar Presidio were planted into each pot and covered with 10 g of sterilized sand. Before sowing, the seeds were surface sterilized in 10% household bleach for 5 min and rinsed with distilled water five times. Pots were maintained at 25±2°C in a growth chamber with a 12-h

photoperiod for 14 days. Each pot was applied with 50 ml of water at the 4-day intervals. Each treatment had five replicated pots and the experiment was conducted twice.

After 2 weeks, seedlings were harvested, and roots and mesocotyls were observed for the disease symptoms. Disease severity was assessed using a modified disease severity rating scale of Carling et al. (1994): 0 = no symptoms, 1 = slight discoloration, 2 = moderate discoloration or small lesions, 3 = girdling lesion, 4 = post-emergence damping-off, and 5 = pre-emergence damping-off.

Percent plants stand loss was calculated using the following formula:

Stand loss (%) = [(No. of plants in the control pot – No. of plants in the inoculated pot) / No. of plants in the control pot] ×100

Statistical analysis

Statistical analyses were carried out in SAS (version 9.4; SAS Institute Inc., Cary, NC). The data on percent stand loss was subjected to analysis of variance (ANOVA) and Tukey honestly significant difference for multiple means comparison was computed at $P = 0.05$. Disease severity rating was analyzed using the nonparametric Kruskal-Wallis rank test and mean separation using the Wilcox rank-sum test.

Results

Symptoms of seedling blight

Seedling blight symptoms usually became evident within a few days after emergence. The most common symptoms observed were pre- and post-emergence damping-off. The rotting symptoms in the embryo area of seeds were visible in the non-germinated seeds, whereas dark brown necrotic lesions were usually present on the radicles, coleoptiles, or mesocotyls in the germinated seedlings (Figure 2B, 2C). Seedling blight caused by *S. rolfsii* killed rice seedlings

after emergence. Cottony white mycelium and small, round, tan sclerotia were present at the base of the affected seedlings. The symptoms caused by *Marasmius graminum* were dark-brown necrotic lesions on the mesocotyls and roots. The necrotic lesions were covered with white superficial mycelium (Figure 2D).

Figure 2. Seedling disease symptoms observed in the drill-seeded rice field: A. Rice seedlings showing post-emergence damping-off symptoms (arrow) in a rice field at Lake village, Arkansas; B. Dark brown necrotic lesion (arrow) on mesocotyl usually caused by *Rhizoctonia solani*; C. Healthy rice seedlings (right) vs. diseased seedlings (left); and D. Symptoms of stem necrotic lesion caused by *M. graminum*, covering with white superficial mycelium in rice seedlings.

Isolation and identification of fungi

A total of 445 fungal isolates were collected from diseased seedlings in this study. The isolates were identified into four genera based on colony morphology, microscopic observation, and molecular identification. *R. solani* and *Fusarium* spp. were the most common fungi isolated from the diseased samples, whereas *S. rolfsii*, and *M. graminum* occurred less frequently (Table 2). *R. solani* accounted for 57% of total no. of the isolates obtained, followed by *Fusarium* spp. (27%).

Frequency and distribution of fungal pathogens

A total of 384 fungal isolates, belonging to *R. solani*, *Fusarium* spp., *S. rolfsii* and *M. graminum*, were found to be pathogenic to rice in pathogenicity tests (Table 2). The percentage of *R. solani*, *Fusarium* spp., *S. rolfsii*, and *M. graminum* recovered from rice seedlings varied by year and state (Table 2). However, *R. solani* was the most frequently isolated fungal pathogen (62 to 83% of total pathogenic isolates) in Arkansas, Mississippi, Missouri, and Texas, whereas *S. rolfsii* was the most frequently isolated pathogen in Louisiana (Figure 4). *Fusarium* spp. were the second most recovered fungal pathogen (Table 2). *S. rolfsii* and *M. graminum* were isolated mainly from Louisiana and Texas (Table 2). The frequency of percentages of *R. solani* and *Fusarium* spp. isolated across 2018 and 2019 increased from 60 to 80 and 11 to 20, respectively (Table 2). No isolates of *S. rolfsii* and *M. graminum* were obtained in 2019. *R. solani* accounted for 72% of total pathogenic fungal isolates, followed by *Fusarium* spp. (17%), *S. rolfsii* (7%), and *M. graminum* (4%).

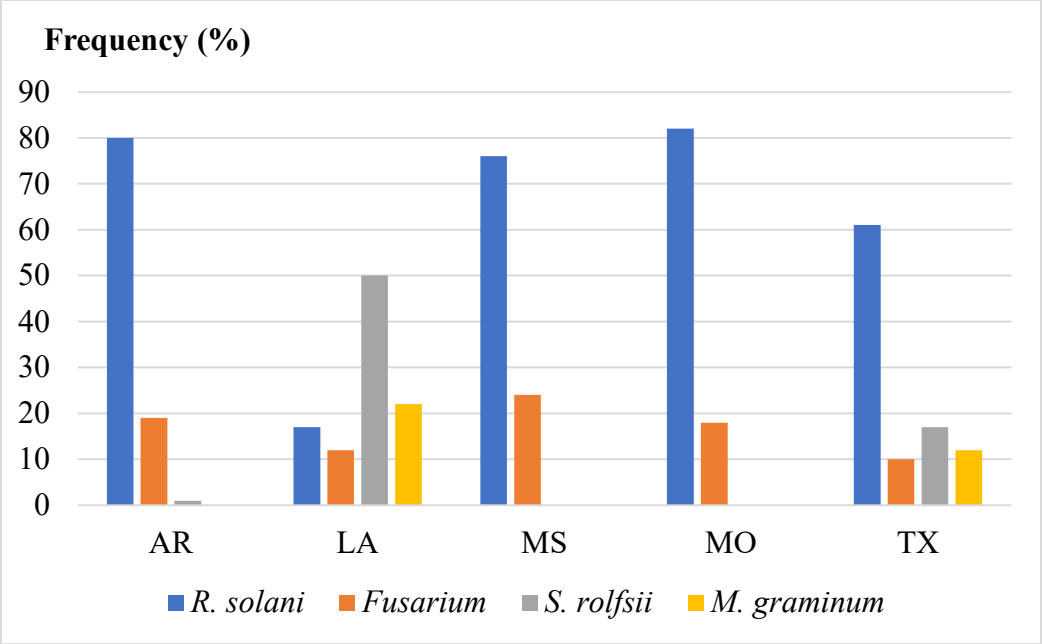

Figure 3. Frequency (%) of fungal pathogens isolated from rice seedlings in Arkansas (n=349), Louisiana (n=70), Mississippi (n=75), Missouri (n=116), and Texas (n= 190).

Table 2 Pathogenic isolates of *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii*, and *Marasmius graminum* from Arkansas, Louisiana, Mississippi, Missouri, and Texas in the 2018 and 2019 cropping seasons

				Number (percentage) of fungal pathogens				
Year	**State[a]**	**County/Parish**	**No. of Fields**	***R. solani***	***Fusarium* spp.**	***S. rolfsii***	***M. graminum***	**Total isolates**
2018	TX	Wharton	2	14 (60.8)	3 (13.0)	0 (0)	6 (26.1)	23
		Chambers	7	25 (80.6)	5 (16.1)	1 (3.2)	0 (0)	31
		Liberty	2	3 (33.3)	0 (0)	6 (66.7)	0 (0)	9
		Matagorda	4	3 (33.3)	0 (0)	3 (33.3)	3 (33.3)	9
		Jefferson	2	7 (77.8)	0 (0)	2 (22.2)	0 (0)	9
		Jackson	2	2 (28.5)	1 (14.3)	3 (42.8)	1 (14.2)	7
	LA	Jefferson						
		Davis	1	0 (0)	0 (0)	4 (57.1)	3 (42.8)	7
		Acadia	2	1 (20.0)	0 (0)	3 (60.0)	1 (20.0)	5
		Concordia	3	1 (33.3)	0 (0)	2 (66.7)	0 (0)	3
	AR	Chicot	2	5 (83.3)	1 (16.7)	0 (0)	0 (0)	6
		Arkansas	2	3 (50.0)	2 (33.3)	1 (16.7)	0 (0)	6
		Prairie	2	4 (100.0)	0 (0)	0 (0)	0 (0)	4
	MS	Bolivar	4	12 (85.7)	2 (14.3)	0 (0)	0 (0)	14
	Total	**13**	**35**	**80 (60.1)**	**14 (10.5)**	**25 (18.8)**	**14 (10.5)**	**168**
2019	AR	Monroe	1	10 (100.0)	0 (0)	0 (0)	0 (0)	10
		Arkansas	6	26 (74.3)	9 (25.7)	0 (0)	0 (0)	35
		Prairie	2	7 (53.8)	6 (46.2)	0 (0)	0 (0)	13
		Greene	1	6 (85.7)	1 (14.2)	0 (0)	0 (0)	7
		Craighead	1	6 (85.7)	1 (14.2)	0 (0)	0 (0)	7
		Poinsett	5	26 (83.9)	5 (16.1)	0 (0)	0 (0)	31
		Cross	3	10 (90.9)	1 (9.1)	0 (0)	0 (0)	11
		Woodruff	3	15 (88.2)	2 (11.7)	0 (0)	0 (0)	17
	MO	Dunklin	4	29 (85.3)	5 (14.7)	0 (0)	0 (0)	34
		Pemiscot	5	22 (78.6)	6 (21.4)	0 (0)	0 (0)	28
	MS	Bolivar	1	2 (50.0)	2 (50.0)	0 (0)	0 (0)	4
		Sunflower	2	12 (75.0)	4 (25.0)	0 (0)	0 (0)	16
	LA	Allen	1	1 (33.3)	2 (66.7)	0 (0)	0 (0)	3
	Total	**13**	**35**	**172 (79.6)**	**44 (20.3)**	**0 (0)**	**0 (0)**	**216**

[a] AR= Arkansas, LA= Louisiana, MS= Mississippi, MO= Missouri, and TX= Texas.

Anastomosis grouping of R. solani

The 252 isolates of *R. solani* were grouped into two morphologically distinct groups based on colony characteristics (Figure 4). Colony of *R. solani* AG-4 isolates in the center of the PDA plates were off-white during early growth stage. Based on sequencing of nuclear ribosomal ITS region of representative isolates from each group (Table 1), these isolates were characterized into two anastomosis groups: *R. solani* AG-11 (n = 245) and *R. solani* AG-4 (n = 7).

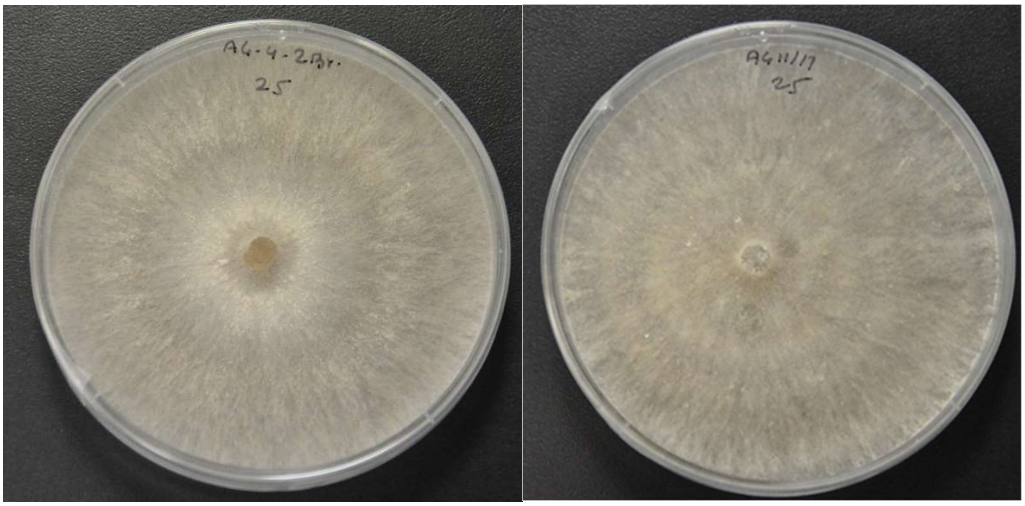

Figure 4. Morphological characteristics of *Rhizoctonia solani* AG-4 (left) and AG-11 (right) on PDA at 25°C for 5 days.

Pathogenicity tests

All recovered fungal isolates were evaluated for their pathogenicity on rice seedlings in the growth chamber. All tested isolates of *R. solani* AG-11 (n = 245), *R. solani* AG-4 (n = 7), *S. rolfsii* (n=25), and *M. graminum* (n=12) were pathogenic to rice seedlings. Approximately 50% isolates of *Fusarium* spp. showed pathogenic reaction to rice seedlings. There were significantly differences ($P < 0.0001$) in median disease severity rating, mean ranks, and percent stand loss among different fungal species (Table 3). Median disease severity rating, mean ranks, and percent stand loss were significantly higher on rice seedlings inoculated with *R. solani* AG-4 and

M. graminum than with *R. solani* AG-11, *Fusarium* spp., and *S. rolfsii* (Table 3 and Figure 5). *R. solani* AG-4 was more aggressive in causing rice seedling blight than *R. solani* AG-11. There was positive correlation between median disease severity rating and percent stand loss ($r = 0.76$, $P < 0.0001$).

Table 3 Median disease rating, mean rank, and stand loss (%) caused by *Rhizoctonia solani* AG-11, *R. solani* AG-4*, Fusarium* spp., *Sclerotium rolfsii*, and *Marasmius graminum* on rice seedlings under controlled conditions

Fungus	**No. of isolates (n)**	**Disease assessment**		
		Median disease rating (0-5) [x]	**Mean rank**	**Stand loss (%)**
R. solani AG-11	245	4	201.1b[y]	60.9 ± 1.25bc[z]
R. solani AG-4	7	5	356.0a	87.4 ± 3.95a
Fusarium spp.	58	3.5	174.3b	52.5 ± 3.65c
S. rolfsii	25	3	143.2bc	41.7 ± 7.19c
M. graminum	12	5	374.0a	80.3 ± 3.97ab
Untreated control		0	2.0d	0 ± 0d
P-value			<0.0001	<0.0001

[x] Disease ratings were collected from 10 treated plants per fungal isolate.
[y] Results from a Kruskal-Wallis test were significant ($\chi 2 = 107.1$, df = 5, $P = < 0.0001$). Pairwise comparison was performed using a Wilcoxon rank-sum test ($\alpha = 0.05$).
[z] Means of two experiments with five replications of n isolates followed by standard error of the mean.
Treatment means followed by the same letter are not significantly different based on the Tukey HSD test at $P = 0.05$.

Figure 5. Aggressiveness of fungal pathogens inoculated with: A. *Rhizoctonia solani* AG-11, B. *R. solani* AG-4, C. *Fusarium* spp., and D. *Marasmius graminum.*

Discussion

This work represents the first multistate survey to identify the fungal pathogens associated with seedling blight of rice and their distribution in the southern United States. Four fungal genera, *R. solani*, *Fusarium* spp., *S. rolfsii* and *M. graminum* were identified as pathogens causing seedling blight and distributed in all five rice growing states in US.

The results of this study reveal that *R. solani* was the most prevalent fungal pathogen causing seedling blight of rice in the southern United States. Although various studies have reported that *R. solani* is an important pathogen causing seedling blight in dry-seeded rice, its anastomosis groups were unknown. The *R. solani* populations causing seedling blight in the current study were further classified into two anastomosis group: AG-11 and AG-4. *R. solani* AG-11 was found to be the most prevalent population in this five-state survey study. The

dominant occurrence of *R. solani* AG-11 in rice in this study is consistent with the dominant occurrence of *R. solani* AG-11 in soybean fields rotated with rice but reported non-pathogenic to the host plant in a previous study conducted by Spurlock et al. (2016). Jones and Carling (1999) and Carling et al. (1994) also reported the presence of *R. solani* AG-11 in rice in Arkansas and Texas, however, this AG has not been recognized as a pathogen causing seedling diseases in rice. So far, *R. solani* AG-11 has been reported to be pathogenic to lily (*Lilium* spp.) in Japan (Misawa et al. 2017), lupine (*Lupinus angustifolius* L.) in Western Australia (Sweetingham et al. 1986), snap bean (*Phaseolus vulgaris*) in Idaho (Woodhall et al. 2020), soybean (*Glycine max* (L.) Merr.) in Arkansas (Ajayi-Oyetunde and Bradley 2016), and sugar beet (*Beta vulgaris*) in Poland (Moliszewska et al. 2020). The results of our pathogenicity tests indicated that *R. solani* AG-4 was more aggressive (stand loss = 87%) toward rice seedlings than *R. solani* AG-11 (stand loss = 61%). The wide occurrence of highly aggressive *R. solani* AG-4 in Arkansas, Missouri, and Texas, indicates that this pathogen may have widely established in the southern United States where rice-soybean rotations are among the most common cropping systems. Although *R. solani* AG-4 has been reported to cause seedling diseases in various crops including soybean, broccoli, cotton, melon, peanut, potato, spinach, and tomato (Ajayi-Oyetunde and Bradley 2017), our study reported rice as a new host of *R. solani* AG-4 and a causal agent of seedling blight of rice (Gaire et al. 2020).

Following *R. solani*, *Fusarium* spp. were the second most prevalent species associated with seedling blight in rice in the current study. *Fusarium* spp. were isolated from all the five states surveyed, and almost 50% of the recovered *Fusarium* isolates were pathogenic to rice seedlings. Due to the complexity of *Fusarium* spp., the *Fusarium* spp. isolates were not identified into the species level in this study. Further studies are needed to identify these isolates

to understand the species of *Fusarium* associated with seedling disease in rice. *F. roseum* and *F. solani* have been reported to be the economically important species causing seedling blight of rice in the nursery box in Japan (Ibaraki 1988). *F. oxysporum* has been reported as a dominant fungal species causing seedling blight in rice in China (Liu et al. 2019).

Sterile white basidiomycetes fungus *M. graminum* has been reported as a new pathogen causing seedling blight in rice in our study (Gaire et al., 2021). In this study, *M. graminum* was found to be present in three counties of Texas and two parishes of Louisiana, accounting for 14% of total fungal isolates from the 2018 survey. We also found that *M. graminum* was one of the most aggressive pathogens (stand loss = 80%) causing seedling blight in rice. Because of its wide distribution and high aggressiveness, the new pathogen may pose a threat to early rice establishment that affects rice productivity in the dry-seeded rice production system. In addition to rice, *M. graminum* has been reported as a causal agent of stem rot of snap bean (*Phaseolus vulgaris*) in Florida (Howard et al. 1977) and Nebraska (Harveson 2002), roots or hypocotyl rot of corn (*Zea mays*), snap bean, squash (*Cucurbita pepo* var. *melopepo*) and peanut (*Arachis hypogaea*) in Georgia (Bell and Sumner 1984), and crown rot of pigeon pea (*Cajanus cajan*) in Puerto Rico (Kaiser et al. 1987).

Sclerotium seedling blight caused by *S. rolfsii* was observed with a high frequency (50%) in Louisiana in the current survey. Groth et al. (1991) and Rush (1992) also reported a similar finding that *S. rolfsii* is a major pathogen causing severe damage to rice seedlings at emergence under the humid and warm weather conditions in Louisiana.

In this multistate survey, no *Pythium* and *Phytophthora* isolates were recovered from any rice samples. Previously, these oomycetes species were thought among the important soil-borne pathogens causing stand loss in rice (Rush 1992; Rush and Schneider 1990; Zhou and Jo 2014).

Rush and Schneider (1990) reported that rice seedling disease caused by the genus *Pythium* was most common in the Louisiana water-seeded rice production system. *Pythium dissotocum*, *P. spinosum*, *P. irregulare*, *P. arrhenomanes*, and *P. graminicola* were among the soil-borne pathogens causing seedling blight in the dry-seeded rice production system in the southern United States (Eberle et al. 2007; Rush, 1992). *Pythium arrhenomanes* was found most important pathogen causing seedling blight in rice in the Australia (Cother and Gilbert 1993). *Pythium graminicola* was the most common pathogen of rice seedling blight in Japan (Kato el al. 1985) and Korea (Sung et al. 1983). No presence of *Pythium* and *Phytophthora* in the surveys of the current study might be due to fungicides-coated seeds planted in most of the rice fields, differences in cropping system, rice cultivar, crop management practice, and other unknown factors as compared to previous studies. In addition, in this study we did not observe any severe seedling blight diseases caused by the seed-borne fungi *Alternaria alternata*, *A. padwickii*, *Aspergillus flavus*, *Cochliobolus miyabeanus* (anamorph*: Bipolaris oryzae*), *Curvularia lunata*, *Penicillium* spp., and *Phoma* spp. that have been reported in other previous studies (Dossou and Silue 2017; Liu et al. 2019; Rush 1992).

Differences in the frequency and distribution of fungal pathogens among the five states surveyed in this study might be due to the differences in cropping systems and soil types. In Arkansas, Mississippi and Missouri, most rice is grown in rotations with soybean (Hardke 2015; Riar et al. 2013). In Texas, most common cropping systems are rice-fallow-rice and rice-fallow-fallow-rice (Liu et al. 2016). In Louisiana, rice is normally rotated with soybean alone or in rotation with soybean and crawfish (Street and Bollich, 2003). These different cropping systems could have a significant impact on the distribution and importance of soilborne pathogens. *R. solani* can infect both rice and soybean, resulting in inevitable accumulation of inoculum in the

rice and soybean production system over years. This can be a reason why *R. solani* was the most prevalent species in the five states surveyed in the current study. However, in Louisiana where crawfish rotation with rice is common, crawfish production can be a factor contributing to the reductions of *R. solani*, as observed in this study with *R. solani* not being the most prevalent species. Crawfish are herbivores, detritivores and omnivores and can consume almost all organic matters in the field, including mycelium and sclerotia of *R. solani* (Lutz 2019).

The results of this study have important implications in fungicide seed treatment for control of stand loss caused by seedling blight in rice in the southern United States. Currently, almost all hybrid rice seeds and approximately 60% of inbred seeds are treated with fungicides, mostly with two or more fungicides, for control of stand loss caused by a broad spectrum of soil- and seed-borne pathogens, including *Pythium* spp. *Phytophthora* spp., *R. solani*, *S. rolfsii*, and *Fusarium* spp. For example, CruiserMaxx® Rice, mostly commonly used seed treatment product, contains three fungicides, azoxystrobin, fludioxonil, and mefenoxam, which targets for control of a wide range of soil-borne pathogens, with mefenoxam targeting for control of *Pythium* spp. NipsIt® Suite Rice, another common seed treatment product, contains fludioxonil and metalaxyl, with fludioxonil targeting for *R. solani* and metalaxyl for *Pythium* spp. and *Phytophthora* spp. However, the current seed treated fungicides are not quite effective to protect seedling blight caused by *R. solani*, *Fusarium* spp. and *M. graminum*.

In conclusion, this is the first systematic report on the distribution and importance of fungal species associated with seedling blight in rice in the southern United States. Four fungal taxonomic groups, *R. solani*, *Fusarium* spp., *S. rolfsii*, and *M. graminum*, were found to be the causes of seedling blight, with *R. solani* being the most prevalent species. There were two anastomosis groups, AG-11, and AG-4, present in the *R. solani* populations, with *R. solani* AG-

11 being more prevalent than *R. solani* AG-4. *R. solani* AG-4 and *M. graminum*, which have not been recognized as causing any diseases in rice, were among the pathogens associated with seedling blight in rice. All these fungal pathogens were of great aggressiveness ranging from moderate to high levels toward rice. These findings from the current study can help develop more effective fungicide seed treatment strategies for control of rice seedling blight by targeting the most prevalent fungal populations in a region.

CHAPTER III

EVALUATION OF RICE HOST RESISTANCE TO SEEDLING BLIGHT CAUSED BY RHIZOCTONIA SOLANI

Synopsis

Seedling blight is one of the most important disease complexes in rice in Texas mainly caused by *Rhizoctonia solani* anastomosis group 11 and 4. Use of resistant cultivars would be an effective and environmentally sound strategy to minimize economic losses caused by this disease. However, there is no information regarding rice genotype resistance against these two important pathogens. Two hundred two rice genotypes, including inbred and hybrid cultivars and elite breeding lines were evaluated for resistance to *R. solani* AG-11 under greenhouse condition in 2018. Forty genotypes against *R. solani* AG-11 under field condition at Beaumont in 2019 and additional five hybrid cultivars against *R. solani* AG-11 and *R. solani* AG-4 at Beaumont and Eagle Lake in 2020 were evaluated for resistance to seedling blight caused by *R. solani* AG-11 and *R. solani* AG-4. Most of the breeding lines and inbred cultivars were highly susceptible to seedling blight. However, the hybrid cultivar RT7521 FP and inbred cultivar CLL15 showed low to moderate stand loss against *R. solani* AG-11 in both cropping years. All rice genotypes evaluated against *R. solani* AG-4 were highly susceptible. Results from this study indicated that no breeding lines or commercial cultivars had a high level of resistance against *R. solani* AG-11 and *R. solani* AG-4. More research is needed for identifying resistant genotypes that can be used to develop seedling disease resistant cultivars.

Introduction

Seedling disease, also known as seedling blight or damping-off, ranks among top five diseases with respect to yield loss in the southern rice-producing states in the United States (Allen et al. 2020). Among the various plant pathogens associated with the seedling disease complex of rice, *Rhizoctonia solani* Kühn (syn. *Thanatephorus cucumeris* (A. B. Frank) Donk) represents one of the important and dominant pathogens. *R. solani* is a species complex, classified into 14 anastomosis groups (AG-1 to 13 and AG-BI) (Carling 1996; Ogoshi 1987), which are non-interbreeding populations (Anderson 1982). *R. solani* AG-11 and AG-4 are two important pathogens causing seedling blight in dry-seeded rice in the southern United States (Gaire et al., 2020).

Rhizoctonia solani is a necrotrophic fungal pathogen. In general, plant resistance against necrotrophic pathogens is quantitative and multilayered, making its genetic dissection challenging. Over the past decades, several scientists have conducted research on rice resistance to *R. solani*. These studies focused on disease resistance to sheath blight caused by *R. solani* AG-1-IA. To date, no rice cultivar has been found to be of high-level resistance to sheath blight disease although some cultivars and wild relatives of rice with varying degree of resistance have been reported (Groth and Novick 1992). Short- and medium-grain rice cultivars grown in the southern United States have moderately resistant against sheath blight (Lee and Rush, 1983).

Due to a lack of high levels of resistance in commercial cultivars, growers largely rely on fungicide seed treatment for control of seedling blight. Seedling blight symptoms were frequently observed even in the fields planted with fungicide treated seeds. Organic rice growers rely only on host resistance to protect from diseases. Thus, cultivar resistance can be the most effective and economical method for the management of this disease. However, no research has

been conducted to understand if there are any resistance available in rice genotypes to seedling blight caused by *R. solani* AG-11 and *R. solani* AG-4. The objective of this research was to evaluate inbred and hybrid cultivars and breeding lines for resistance to seedling blight caused by *R. solani* AG-11 and AG-4 under the greenhouse and field conditions.

Materials and methods

Inoculum preparation

R. solani AG-11 isolate SG_R15 and *R. solani* AG-4 isolate SG_2Br originally isolated from rice seedlings in Texas were used in this study. Anastomosis group confirmation was performed by sequencing nuclear ribosomal internal transcribed spacer (ITS) region of both isolates. Inoculum of both anastomosis groups were prepared separately in wheat bran as described by Zhang et al. (2014). Briefly, *R. solani* cultures were grown on petri dishes containing potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) and incubated at 25°C with 12-h light/dark cycles. Conical flask (500 ml) containing 250 g wheat bran (Bob's Red Mill, OR, US) mixed with 250 ml distilled water was autoclaved at 121°C for 40 min each on two successive days. Ten agar plugs (4-mm-diameter) from the edge of 5-day-old actively growing culture were transferred to each conical flask containing sterilized wheat bran and incubated at 25°C with 12-h light/dark cycles for 14 days. The flasks were stirred every 4 days for the uniform growth of the fungus. After two weeks, the inoculum was removed from the conical flasks and overnight air dried in a laminar air flow.

Greenhouse experiment

Initial resistance screenings against *R. solani* AG-11 were conducted on 202 rice genotypes consisting of 18 commercial cultivars, and 21 hybrid and 163 inbred breeding lines developed by Arkansas, Louisiana, Mississippi, Missouri, and Texas (APPENDIX B). Most of

these genotypes were from the 2018 Uniform Regional Rice Nursery (URRN). There were six replicated pots per genotype treatment, and pots were arranged in a completely randomized design (CRD). Ten seeds were sown in each plastic pot (10 cm in diameter) filled with 500 g of sterilized soil inoculated with 1 g of inoculum prepared in wheat bran colonized with a *R. solani* AG-11 isolate. Pots amended with sterilized wheat bran without the fungus served as the controls. Treated pots were placed in a growth chamber at 25ºC with a 14-h of artificial light per day. After 5 days of growth, pots were transferred to a greenhouse. Stand loss was assessed at 2 weeks after planting. The initial evaluation was conducted with the 202 genotypes and subsequent evaluation was conducted with 51 genotypes (17 hybrid and 34 inbred breeding lines) that had the least stand loss in the initial evaluation.

Field trials

A field experiment was conducted at the Texas A&M AgriLife Research Center, Beaumont, Texas in 2019 to evaluate the response of rice genotypes to seedling blight caused by *R. solani* AG-11. Forty rice genotypes, including 17 elite breeding lines that had the least stand loss in the greenhouse evaluation, 2 elite breeding lines from the 2019 Uniform Regional Rice Nursery (URRN), 13 inbred and 8 hybrid cultivars, were arranged in randomized complete block design with four replications (Table 6). Plots consisted of eight 8 ft long rows with 7-in. spacing between rows. Each genotype was planted into each row of the plots except two border rows. Rice cultivar Presidio was planted in all border rows. The genotypes were tested for their germination rates before planting and seedling rates were adjusted to maintain 10 germinated seeds per ft of row. Rice was manually seeded on 15th May. *R. solani* AG11 inoculum that had been prepared in wheat bran was inoculated @ 8 grams per row at the time of seeding. Seedling

stands per row were counted at 30 days after planting and the percentage of stand loss was calculated using the following formula:

Stand loss (%) = [(80 – no. of plants per row) / 80] ×100

where the number 80 was calculated based on 10 germinated seed per ft of row multiplied by 8 ft of row per plot.

In 2020, two field experiments were conducted at the Texas A&M AgriLife Research Center, Beaumont and one filed experiment at Texas A&M AgriLife Research's Wintermann Rice Research Station, Eagle Lake, TX to evaluate the response of rice genotypes to seedling blight caused by *R. solani* AG-11 and *R. solani* AG-4. At the Beaumont site, one trial was setup in one field inoculated with *R. solani* AG-11 and another trial was setup in another field inoculated with *R. solani* AG-4. The same field experiment that was inoculated with *R. solani* AG-4 was repeated at the Eagle Lake site. Forty rice genotypes that were used in the 2019 field experiment and five additional hybrid cultivars were included to evaluate for resistance to *R. solani* AG-11 and AG-4 in 2020. Field set up, inoculations, plantings, and disease assessment were conducted as previously described. The trial at Beaumont was seeded on 2nd April and Eagle Lake on 23rd April.

Data analysis

Percent stand loss data in the 2019 and 2020 field experiments were analyzed separately due to significant differences in stand loss of genotypes by year. Analysis of variance (ANOVA) was conducted using general linear models' procedure (PROC GLM) of SAS statistical software (version 9.4, SAS Institute, Cary, NC). There were no significant genotypes by location interactions, therefore data of the *R. solani* AG-4 inoculated field experiments at Beaumont and Eagle Lake in 2020 were pooled and analyzed together using mixed models' procedure (PROC

MIXED) of SAS statistical software (version 9.4, SAS Institute, Cary, NC). The location and replication were considered as random and genotypes as fixed variable during the analysis. Significant differences among genotypes were determined using a Duncan multiple range test (DMRT) at $P = 0.05$.

Results

Greenhouse experiment

A total of 202 rice genotypes against *R. solani* AG-11 were evaluated in the initial greenhouse screening. Almost 75% of the genotypes evaluated had a stand loss greater than 90% (Figure 6). In the subsequent evaluation of 51 genotypes that had least stand loss in the initial evaluation, most genotypes were susceptible or very susceptible to *R. solani* AG-11, with a stand loss greater than 40% (Table 5). Only breeding lines RU1805233, RU1805236, RU1805203, RU1805223, and RU1805213 showed partial resistance to the seedling blight caused by *R. solani* AG-11 (Figure 7). Mean stand loss of hybrid breeding lines was comparatively lower than the inbred breeding lines.

Table 4 Analysis of variance (ANOVA) for screening of rice genotypes against *Rhizoctonia solani* AG-11 in the greenhouse initial and subsequent screenings

Source of variation	**df**	**MS**	***F***	**P>*F***
Initial screening				
Replication	2	94.73	47.36	0.7028
Genotypes	201	486.55	3.63	0.0001
Subsequent screening				
Replication	2	44.41	0.15	0.858
Genotypes	50	1285.19	4.44	<0.0001

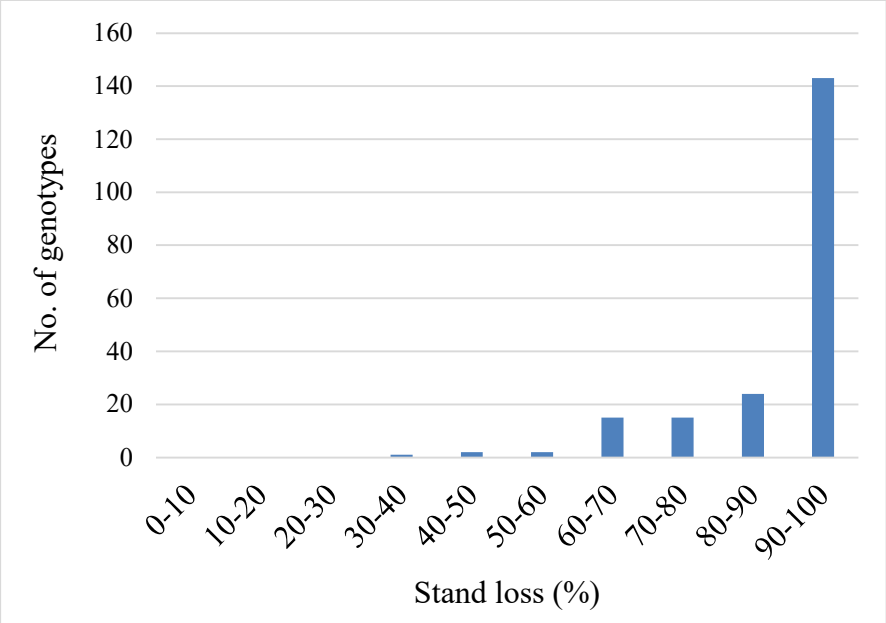

Figure 6. Frequency distribution of percent stand loss caused by *R. solani* AG-11 of 202 rice genotypes.

Table 5 Stand loss of 51 rice genotypes caused by *Rhizoctonia solani* AG-11 in the greenhouse experiment

Genotype	**Stand loss (%)[y]**
RU1805233	12.0± 4.2m[z]
RU1805236	14.0±2.3lm
RU1805203	28.3±13.6k-m
RU1805223	36.5±4.6j-m
RU1805213	39.6±10.2i-m
RU1805228	42.6±2.8g-m
RU1802202	45.8±6.0f-l
RU1805198	50.4±12.7e-k
RU1802094	51.8±1.0d-k
RU1802086	60.7±4.3b-k
RU1702183	61.0±8.0b-k
RU1805208	68.0±2.1a-j
RU1804147	68.4±7.6a-j
RU1602082	68.5±6.5a-j
RU1804139	68.5±3.7a-j
RU1504197	71.7±6.5a-i
RU1804063	71.7±6.5a-i
RU0703144	71.7±2.7a-h
RU1802082	73.8±4.2a-h
RU1804219	75.4±4.4a-h
RU1704154	76.3±6.8a-g
RU1702140	78.5±0.4a-f
RU1804087	79.2±6.6a-f
RU1703126	80.0±2.9a-f
RU1804115	80.5±4.0a-e
RU1804143	81.5±7.3a-e
RU1804107	84.2±4.6a-e

Table 5 continued

Genotypes	Stand loss (%)[y]
RU1804224	87.5±7.2a-c
RU1303181	91.6±4.8a-c
RU1704198	91.6±2.4a-c
RU1803156	91.6±4.8a-c
RU1803140	91.6±4.8a-c
RU1804214	92.1±2.3a-c
RU1804127	100.0±0.0 a
RU1803112	100.0±0.0 a
TH723	41.0±1.0h-m
TH783	43.9±6.2g-m
TH743	50.9±7.7e-k
TH730-1	59.2±6.8c-k
TH730-2	64.3±3.2b-j
TH793	65.4±1.7a-j
TH720	72.2±4.4a-h
TH733	73.2±3.9a-h
TH756	74.6±4.8a-h
TH750	75.4±4.4a-h
TH763	76.7±10.0a-g
TH770	82.7±2.3a-e
TH753	83.3±4.8a-e
TH740	86.3±4.7a-d
TH760-1	87.5±4.2a-c
TH710	95.2±2.7ab

[y] Stand loss (%) = [(No. of plants in control pot – No. of plant in inoculated pot) / No. of plant in control pot] *100.

[z] Means in a row with the same letters are not significantly different at 5% level.

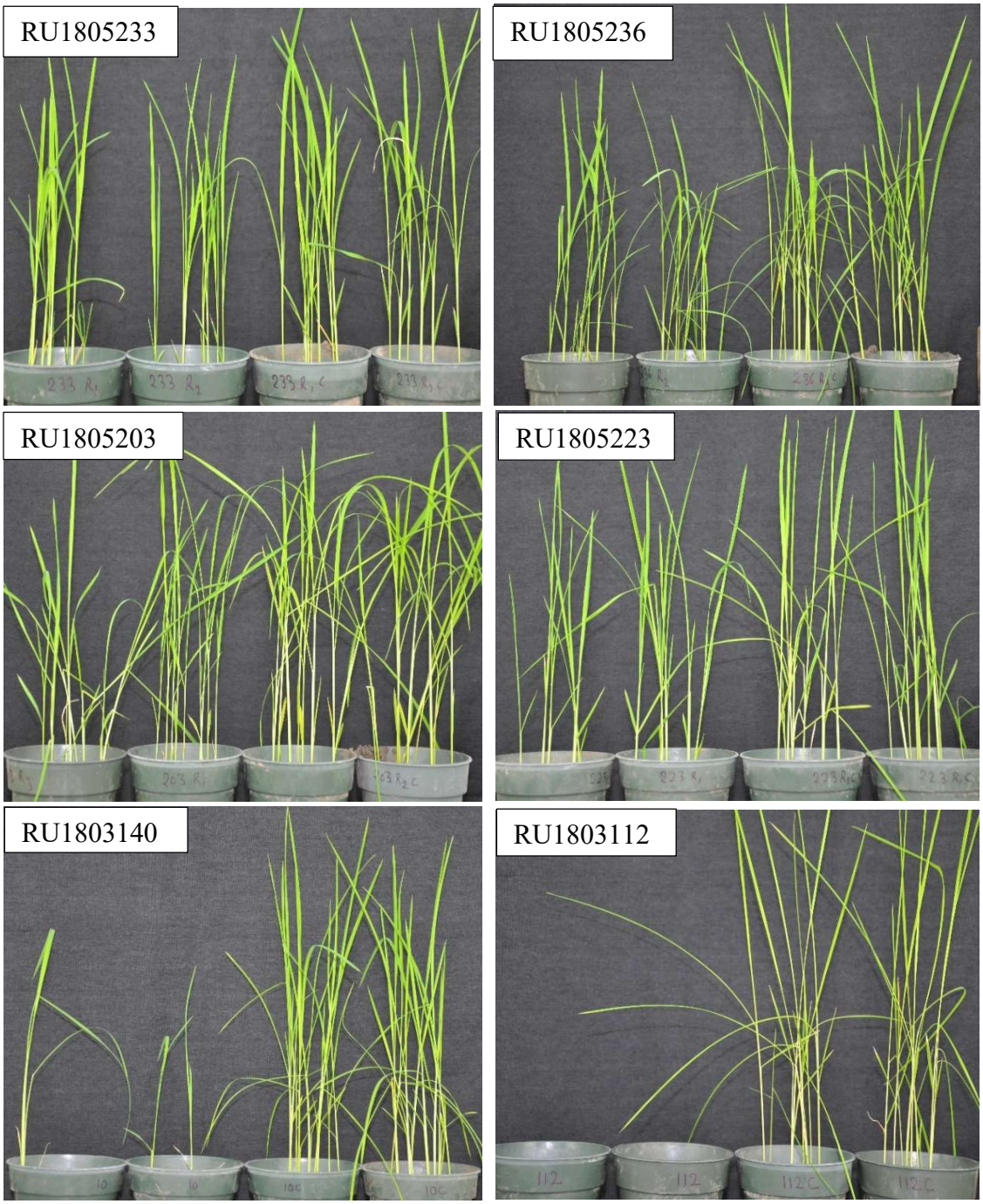

Figure 7. Plant stands on partially resistance (RU1805233, RU1805236, RU1805203, RU1805223) and highly susceptible (RU1803140, RU1803112) genotypes in a greenhouse experiment evaluating rice genotypes against *R. solani* AG-11. First two pots from the left in each picture were inoculated with the seedling blight pathogen *Rhizoctonia solani* AG-11 and the remaining two were non-inoculated control pots.

Field trials

In the 2019 cropping season, the rice genotypes inoculated with *R. solani* AG-11 had stand loss ranging from 15-63% (Table 6). Several inbred cultivars, including Rondo, Jupiter, and Clearfield had low to moderate stand loss (20 to 37%). Most of the hybrids had low to moderate stand loss (15 to 35%). In the 2020 cropping season, most of the genotypes evaluated had higher percent stand loss to *R. solani* AG-11. The rice genotypes had stand loss ranging from 47-96% (Table 6). However, hybrid cultivar RT7521 FP and inbred cultivar CLL15 had moderate stand loss (47-50%) (Table 6).

Rice genotypes inoculated with *R. solani* AG-4 had higher percent stand loss ranging from 80-97% (Table 6). All rice genotypes evaluated against *R. solani* AG-4 were highly susceptible. Hybrid cultivars had a significantly lower stand loss compared to the breeding lines and inbred cultivars.

Table 6 Stand loss (%) in rice elite breeding lines and inbred and hybrid cultivars inoculated with *Rhizoctonia solani* AG-11 and *R. solani* AG-4 under filed conditions

Genotypes	Stand loss (%)[x]		
	2020[y]	2020	2019
	AG-4	AG-11	AG-11
RU1905236	96.3±1.4ab[z]	92.9±0.4a-e	29.6 i-n
RU1805208	96.3±1.2ab	92.9±1.6a-e	52.8 abc
RU1804139	95.6±1.7abc	90.0±3.3a-g	39.0 c-j
RU1804067	95.3±1.2abc	82.1±1.6f-k	34.0 g-m
RU1805233	95.2±0.4abc	96.7±0.7a	44.6 b-h
RU1804187	94.7±0.9abc	73.3±4.3klm	23.1 k-o
RU1805203	94.2±2.0a-d	91.7±1.3a-f	35.0 f-m
RU1802094	94.1±1.3a-e	95.4±1.0a	62.5 a
RU1805213	93.9±1.7a-e	90.8±2.6a-f	48.7 b-f
RU1905233	93.9±0.9a-e	93.3±1.6a-d	50 a-e
RU1804063	93.6±1.1a-f	90.4±2.2a-f	54.3 ab
RU1805198	93.4±0.8a-g	85.0±3.3b-h	45.3 b-h
RU1805236	93.1±1.5a-g	84.6±1.9c-i	36.8 d-k
RU1802202	92.9±1.9a-g	73.8±1.9j-m	30.9 h-n
RU1805228	92.7±2.7a-g	93.8±1.3abc	39.3 c-j
RU1804107	92.5±1.4a-g	95.8±1.0a	37.1 d-k
RU1804147	91.6±1.6a-g	94.2±1.6abc	47.1 b-g
RU1804087	91.1±2.7a-g	76.7±2.5h-l	35.3 f-l
RU1805223	90.9±2.4a-g	95.0±0.0ab	50.9 a-d
Jupitar	97.2±0.9 a	94.6±2.2abc	20.3 m-o
Rondo	95.9±0.9abc	96.3±0.6a	37.1 d-k
Presidio	87.0±1.3gh	90.0±1.3a-g	40.0 c-i
PVL01	94.5±2.3a-d	92.5±1.3a-e	35.9 e-k
CLL15	94.8±1.4abc	49.6±8.2o	25.3 i-o
CL172	94.2±0.7a-d	88.3±2.8a-g	23.4 k-o
CL272	94.1±1.6a-e	74.6±5.8j-m	24.0 k-o
CLM04	94.1±1.4a-e	75.0±5.1i-m	20.9 l-o
CL153	93.4±0.8a-g	93.3±0.7a-d	22.8 k-o
CL111	92.8±1.9a-g	88.3±1.0a-g	31.5 h-n
CL151	92.5±1.1a-g	89.2±3.0a-g	27.1 i-o
CLJ01	90.9±1.5a-g	66.3±5.0mn	27.5 i-o
CL163	90.6±2.2a-g	95.0±0.6ab	24.6 j-o
CLXL745	87.9±2.1d-h	73.3±1.6klm	34.0 g-m

Table 6 Continued

Genotypes	Stand loss (%)[x]		
	2020[y]	2020	2019
	AG-4	AG-11	AG-11
CL Gemini 214	90.3±2.2b-h	70.0±4.5 lmn	15.0 o
RT7311CL	95.6±1.4abc	94.2±0.7abc	-
RT7401	84.0±3.9hi	82.5±0.6 f-k	-
RT3201	87.3±2.7fgh	62.5±3.1mn	-
RT7801	90±3.2b-h	80.4±4.2g-k	33.1 g-m
RT7501	89.8±2.5b-h	77.9±4.0 h-l	35.3 f-l
RT7812CL	89.4±2.3c-h	82.9±1.0e-k	-
RT7301	91.9±2.2a-g	93.7±0.6abc	-
RT7321 FP	87.5±2.3e-h	70.4 ±3.0mn	18.1 no
RT7521 FP	80.0±5.4i	47.1±4.2 o	17.5 o
XP760	92.2±2.1a-g	90.8±1.6a-f	30.9 h-n
XL753	87.0±3.5gh	83.3±1.3d-j	31.8 h-n

[x] Stand loss (%) = [(80 – no. of plants per row) / 80] *100.

[y] Data represent the means combined over two locations of 2020 for analysis

[z] Means in a row with the same letters are not significantly different at 5% level.

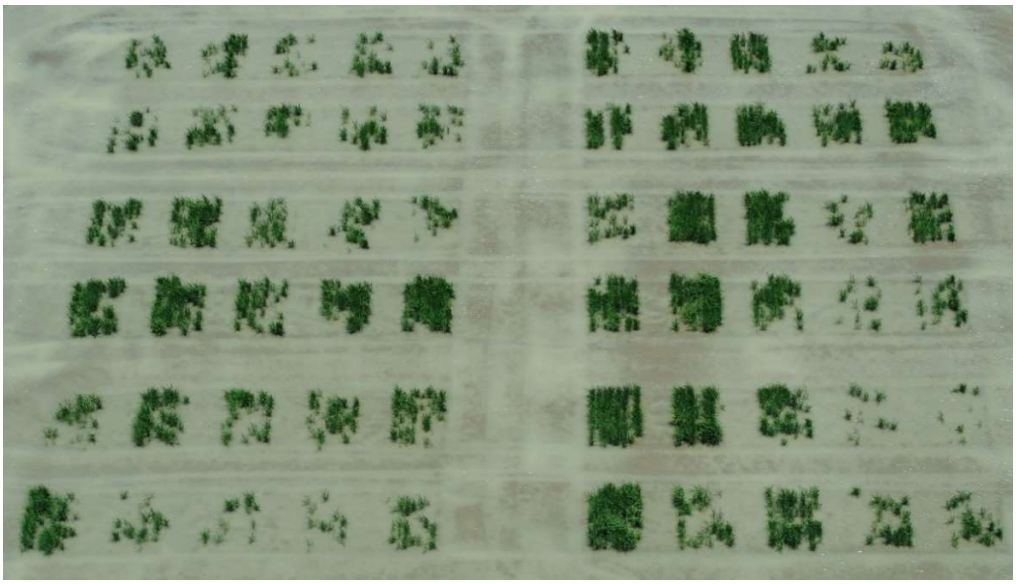

Figure 8. Field plots containing 45 rice genotypes inoculated with *Rhizoctonia solani* AG-4 (left field) and *Rhizoctonia solani* AG-11 (right field) at Beaumont, TX during the 2020 cropping season.

Correlation between resistance of rice genotypes against R. solani AG-11 and AG-4

There is a significant positive correlation ($r = 0.5$, $P = 0.0004$) between the seedling blight resistance against *R. solani* AG-4 and *R. solani* AG-11 in the rice genotypes evaluated in this study.

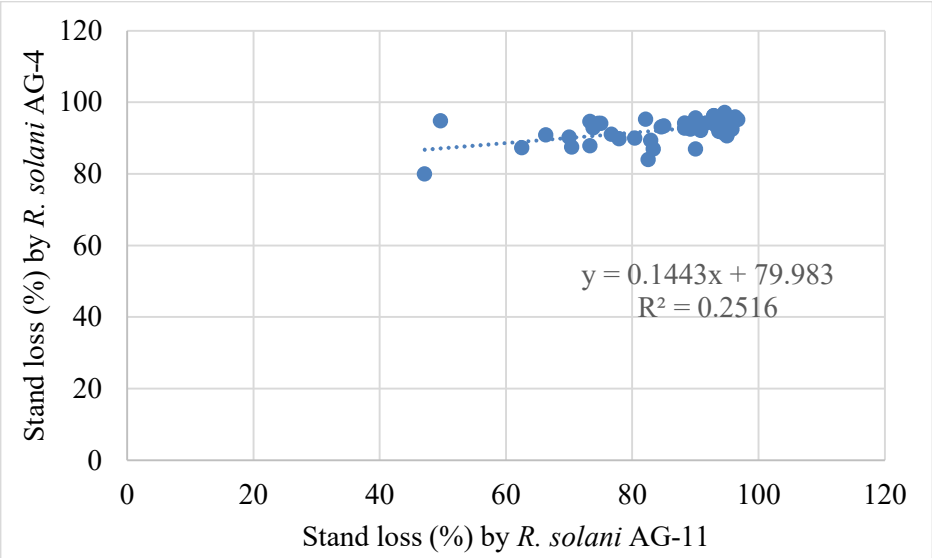

Figure 9. Correlation between stand loss (%) caused by *R. solani* AG-4 and *R. solani* AG-11 on 45 rice genotypes.

Discussion

In this study, rice elite breeding lines, and inbred and hybrid cultivars were screened against the seedling blight pathogens *Rhizoctonia solani* AG-11 and *R. solani* AG-4 over a 3-year period in the greenhouse and field experiments. Most genotypes evaluated were susceptible or very susceptible (>50% stand loss) to *R. solani* AG-11 and *R. solani* AG-4. However, there were few genotypes showing partial resistance (<50% stand loss) to these pathogens. The significant positive correlation of resistant traits between *R. solani* AG-4 and *R. solani* AG-11 provided evidence of similar resistant genes associated with seedling blight.

Some elite breeding lines showed partial resistant to *R. solani* AG-11 in the greenhouse experiment (12 – 40 % stand loss) (Figure 7) and field experiment in 2019 (<50% stand loss) (Table 6). In the field experiments inoculated with *R. solani* AG-11, the stand loss in 2019 were comparatively lower than those in 2020. This could be contributed to the low soil temperature in 2019 that inhibited the germination and growth of rice that favored the development of seedling blight (Groth et al. 1991). The daily average soil temperatures were higher during the planting month in 2019 than in 2020 (Figure 10).

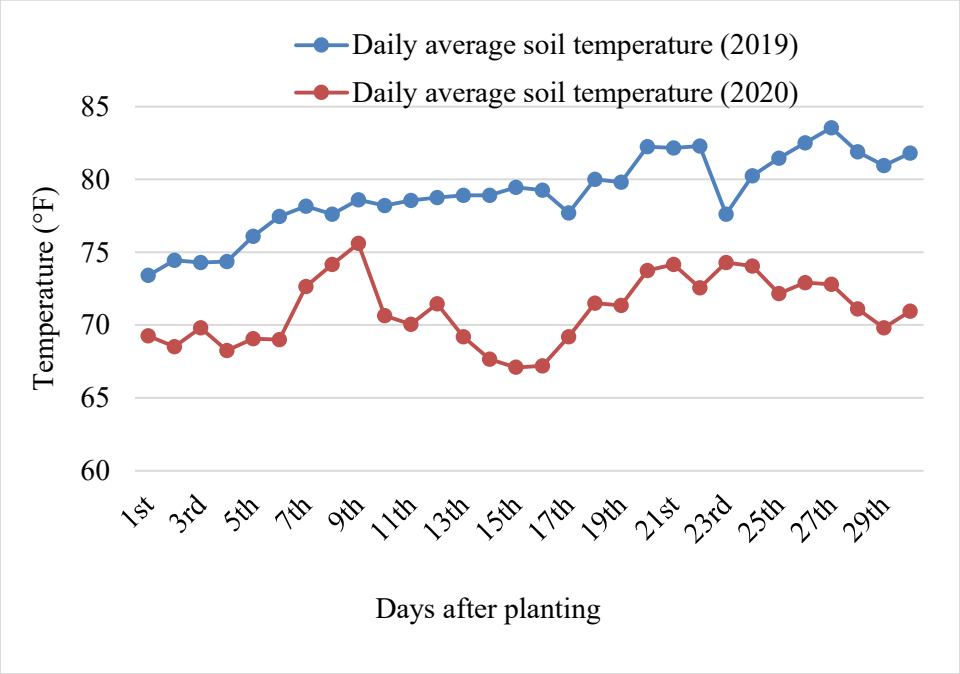

Figure 10. Average daily soil temperatures (°F) for 30 days after planting at Beaumont, TX in 2019 and 2020 (Source: https://beaumont.tamu.edu/ClimaticData/)

One of the goals of this research was to identify commercial rice cultivars grown in Texas that would show resistance to *R. solani* AG-11 and AG-4, so that growers will choose resistant rice cultivars for managing seedling blight caused by *R. solani*. Based on the results of

this study, most of the commercial inbred and hybrid cultivars were highly susceptible (> 80% stand loss) to *R. solani* AG-4 and susceptible (50 – 80% stand loss) to *R. solani* AG-11. However, hybrid cultivar RT7521 FP and inbred cultivar CLL15 showed partial resistance (< 50% stand loss) to *R. solani* AG-11. The hybrids and Clearfield cultivars appear to be the sources of resistance to *R. solani* in commercial cultivars tested. Rondo is a long grain *indica* cultivar with resistance with multiple diseases including rice blast (*Magnaporthe grisea* (Hebert) Barr) (Yan and McClung 2010). However, in our study Rondo was also highly susceptible (~96% stand loss) to *R. solani* AG-11 and *R. solani* AG-4. *Indica* rice is less tolerant than *japonica* to cold temperature. Early planting (first week of April) of rice genotypes in the field experiment in 2020 might be the factor contributing to the low plant stand.

Medium-grain rice cultivars are usually moderately resistant to *R. solani* AG-1-IA whereas long-grain cultivars are highly susceptible (Marchetti, 1983). Breeding lines RU1802202, RU1805228 and cultivar Jupitar are medium-grain cultivars used in this study. However, they showed highly susceptible reaction against seedling blight pathogens *R. solani* AG-11 and AG-4. Medium-grain cultivars are comparatively taller than long-grain cultivars (semi-dwarf). Sheath blight develops more extensively in semi-dwarf cultivars than in the standard (relatively taller) cultivars (Marchetti, 1983).

Soybeans (*Glycine max* (L.) Merr.) have emerged in recent years as a major rotation crop in the southern rice belt. However, the same anastomosis groups (AG-11 and AG-4) of *R. solani* fungus that cause rice seedling blight can also attack soybeans. This could be another factor causing high seedling blight disease severity.

The mechanism of resistance in rice against this necrotrophic pathogen *R. solani* is un-known. Rice resistance to the sheath blight pathogen *R. solani* AG-1-IA is generally believed to

be a typical quantitative trait controlled by several genes (Sha and Zhu 1989). Several studies reported the agronomic traits, such as plant height and heading date, were associated with sheath blight resistance (Groth and Novick 1992; Hashiba et al. 1981). However, these traits may not be applied in seedling blight resistance because the disease is developed at the seedling stage. Further research is needed to determine the mechanism of resistance in the partially resistant rice genotypes identified in this study and to determine the nature of inheritance of this partial resistance.

The results of this study demonstrate that current U.S. rice cultivars and breeding lines, except for a few genotypes, are susceptible or highly susceptible to the seedling blight pathogens *R. solani* AG-11 and *R. solani* AG-4. Hybrid cultivar RT7521 FP and Clearfield cultivar CLL15 showed partial resistance to *R. solani* AG-11 in the current study. More research is needed for identifying resistant genotypes that can be used to develop seedling disease-resistant cultivars.

CHAPTER IV

POPULATION DIVERSITY OF RHIZOCTONIA SOLANI AG-11 ASSOCIATED WITH RICE SEEDLING BLIGH IN THE SOUTHERN UNITED STATES

Synopsis

Rhizoctonia solani AG-11 is a most prevalent fungal pathogen causing seedling blight of rice in the southern United States. We used whole-genome sequencing to characterize genetic structure of *R. solani* AG-11 populations from Arkansas, Mississippi, Missouri, and Texas and examine the reproductive biology of this pathogen. Demographic analysis showed evidence for two clades with the Texas population clustering in one clade and the Arkansas, Mississippi, and Missouri populations in another clade. The results from the dendrogram and discriminate analysis of principal components grouped the Arkansas, Mississippi and Missouri isolates in a single cluster suggesting the possibility of genotype flow among these populations. Phylogenetic analysis revealed long terminal multifurcating branches for some members of Arkansas, Missouri, and Texas populations, providing the evidence of sexual reproduction. Furthermore, Texas populations were further divided by geographical zone. This research provides new insights into the structure of southern United States populations of the rice seedling blight pathogen *R. solani* AG-11.

Introduction

The soilborne necrotrophic fungus *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris* (A. B. Frank) Donk) anastomosis group (AG) 11 has been reported to be pathogenic to rice in Arkansas and Texas (Carling et al. 1994; Jones and Carling 1999), lily (*Lilium* spp.) in Japan (Misawa et al. 2017), lupine (*Lupinus angustifolius* L.) in Western Australia (Sweetingham et al. 1986), snap bean (*Phaseolus vulgaris*) in Turkey and Idaho (Eken and Demirci 2004; Woodhall et al. 2020), soybean (*Glycine max* (L.) Merr.) in Arkansas and Illinois (Ajayi-Oyetunde and Bradley 2016; Carling et al. 1994), sugar beet (*Beta vulgaris*) in Poland (Moliszewska et al. 2020), and vegetables and sweet corn in New York (Ohkura et al. 2009). *R. solani* AG-11 was first described by Carling et al. (1994), and was mildly pathogenic to wheat, cotton, radish, and potato but was non-pathogenic to rice seedlings. In our rice seedling disease survey in the southern United States, *R. solani* AG-11 was found to be the dominant pathogen causing seedling blight. Disease symptoms include necrotic dark brown lesions on the mesocotyl and the coleoptile, eventually resulting in damping-off.

The population structures of several AGs of *R. solani* have been studied using different molecular markers. Rosewich et al. (1999) provided evidence of sexual reproduction and high degree of gene flow/migration between geographical populations of *R. solani* AG-1-IA from Texas using restriction fragment-length polymorphic (RFLP) markers. Using simple sequence repeat (SSR) markers, Bernardes de Assis et al. (2008) found that the population structure of *R. solani* AG-1-IA isolates from soybean and rice in Louisiana have been significantly differentiated with respect to host species and characterized by both sexual and asexual reproduction. High genetic diversity, no population differentiation and a mixed reproductive system have been reported for *R. solani* AG-3 PT (Ceresini et al. 2002; Ferrucho et al. 2013).

Using genotyping by sequencing (GBS), Ajayi-Oyetunde et al. (2019) provided evidence of high genetic diversity and mixed reproduction among the populations of *R. solani* AG-2-2IIIB from soybean in Illinois, Ohio, and Ontario. However simple sequence repeat, and single nucleotide polymorphism (SNP) marker are preferred markers for population genetics studies in most fungi including *R. solani* (Milgroom 2014). Use of SNPs for the study of genetic diversity among fungal populations is a new approach. Whole-genome sequencing using next generation sequencing technologies allows simultaneous discovery of SNPs and the application of genotyping in population genetic studies of fungi.

Several studies have been conducted for understanding the host range and pathogenicity of *R. solani* AG-11; however, there is no information regarding ecology and the genetic structure of *R. solani* AG-11. Although, Jones and Belmar (1989) observed basidiospores of *R. solani* AG 11 in the Texas rice field, the role of basidiospores in the disease cycle and creating genetic diversity remains largely unknown.

The objective of this research was to characterize the genetic structure of populations of *R. solani* AG-11 using genome-wide SNPs obtained from whole-genome sequencing. We tested the hypotheses that the populations of *R. solani* AG-11 are differentiated by geographical region.

Materials and Methods

Fungal isolates

Eighty *R. solani* AG-11 isolates (APPENDIX B) recovered from symptomatic rice seedling samples in Arkansas (n = 29), Mississippi (n = 4), Missouri (n = 19), and Texas (n = 23) and 4 tester isolates (2 from rice and peanut in Arkansas, 1 from rice in Louisiana and 1 from rice in Texas) were used in this study. These isolates were evaluated for their pathogenicity on rice seedlings in the growth chamber. All tested isolates of *R. solani* AG-11 were pathogenic to

rice seedlings with varying degree of aggressiveness. The Texas (except one isolate) and Mississippi isolates were collected in 2018, and the isolates from Missouri were collected in 2019. The Arkansas isolates were collected in 2018 and 2019. Isolates were stored by transferring PDA plugs from 5-days-old growing cultures of each isolate in a 1.5-ml microcentrifuge tube containing 750 µl of 15% glycerol and maintained at -80°C.

DNA extraction and whole-genome sequencing

For genomic DNA extraction, each isolate was grown in PDA medium at 25°C. Fungal mycelia were harvested from 7-day-old culture and DNA was extracted using Zymo Research Quick-DNA™ Fungal/Bacterial Miniprep Kit following the manufacturer's protocol (Zymo Research Corp.). The library preparation and sequencing were done at Texas A&M AgriLife Research's Genomics and Bioinformatics Service, College Station, TX. Sample libraries have been sequenced using the Illumina NovaSeq 6000 platform with paired end read lengths of 150 bp.

De novo genome assembly

The quality check of all sequence read obtained as FASTQ files was conducted using FastQC version 0.11.8 (Andrews, 2010) to remove low quality reads and residual adaptor sequence and were subsequently trimmed using Trimmomatic version 0.38 (Bolger et al. 2014). The draft genome assembles of *R. solani* AG1-IA (GenBank: GCA_000334115), AG1-IB (GenBank: GCA_000350255), AG3 (GCA_000524645), and AG8 (GCA_000695385) are available. Although we tried to align our sequenced reads with the available draft genomes of *R. solani*, less than 30% reads were mapped. To get high percentage of alignment, we did *de novo* genome assembly of *R. solani* AG-11 isolate M19_06, one of the tester AG-11 isolate from rice seedling in Missouri. The high-quality paired-read ends of isolate M19_06 were assembled with

SPAdes version 3.13.0 (Bankevich et al. 2012) to generate primary contigs. To reduce sequence redundancies caused by the assembly of heterozygous homeologs, we used program Redundans version 0.13 (Pryszcz and Gabaldon 2016) before scaffolding. The assembly was scaffolded with SSPACE-STANDARD version 3.0 (Boetzer et al. 2011) using the parameters (end extension, minimum contig length 500 bp). Pilon version 1.20 (Walker et al. 2014) was used to improve draft genome assembly by correcting bases, fixing-mis-assembly, and filling gaps. The *R. solani* AG-11 draft assembly contains 3,462 scaffolds and has a total length of 36.11 Mbp (Table 2). The completeness of the assembly was evaluated using BUSCO version 3.0.2 with the lineage-specific profile library fungi_odb9 (Simao et al. 2015), which showed that the genome assembly contain 43% of the 1335 conserved fungal orthologs.

Table 7 Assembly statistics of draft genome assembly of *Rhizoctonia solani* AG-11

Fungi	*R. solani* AG-11
Isolate	M19_06
Sequencing method	Illumina NovaSeq 6000: 2×150 bp.
Assembly method	SPAdes 3.13.0 Redundans 0.13 SSPACE 3.0 Pilon 1.20
Total assembly length	36.11 Mbp
Maximum scaffold length	144,249 bp
Minimum scaffold length	501 bp
G:C content	49.03
N50	462
N50 length	25,553 bp
Total number of scaffolds	3,462

Read mapping, SNP calling and quality filtering

Paired-end sequencing reads of *R. solani* AG-11 isolates were aligned to the assembled draft genome of *R. solani* AG-11 using the Bowtie2 version 2.3.5.1 (Langmead and Salzberg, 2012). SAMtools version 0.1.18 (Li et al. 2009) and Picard version 1.18.27 (http://broadinstitute.github.io/picard/) were used for sorting and merging mapped reads. Sorted reads were further proceed following FreeBayes version 0.9.9 (Garrison and Marth, 2012) for the variant calling. Variants were filtered using VCFtools version 0.1.16 (Danecek et al. 2011) based on a minimum read depth of 15, minimum mapping quality of 44, minimum minor allele frequency set to 5%, and sites with more than 3% missing data were removed from the datasets.

Population structure of R. solani AG-11

The SNP data were further proceeded in R using the R package *vcfR* (Knaus & Grünwald, 2017). Pairwise genetic distance between genotypes was calculated using genetic distance and distance tree was constructed based on the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm, with 1000 bootstrap replicates to assess branch support. The genotypes clustered together were considered as clone. This clustering was constructed in R using the package poppr version 2.8.7 (Kamvar et al. 2014).

The genetic structures of the combined Arkansas, Mississippi, Missouri, and Texas populations were assessed using the uncensored data. Population structure was examined by conducting the discriminant analysis of principal component (DAPC). Number of clusters of genetically related individuals can be identified by DAPC (Jombart et al. 2010). DAPC is a multivariate analysis method in which data were first transferred using a principal component analysis and subsequently clustering using discriminant analysis. The R package *ade4* (Dray and Dufour 2007) was used for this analysis. The posterior membership probability of isolates to

each geographical origin was plotted to determine how well individuals were assigned back to their respective geographical origin. To visualize the posterior assignments of each sample, we use a composite stacked bar plot (compoplot). A compoplot illustrates the probability of population membership on the y-axis and each sample is a bin on the x-axis. Mixed-membership model can account for the fact that individual genotypes may come from different subpopulations according to proportions of an individual's ancestry.

To determine subpopulation differentiation of *R. solani* AG-11 Texas population among two geographical regions (east and northwest), we reconstructed a UPGMA dendrogram for clustering of genotypes. Each region was considered as a subpopulation. Subpopulation structure was examined by conducting a DAPC scatterplot and used a composite stacked bar plot to visualize the posterior assignments of each isolate.

Results

The heterozygosity and multinuclearity nature of *R. solani* posed considerable challenges for genome assembly. Genomic sequencing was performed on an Illumina NovaSeq 6000 platform with 2 × 150-bp paired-end reads. High-quality reads with ≥ Q30 were selected from raw data using FastQC version 0.11.8 (Andrew 2010) and Trimmomatic version 0.38 (Bolger et al. 2014) to trim paried-end read. The Illumina sequencing run (2 × 150 bp) resulted in 6997175 reads yielding approximately 1 Gb sequence information representing on average a coverage of 30-fold. The final draft of 36,583,673 bp was generated and assembled into scaffolds with 49.03 % G:C content.

Number of variants obtained by whole-genome sequence

A total of 79 isolates were genotyped using whole-genome sequence (APPENDIX B). Of the total 1,579,693 raw SNP variants, 171,970 variants were used for final data analysis after

filtering based on read depth, mapping quality, minor allele frequency and sites with missing data.

Population structure of R. solani AG-11 in the southern United States

We tested the hypothesis that populations of *R. solani* AG-11 in the southern United States are differentiated with respect to geographical locations (Arkansas, Mississippi, Missouri, and Texas). The UPGMA dendrogram based on genetic distance and DAPC showed clustering by the geographical location of Texas isolates (Figure 11 and Figure 12). The UPGMA dendrogram showed two clades with the isolates from Texas being clustered in one clade and the isolates from Arkansas, Missouri and Mississippi were into another clade (Figure 11). A discriminant analysis of principal components (DAPC) further supported the differentiation of the Texas population structure from those of Arkansas, Mississippi, and Missouri by showing non-overlapping ellipses representing 95% of the isolates from each of the populations (Figure 12).

The posterior membership probabilities of individuals to their respective geographical origin were relatively high with DAPC. *R. solani* AG-11 isolates from Texas are very well assigned to their geographical origin. Nearly all individuals from Arkansas and Missouri have the high probability of being assigned to their geographical origin (Figure 13). However, Mississippi isolates have the low probability of being assigned to their geographical origin. The results from the dendrogram and DAPC grouped the Arkansas, Mississippi, and Missouri isolates in a single cluster, suggesting the possibility of genotype flow between these populations.

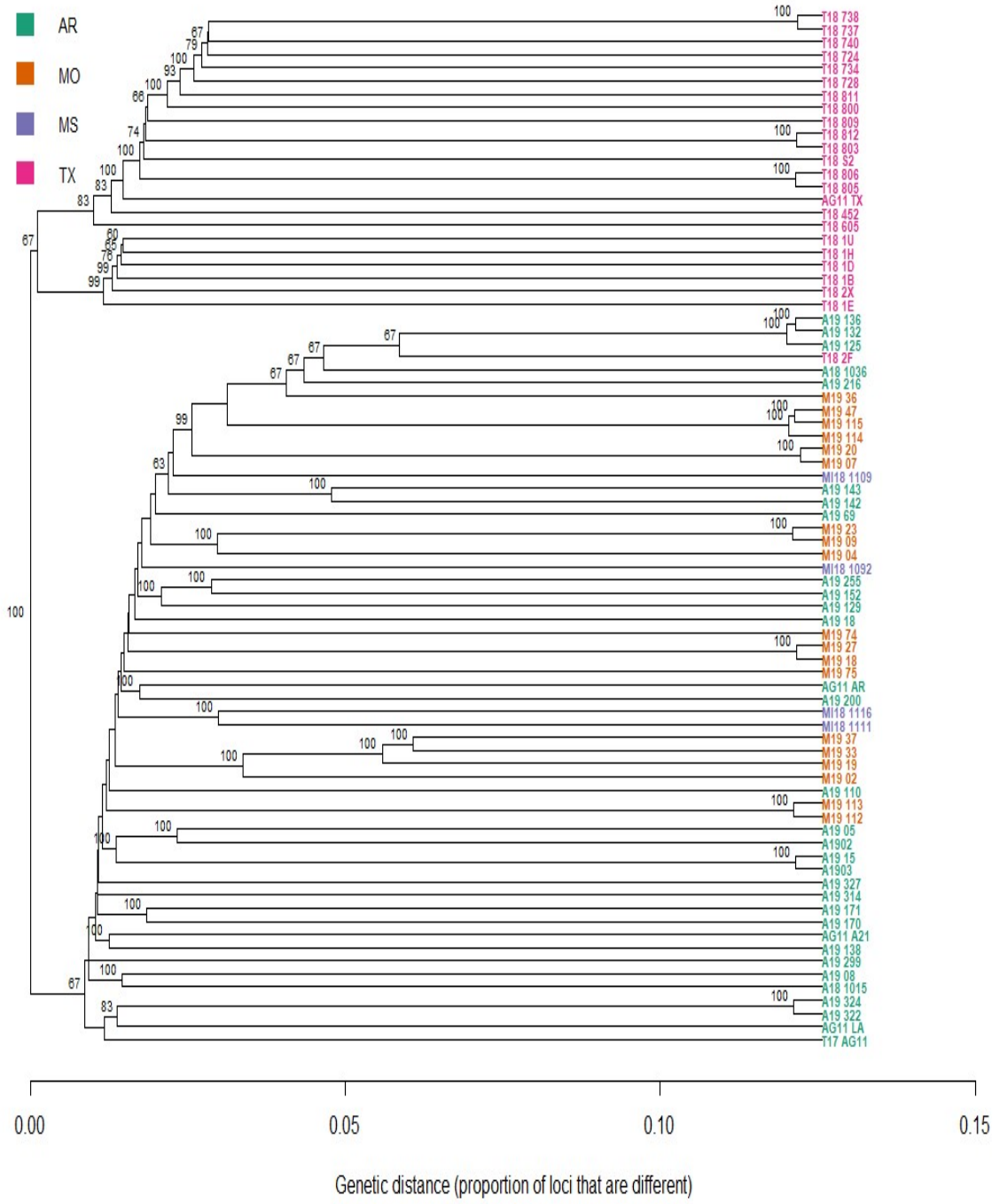

Figure 11. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of *Rhizoctonia solani* anastomosis group 11 from Arkansas, Mississippi, Missouri, and Texas based on genetic distance. Bootstrap support values >65% using 1000 bootstrap samples were shown at the nodes.

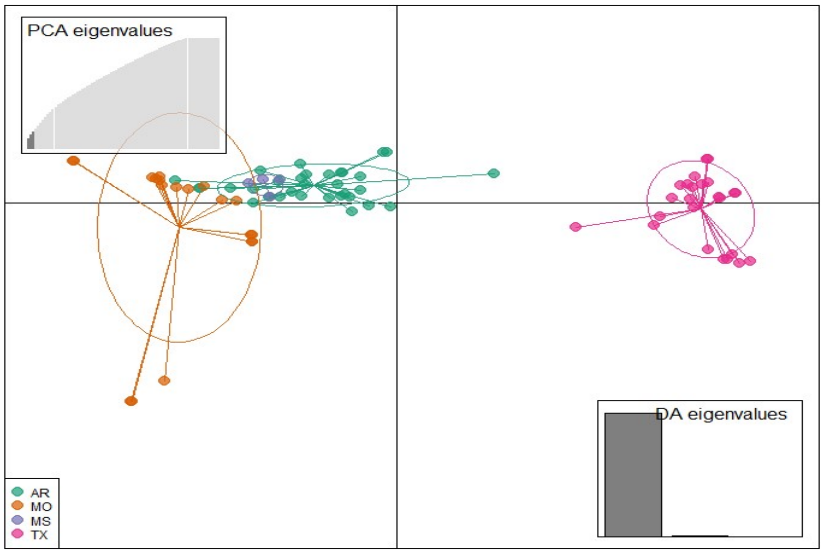

Figure 12. Discriminate analysis of principal components (DAPC) scatterplot showing *Rhizoctonia solani* anastomosis group 11 from Arkansas, Mississippi, Missouri, and Texas. The ellipses represent the maximum area spanned by 95% of the data in a population by state of origin. The amount of variance explained by each principal component (PC) is displayed on the inset bar graphs, and the number of PCs retained is indicated in black color.

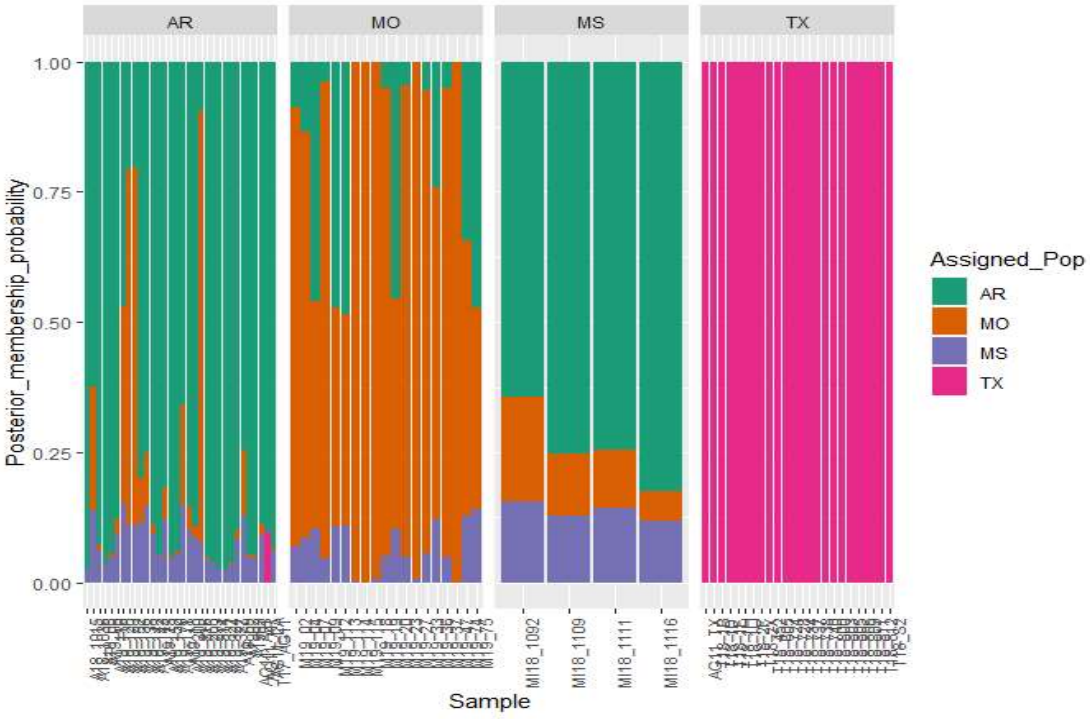

Figure 13. Posterior membership probabilities of individuals to each geographical origin (AR, MS, MO, and TX) from DAPC analyses.

Subpopulation structure of R. solani AG-11 from Texas

We also tested the hypothesis of genetic differentiation across subpopulations of *R. solani* AG-11 in Texas. We consider geographic zone in Texas (east and northwest zone) as a sub-population. A UPGMA dendrogram showed that the Texas isolates were clustered by geographical zone (Figure 14). A principal component analysis further supported subpopulation differentiation by showing non-overlapping ellipses from each of the subpopulations (Figure 15). The composite stacked bar plot to visualize the posterior subpopulation membership probability assignments shows that the population from two zones in Texas were very well assigned to their geographical zone (Figure 16). The results from the dendrogram, PC, and composite plot analysis showed that the two Texas subpopulations were differentiated.

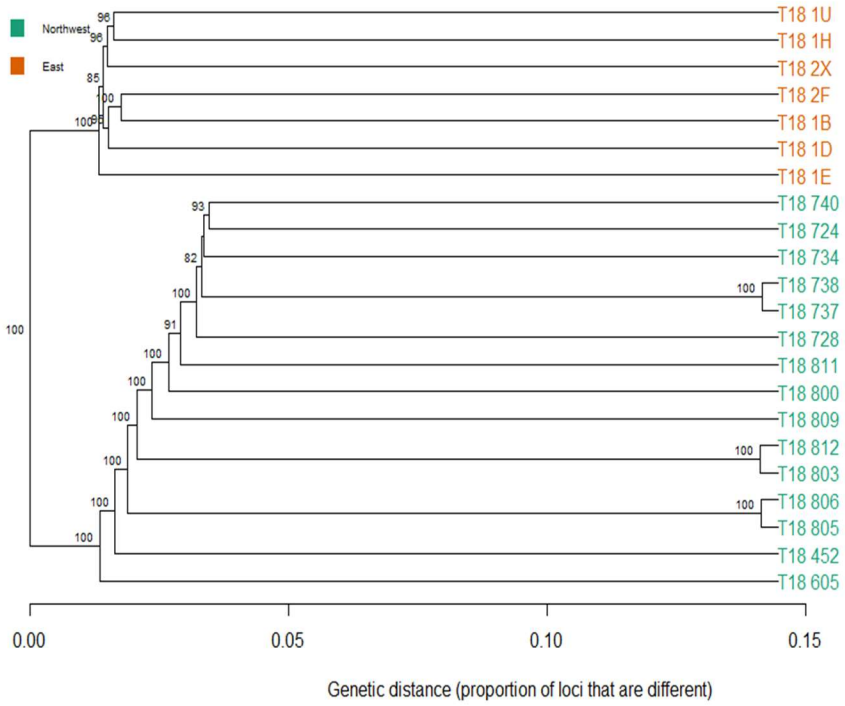

Figure 14. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of *Rhizoctonia solani* anastomosis group 11 from east and northwest of Texas based on genetic distance. Bootstrap support values >80% using 1000 bootstrap samples were shown at the nodes.

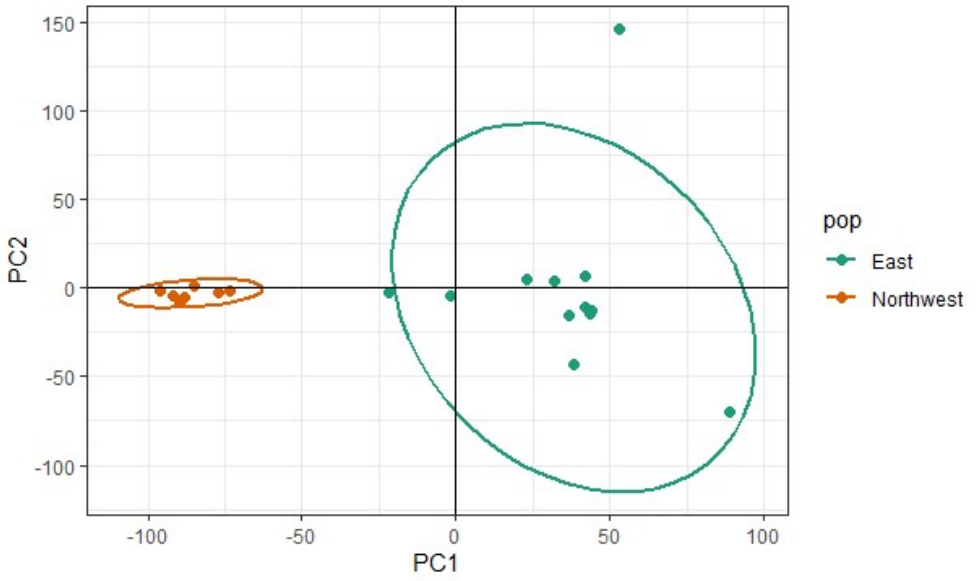

Figure 15. Discriminate analysis of principal components (DAPC) scatterplot showing *Rhizoctonia solani* anastomosis group 11 from east and northwest zone of Texas. The ellipses represent the maximum area spanned by 95% of the data in a subpopulation by zone of origin.

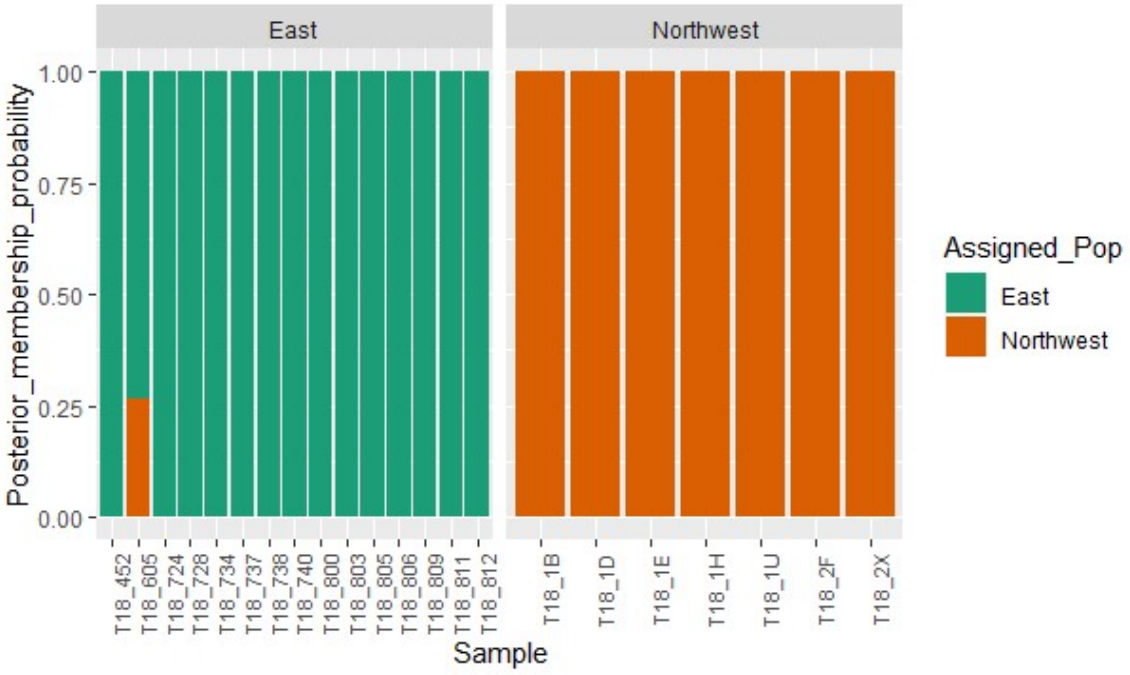

Figure 16. Composite stacked bar plot of *Rhizoctonia solani* AG-11 isolates from east and northwest zones in Texas to visualize the posterior subpopulation membership probability assignments.

Discussion

This work provides new information on the genetic structure of *R. solani* AG-11 populations associated with seedling blight of rice in the southern United States. We examined 80 *R. solani* AG-11 isolates from four states (AR= 29, MS = 4, MO= 19, and Texas =23 and 4 tester AG-11 isolates) in the southern United States. The result from this study showed that the *R. solani* AG-11 populations in close geographical proximity (Arkansas, Mississippi, and Missouri populations) were not genetically differentiated, however, distant population (Texas) was genetically differentiated. We propose that the low level of genetic differentiation of Arkansas, Mississippi, and Missouri populations in this study may be attributed to genotype flow via dispersal of asexual propagules. In pathogens reproducing mainly asexually, entire genotypes can be transferred from one population to another know as genotype flow. Genotype flow may have occurred through the interconnected irrigation network, movement of contaminated vehicle or machinery that move soil and fungal material (sclerotia, mycelia etc.). Mississippi river is one of the major water sources in Arkansas, Mississippi, and Missouri.

This study provided first information about the genetic structure of *R. solani* anastomosis group 11. We found geographically distinct populations of *R. solani* AG-11 in the southern United States. This finding is different from previous population genetic study about other AGs. Lack of population differentiation was reported for the population of *R. solani* AG-3 PT in eastern North Carolina (Ceresini et al. 2002), Denmark (Justesen et al. 2003), *R. solani* AG-1-IA from rice in China (Bernardes-de-Assis et al. 2008; Wang et al. 2013), North India (Goswami et al. 2017), Japan, Philippines (Cumagun et al. 2020), Texas (Rosewich et al. 1999), and *R. solani* AG-2-2IIIB from soyabean in Illinois, Ohio, and Ontario (Ajayi-Oyetunde et al. 2019).

R. solani AG-11 populations of Texas were differentiated from the populations of other states. This can be explained by the fact that the populations in Texas were geographically more distant from other sates' populations and that the chances of long-distance dispersal of the fungal propagules through contaminated soil or machinery might be much less. Cropping systems could also play a role in the selection of host-specialized pathogen populations. In Texas, rice-fallow-rice or rice-fallow-fallow-rice is common cropping systems (Liu et al. 2016), whereas in Arkansas, Mississippi and Missouri, most rice is grown in rotations with soybean (Hardke 2015; Riar et al. 2013). Results from other studies yielded similar findings on genetically distinct geographic population of *R. solani* AG 3-PT in South Africa (Muzhinji et al. 2016), Colombian Andes (Ferrucho et al. 2013), *R. solani* AG-1-1A from rice in China (Cumagun et al. 2020), and in Iran (Padasht-Dehkaei et al. 2012).

We also further evaluated the two subpopulations of *R. solani* AG-11 in Texas based on their geographical zones (northwest =15 and east =7) and found that the two subpopulations within Texas were genetically differentiated. This contrasts with the results of Rosewich et al. (1999) who found lack of population structure among the Texas isolates of *R. solani* AG-1-IA on rice. The east and northwest rice producing areas in Texas are separated geographically by Houston city. The big metropolitan area of Houston might serve as a geographical barrier to prevent the potential exchange of fungal materials (sclerotia, mycelium etc.) via. contaminated soil or machinery between these two major rice-growing areas.

Sexual populations produce multifurcating branches with long tree length while constructing phylogenetic tree. Some members of Arkansas, Missouri, and Texas populations have these characteristics, providing possibility of sexual reproduction.

In conclusion, this is the first study on the population genetics of *R. solani* AG-11. We used whole-genome sequence using next generation sequencing techniques to characterize the population structure of *R. solani* AG-11. The results showed that the populations of *R. solani* AG-11 isolated from the rice seedlings in the southern United States are genetically differentiated among the geographically distant populations and lack of differentiation with geographical proximity. Movement through contaminated soil, machinery or irrigation water in Arkansas, Mississippi, and Missouri can be the most plausible hypothesis that explain the low population differentiation. So far, no commercial rice cultivar resistant to *Rhizoctonia* seedling blight is available, thus growers must rely only on fungicide seed treatment and agronomical measures for management of seedling blight in the southern United States. Considering the differentiated population structure of *R. solani* AG-11 in the southern United States, isolates representing different populations should be used to evaluate seed treatment fungicides and rice genotypes to develop effective fungicide seed treatment and host resistance strategies for control of seedling blight in rice.

CHAPTER V

CONCLUSIONS

Seedling blight of rice is one of the important disease complexes in the southern rice growing belts of the United States. The overall goal of this research was to identify pathogens causing seedling blight in rice and their effective management strategy. The first objective was to identify the fungal pathogens associated with rice seedling blight and their distribution and importance in the southern United States. Four fungal taxonomic groups, *R. solani*, *Fusarium* spp., *S. rolfsii*, and *M. graminum*, were found to be the causes of seedling blight, with *R. solani* being the most prevalent species. Two anastomosis groups (AG-11 and AG-4) were present in the *R. solani* populations, with *R. solani* AG-11 being dominant and *R. solani* AG-4 being the most aggressive pathogen. *R. solani* AG-4 and *M. graminum* were identified as new pathogens causing seedling blight of rice. These findings support the hypothesis that the soil-borne fungi along with new fungal pathogens resulted seedling stand loss of rice in the southern United States.

The second objective was to evaluate inbred and hybrid cultivars and elite breeding lines against seedling blight caused by *R. solani* AG-11 and AG-4. Results from this study indicated that no inbred and hybrid cultivars and breeding lines had a high level of resistance against *R. solani* AG-11 and *R. solani* AG-4. However, hybrid cultivar RT7521 FP and inbreed cultivar CLL15 showed partial resistance to *R. solani* AG-11. These findings from this experiment therefore support the hypothesis that the US rice genotypes have certain degree of resistance to seedling blight caused by *R. solani* AG-11 and *R. solani* AG-4.

The third objective was to study the population structure of *R. solani* AG-11 from the southern United States. Our results revealed that the *R. solani* AG-11 isolates from the southern

United States were being clustered with geographical locations and we found the evidence of the differentiation of the Texas population from Arkansas, Mississippi, and Missouri populations. These findings support the hypothesis that the populations of *R. solani* AG-11 from rice seedling blight in the southern United States are differentiated with respect to geographical locations (AR, MS, MO, and TX).

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APPENDIX A

ENTRY NUMBER, SYMBOL, PEDIGREE, GRAIN TYPE AND STATE OF RICE

GENOTYPES EVALUATED

ENTRY	RU	PEDIGREE	GT	TYPE	ST
2	RU1702103	CCDR/JEFF//CFX-26/9702128/3/WELLS/CFX-18//DREW/CFX-18	L	CL	LA
3	RU1504083	CL131/PSCL	L		MS
6	RU1602097	CL 131/TRNS	L	CL	LA
7	RU1504197	RSMT/3/MARS/NWRX//TBNT/4/CL151	L		MS
8	RU1503175	L202/LQ39a//SABR	L		TX
10	RU1702140	CHNR/MRMT	L	CONV	LA
11	RU1604197	CL151//COLUMBIA2/BENGAL	L		MS
12	RU1003098	CPRS/NWBT//KATY/3/CCDR	L		TX
13	RU1601133	RU1102192/4/WLLS/CFX-18/3/CFX-18//CCDR/9770532 DH2	L	CL	AR
14	RU1702162	BNGL//MERC/RICO/3/EARL/4/BNGL/CL161	M	CL	LA
15	RU1704055	CL151/JSMN85//CL161	L		MS
16	RU0803153	CPRS/CCDR	L		TX
18	RU1402134	CL153	L		LA
19	RU9903092	PRESIDIO	L		TX
20	RU1002017	CL111	L		LA
21	RU1601111	RU1302048/RU1302045	L	CL	AR
22	RU1702165	CAFFEY/CL261	M	CL	LA
23	RU1704122	CL151/JSMN85//CL161	L		MS
24	RU1303153	IR64/IR 1321-12	L		TX
25	RU1601099	RU0502068/RU1202088	L	CL	AR
26	RU1702168	CL 131/3/CPRS/KBNT//9502008-A/4/LGRU/CLR 11/4/9602065/3/CFX-29/AR 1142/LA 2031	L	CL	LA
27	RU1704154	Rex/CL151	L		MS
28	RU1603138	WAB 450-11-1-1-P31-HB (NERICA 5)/RSMT	L		TX
29	RU1701124	JPTR/TITN	M		AR
30	RU1602195	9502008-A/DREW//CLR 20/4/CPRS/KBNT//9502008-A	L	CL	LA
31	RU1704196	BOWMAN/CL131	L		MS
32	RU1303138	IR64/IR 1321-12	L		TX
33	RU1601030	RU1202168/JPTR	CM	CL	AR
35	RU1704198	BOWMAN/CL131	L		MS
36	RU1403141	AC110DH2/AC108DH2//CHEN	L		TX

ENTRY	RU	PEDIGREE	GT	TYPE	ST
37	RU1802037	CCDR/CL131	L	CL	LA
42	RU1802042	MERMENTAU/3/FRANCIS/CLR 13//9502008-A/DREW	L	CL	LA
43	RU1804043	CL151/JSMN85//CL161	L		MS
44	RU1403089	CPRS/9901081	L		TX
45	RU1601124	MRMT/RU0502068	L		AR
46	RU1802046	CCDR/JEFF//CFX-26/9702128/3/CL151	L	CL	LA
47	RU1604193	Cheniere/Banks	L		MS
48	RU1603144	WAB 450-11-1-1-P31-HB (NERICA 5)/RSMT	L		TX
50	RU1802050	CCDR/JEFF//CFX-26/9702128/3/CL151	L	CL	LA
51	RU1704077	Texmont/TeQing(BF7-46)/Tranesse	L		MS
52	RU1303181	043752/0047277/CHEN	L		TX
53	RU1701136	EARL/9902028//RU1202068	CM	CL	AR
54	RU1802054	CCDR/JEFF//CFX-26/9702128/3/CL151	L	CL	LA
55	RU1704114	Bowman//RSMT/KATY	L		MS
56	RU1603178	SABR/CCDR//PRESIDIO	L		TX
57	RU1704157	Cheniere/Banks	L		MS
58	RU0002174	CHENIERE	L		LA
59	RU9502008	COCODRIE	L		LA
60	RU1402008	CL272	M		LA
61	RU1701096	CL172/RU1102192	L	CL	AR
62	RU1802062	CL152/5/9502008-A/DREW//CLR 20/4/CPRS/KBNT//9502008-A	L	CL	LA
63	RU1804063	RSMT/KATY//Bowman	L		MS
64	RU1603113	043752/0047277/CHEN	L		TX
67	RU1804067	RSMT/KATY//Bowman	L		MS
68	RU1603086	CL161/CPRS	L		TX
71	RU1804071	JODON/3/KATY//GFMT/PCOS/Templeton	L		MS
72	RU1403153	L202/LQ39a//SABR	L		TX
74	RU1602082	LAH169	L	HYB	LA
75	RU1804075	JODON/3/KATY//GFMT/PCOS/Templeton	L		MS
76	RU1703129	IR64/IR 1321-12	L		TX
77	RU1403138	043752/0047277/CHEN	L		TX
78	RU0202183	JUPITER	M		LA
80	RU1301021	TITAN	M		AR
82	RU1802082	TRNS/BASF 1-10	L	PV	LA
83	RU1804083	CL151/JSMN85//CL151	L		MS
84	RU1703098	CPRS/3/CPRS/NWBT/KATY	L		TX
86	RU1802086	CHENIERE/BASF 1-2	L	PV	LA
87	RU1804087	Rex/CL151	L		MS
88	RU1703147	CPRS/NWBT//KATY/3/CCDR	L		TX

ENTRY	RU	PEDIGREE	GT	TYPE	ST
90	RU1802090	CHENIERE/BASF 1-2	L	PV	LA
91	RU1804091	CL151/JSMN85//CL161	L		MS
92	RU1703163	Hayakogane/BALDO	L		TX
94	RU1802094	CHENIERE/BASF 1-6	L	PV	LA
95	RU1804095	CL151/JSMN85//CL161	L		MS
96	RU1603166	CPRS/3/CPRS/NWBT/KATY	L		TX
97	RU1801097	RU1102034/RU1302045	L	CL	AR
99	RU1804099	CL151/JSMN85//CL161	L		MS
100	RU1503169	Hayakogane/BALDO	L		TX
101	RU1801101	CL172/RU1102034	L	CL	AR
103	RU1804103	CL151/JSMN85//CL161	L		MS
104	RU1703175	CPRS/CCDR	L		TX
107	RU1804107	GFMT/KDM105//CL151/JSMN85	L		MS
108	RU1703126	CarolinaGoldSelect/Presidio	L		TX
111	RU1804111	GFMT/KDM105//CL151/JSMN85	L		MS
112	RU1803112	CarolinaGoldSelect/Presidio	L		TX
113	RU1701111	RU1002128/RU1202097	L	CL	AR
114	RU1802114	PVL01Sub	L	PV	LA
115	RU1804115	Rex/CL181-AR	L		MS
116	RU1703138	CL161/CPRS	L		TX
117	RU1805117	Bolivar/Drew	L		MO
118	RU1805118	Mo0327005/CL161	L	CL	MO
120	RU1104122	CL163	L		MS
121	RU1701121	EARL/9902028//JPTR	M		AR
123	RU1804123	Rex/CL151	L		MS
124	RU1703178	Hayakogane/BALDO	L		TX
125	RU1801125	RU1302045/CL111	L	CL	AR
127	RU1804127	Rex/CL181-AR	L		MS
128	RU1703181	AC110DH2/AC108DH2//CYBT	L		TX
129	RU1701127	JPTR/J062	M		AR
131	RU1804131	Rex/CL151	L		MS
132	RU1703132	CPRS/3/CPRS/NWBT/KATY	L		TX
133	RU1801133	CL172/4/9502008-A//AR1188/CCDR/3/CFX-26/9702128	L	CL	AR
135	RU1804135	Taggart/CL111	L		MS
136	RU1603187	CPRS/3/CPRS/NWBT/KATY	L		TX
137	RU1801137	14SIT818/RU1501096	CM	CL	AR
139	RU1804139	CL161/Priscilla//CL151/JSMN85	L		MS
140	RU1803140	4579	L		TX

ENTRY	RU	PEDIGREE	GT	TYPE	ST
142	RU1802142	9502008-A/DREW//CFX 26/WELLS/4/CPRS/3/CFX 29//AR 1142/LA 2031/5/CL 161	L	CL	LA
143	RU1804143	CL161/Priscilla//CL151/JSMN85	L		MS
144	RU1803144	9302065/LMNT	L		TX
147	RU1804147	Rex/CL151	L		MS
148	RU1803148	CPRS/3/CPRS/NWBT/KATY	L		TX
151	RU1804151	Trenasse/Bowman	L		MS
152	RU1803152	AC110DH2/AC108DH2//CYBT	L		TX
154	RU1702143	CAFFEY/3/BNGL/9502065//EARL	M	CONV	LA
155	RU1804155	Trenasse/Bowman	L		MS
156	RU1803156	CCDR/MILL	L		TX
157	RU1805157	NIL219_2-9/Mo0212002	L		MO
158	RU1805158	Mo0204044/Kataki	M		MO
159	RU0703144	CPRS/CCDR (ANTONIO)	L		TX
160	RU1104077	Thad	L		MS
163	RU1804163	CPRS//NWBT/KATY/3/Bowman	L		MS
164	RU1803164	CPRS/3/CPRS/NWBT/KATY	L		TX
165	RU1801165	RU1102034/RU1302045	L	CL	AR
167	RU1804167	RSMT/KATY//Bowman	L		MS
168	RU1803168	CPRS/NWBT//KATY/3/CCDR	L		TX
169	RU1801169	ROYJ/RU1501024	L	CL	AR
171	RU1804171	RSMT/KATY//Bowman	L		MS
172	RU1703172	AC110DH2/AC108DH2//CHEN	L		TX
173	RU1801173	ROYJ/RU1102192	L		AR
175	RU1804175	RSMT/KATY//Bowman	L		MS
176	RU1803176	CPRS/CCDR//WELLS	L		TX
178	RU1802178	NEPTUNE//BNGL/CL 161/3/BNGL/CL 161	M	CL	LA
179	RU1804179	RSMT/KATY//Bowman	L		MS
180	RU1803180	CPRS/CCDR//CCDR	L		TX
183	RU1804183	DXBL//NWBT/KATY/3/Bowman	L		MS
184	RU1803184	CPRS/SABR//MADISON	L		TX
185	RU1701185	CHNR/CTHL	L		AR
187	RU1804187	DXBL//NWBT/KATY/3/Bowman	L		MS
188	RU1803188	Jangseongbyeo/IR 1321-12	L		TX
190	RU1802190	NEPTUNE//BNGL/CL 161/5/BNGL//MERC/RICO/3/MERC/RICO//BNGL/4/9502065/3/MERC//MERC/3/9902028	M	CL	LA
191	RU1804191	Bowman/L201//TBNT/BLMT/3/RXMT/IR36	L		MS
192	RU1803192	Jangseongbyeo/IR 1321-12	L		TX
194	RU1802194	CL272 sub A	M	CL	LA
195	RU1804195	Bowman/L201//TBNT/BLMT/3/RXMT/IR36	L		MS

ENTRY	RU	PEDIGREE	GT	TYPE	ST
196	RU1803196	CCDR/L202//TRENASSE	L		TX
197	RU1805197	Soberana Q241-1/Francis	L		MO
198	RU1805198	Mo0204074/Nil16_2-1	L		MO
199	RU0603075	RONDO	L		TX
200	RU0902018	CL151	L		LA
202	RU1802202	CL272 sub B	M	CL	LA
203	RU1805203	Soberana Q241-2/Wells	L		MO
204	RU1804204	Bowman/L201//TBNT/BLMT/3/RXMT/IR36	L		MS
207	RU1702183	TRNS//CCDR/JEFF/5/9502008-A/DREW//CLR 20/4/CPRS/KBNT//9502008-A	L	CL	LA
208	RU1805208	Mo0239718/CL161	L	CL	MO
209	RU1804209	JODON/3/KATY//GFMT/PCOS/Templeton	L		MS
211	RU1801211	9865216DH2/EARL//JPTR	M		AR
212	RU1402091	CL 131/3/CPRS/KBNT//9502008-A	L	CL	LA
213	RU1805213	NIL219_2-9/RU0001108	L		MO
214	RU1804214	REX/Templeton	L		MS
215	RU1803218	CPRS/SABR//Gulfmont	L		TX
217	RU1402174	9502008/3/MBLE//LMNT/20001-5/4/WELLS/CFX18/5/TAGGART	L	CL	LA
218	RU1805218	NIL43_2-1/Mo0205014	L		MO
219	RU1804219	REX/Templeton	L		MS
220	RU1803232	CPRS/SABR//MADISON	L		TX
221	RU1801221	CTHL/CL172	L	CL	AR
222	RU1702195	CPRS/KBNT//9502008-A/3/CCDR/4/CL131	L	CL	LA
223	RU1805223	Mo0215035 / CIRAD141Q244-3	L		MO
224	RU1804224	REX/Templeton	L		MS
225	RU1803233	CPRS/SABR//MADISON	L		TX
227	RU1802227	CHENIERE/6/CPRS/KBNT//9502008-A/5/KATY/CPRS//NWBT/KATY/3/9502008/4/CLR9	L	CL	LA
228	RU1805228	RU0403166/Spring	M		MO
229	RU1804229	REX/Templeton	L		MS
230	RU1803234	FRAN/WELLS//BANKS	L		TX
232	RU1802232	KATY/CPRS//NWBT/KATY/3/9502008/4/CLR 9/5/KATY/CPRS//NWBT/KATY/5/9502008-A/DREW/3/NWBT/KATY//9902207X2	L	CL	LA
233	RU1805233	STGL01L-49-173/RU0001108	L		MO
234	RU1804234	REX/Templeton	L		MS
235	RU1803236	CPRS/SABR//MADISON	L		TX
236	RU1805236	NIL219_1-5/Mo0205014	L		MO
237	RU1801237	JPTR/EARL	M		AR
238	RU1801238	EARL/9902028//RU1202068	CM	CL	AR
239	RU1202158	DELLA 2	L		LA
240	RU0804083	Rex	L		MS
241	TH710			Hybrid	TX
242	TH720			Hybrid	TX
243	TH730-1			Hybrid	TX

ENTRY	RU	PEDIGREE	GT	TYPE	ST
244	TH730-2			Hybrid	TX
245	TH740			Hybrid	TX
246	TH750			Hybrid	TX
247	TH760-1			Hybrid	TX
248	TH760-2			Hybrid	TX
249	TH770			Hybrid	TX
250	TH780			Hybrid	TX
251	TH723			Hybrid	TX
252	TH733			Hybrid	TX
253	TH743			Hybrid	TX
254	TH753			Hybrid	TX
255	TH763			Hybrid	TX
256	TH773			Hybrid	TX
257	TH783			Hybrid	TX
258	TH793			Hybrid	TX
259	TH756			Hybrid	TX
260	TH766			Hybrid	TX

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APPENDIX B

ISOLATE ID, ORIGIN, AND YEAR OF ISOLATION OF RHIZOCTONIA SOLANI ANASTOMOSIS GROUP 11 ISOLATES RECOVERED FROM RICE SEEDLINGS IN ARKANSAS, MISSISSIPPI, MISSOURI, AND TEXAS

SN	Isolate ID	Origin	Year
1	T18_1B	Wharton Co., Texas	2018
2	T18_2X	Wharton Co., Texas	2018
3	T18_1U	Wharton Co., Texas	2018
4	T18_1H	Wharton Co., Texas	2018
5	T18_1D	Wharton Co., Texas	2018
6	T18_1E	Wharton Co., Texas	2018
7	T18_2F	Wharton Co., Texas	2018
8	T18_800	Chamber Co., Texas	2018
9	T18_803	Chamber Co., Texas	2018
10	T18_805	Chamber Co., Texas	2018
11	T18_806	Chamber Co., Texas	2018
12	T18_809	Chamber Co., Texas	2018
13	T18_811	Chamber Co., Texas	2018
14	T18_812	Chamber Co., Texas	2018
15	T18_724	Chamber Co., Texas	2018
16	T18_728	Chamber Co., Texas	2018
17	T18_734	Chamber Co., Texas	2018
18	T18_737	Chamber Co., Texas	2018
19	T18_738	Chamber Co., Texas	2018
20	T18_740	Chamber Co., Texas	2018
21	T18_S2	Jefferson Co., Texas	2018
22	T18_605	Jefferson Co., Texas	2018
23	T18_452	Liberty Co., Texas	2018
24	T17_AG	Arkansas Co., Arkansas	2019
25	MI18_1116	Bolivar Co., Mississippi	2018
26	MI18_1109	Bolivar Co., Mississippi	2018
27	MI18_1111	Bolivar Co., Mississippi	2018
28	MI18_1092	Bolivar Co., Mississippi	2018
29	A18_1036	Jefferson Co., Arkansas	2018

SN	Isolate ID	Origin	Year
30	A18_1015	Jefferson Co., Arkansas	2018
31	A19_02	Monroe Co., Arkansas	2019
32	A19_03	Monroe Co., Arkansas	2019
33	A19_05	Monroe Co., Arkansas	2019
34	A19_08	Monroe Co., Arkansas	2019
35	A19_15	Monroe Co., Arkansas	2019
36	A19_18	Monroe Co., Arkansas	2019
37	A19_69	Arkansas Co., Arkansas	2019
38	A19_110	Greene Co., Arkansas	2019
39	A19_125	Poinsett Co., Arkansas	2019
40	A19_129	Poinsett Co., Arkansas	2019
41	A19_132	Poinsett Co., Arkansas	2019
42	A19_136	Poinsett Co., Arkansas	2019
43	A19_138	Poinsett Co., Arkansas	2019
44	A19_142	Poinsett Co., Arkansas	2019
45	A19_143	Poinsett Co., Arkansas	2019
46	A19_152	Poinsett Co., Arkansas	2019
47	A19_170	Poinsett Co., Arkansas	2019
48	A19_171	Poinsett Co., Arkansas	2019
49	A19_200	Poinsett Co., Arkansas	2019
50	A19_216	Cross Co., Arkansas	2019
51	A19_255	Woodruff Co., Arkansas	2019
52	A19_299	Woodruff Co., Arkansas	2019
53	A19_314	Arkansas Co., Arkansas	2019
54	A19_322	Arkansas Co., Arkansas	2019
55	A19_324	Arkansas Co., Arkansas	2019
56	A19_327	Arkansas Co., Arkansas	2019
57	M19_02	Dunklin Co., Missouri	2019
58	M19_04	Dunklin Co., Missouri	2019
59	M19_06	Dunklin Co., Missouri	2019
60	M19_07	Dunklin Co., Missouri	2019
61	M19_09	Dunklin Co., Missouri	2019
62	M19_18	Dunklin Co., Missouri	2019
63	M19_19	Dunklin Co., Missouri	2019
64	M19_20	Dunklin Co., Missouri	2019
65	M19_23	Dunklin Co., Missouri	2019
66	M19_27	Dunklin Co., Missouri	2019
67	M19_33	Dunklin Co., Missouri	2019

SN	Isolate ID	Origin	Year
68	M19_36	Dunklin Co., Missouri	2019
69	M19_37	Dunklin Co., Missouri	2019
70	M19_47	Dunklin Co., Missouri	2019
71	M19_74	Pemiscot Co., Missouri	2019
72	M19_75	Pemiscot Co., Missouri	2019
73	M10_112	Pemiscot Co., Missouri	2019
74	M19_113	Pemiscot Co., Missouri	2019
75	M19_114	Pemiscot Co., Missouri	2019
76	M19_115	Pemiscot Co., Missouri	2019
77	AG11_LA	Tester AG-11 isolate from Louisiana	-
78	AG11_AR	Tester AG-11 isolate from Arkansas	-
79	AG11_A21	Tester AG-11 isolate from Arkansas	-
80	AG11_TX	Tester AG-11 isolate from Texas	-

APPENDIX C

BIOINFORMSTIC PROCESSING SCRIPTS

a. *De novo* genome assembly using SPAdes

```
#BSUB -L /bin/bash              # uses the bash login shell to initialize the job's execution
#BSUB -J spades_pe_2mp          # job name
#BSUB -n 20                     # assigns 20 cores for execution
#BSUB -R "span[ptile=20]"       # assigns 20 cores per node
#BSUB -R "rusage[mem=2700]"     # reserves 2700MB memory per core
#BSUB -M 2700                   # sets to 2700MB (~2.7GB) the per process enforceable memory
#BSUB -W 48:00                  # sets to 48 hours the job's runtime wall-clock limit.
#BSUB -o RhizAssemblyRun02.stdout.   # directs the job's standard output to stdout.jobid
#BSUB -e RhizAssemblyRun02.stderr    # directs the job's standard error to stderr.jobid

module load SPAdes/3.13.0-foss-2018b

<<README
   - SPAdes manual: http://spades.bioinf.spbau.ru/release3.5.0/manual.html
README

#############################################################################
thread=20       # make sure this is <= your BSUB -n value
max_memory=52    # max memory used in Gb, make sure this is less than the BSUB total job
cd /scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20
output_dir='RhizoctoniaM1906onlyAssembly'
mkdir -p $output_dir
# sample dataset estimated run time: ~10 hours; max memory ~51Gb; ~200 SUs
pe1_1='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo_1P.fastq.gz'
pe1_2='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo_2P.fastq.gz'

# command to run with defaults and the --cafeful option
spades.py --threads $thread --tmp-dir $TMPDIR --careful --memory $max_memory -o $output_dir --pe1-
1 $pe1_1 --pe1-2 $pe1_2
```

b. Redundans for assembly of heterozygous genome

```
#BSUB -L /bin/bash        # uses the bash login shell to initialize the job's execution environment.
#BSUB -J redundans              # job name
#BSUB -n 20                   # assigns 20 cores for execution
#BSUB -R "span[ptile=20]"       # assigns 20 cores per node
#BSUB -R "rusage[mem=2700]"     # reserves 2700MB memory per core
#BSUB -M 2700                 # sets to 2700MB per process enforceable memory limit. (M * n)
#BSUB -W 48:00                # sets to 48 hours the job's runtime wall-clock limit.
#BSUB -o stdout.%J             # directs the job's standard output to stdout.jobid
#BSUB -e stderr.%J             # directs the job's standard error to stderr.jobid

module load Redundans/0.13c-intel-2017b-Python-2.7.14

<<README
   - Redundans manual: https://github.com/Gabaldonlab/redundans
README

########## INPUTS ##########
assembly_contigs='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/RhizoctoniaM1906onlyAsse
mbly/contigs.fasta'
pe_mp_reads="/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo*"

######## PARAMETERS ########
threads=20

########## OUTPUTS #########
out_dir='run_short-scaffolding-closing'

##################################### COMMANDS ######################
# scaffolding and gap closing with paired-end and mate pairs (no reduction)
redundans.py -v -t $threads -i $pe_mp_reads -f $assembly_contigs -o $out_dir
```

c. Scaffolding using SSPACE

```
#BSUB -L /bin/bash              # uses the bash login shell to initialize the job's execution envir
#BSUB -J sspace_mp2kb           # job name
#BSUB -n 8                      # assigns 8 cores for execution
#BSUB -R "span[ptile=8]"        # assigns 8 cores per node
#BSUB -R "rusage[mem=2500]"     # reserves 2500MB memory per core
#BSUB -M 2500                   # sets to 2500MB (~1GB) per process enforceable memory limit.
#BSUB -W 2:00                   # sets to 2 hours the job's runtime wall-clock limit.
#BSUB -o stdout.%J              # directs the job's standard output to stdout.jobid
#BSUB -e stderr.%J              # directs the job's standard error to stderr.jobid

module load SSPACE-STANDARD/3.0

########## INPUTS ##########
#FR 2kb mate pairs
mp2kb1_1='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo_1P.fastq.gz'
mp2kb1_2='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo_2P.fastq.gz'

config_file="params_mp2kb.conf"
contigs='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/RhizoctoniaM1906onlyAssembly/run_
short-scaffolding-closing/contigs.reduced.fa'

######## PARAMETERS ########
threads=8                       # make sure this is <= your BSUB -n value

########## OUTPUTS #########
out_prefix='sspace_M1906_1000'

##################################### COMMANDS ############################
#
echo "lib1 bwa $mp2kb1_1 $mp2kb1_2 2000 0.8 FR" > $config_file

SSPACE_Standard_v3.0.pl -l $config_file -s $contigs -x 0 -z 1000 -T $threads -k 5 -a 0.7 -b $out_prefix
```

d. Improve assemblies using Pilon

```
#BSUB -L /bin/bash              # uses the bash login shell to initialize the job's execution
#BSUB -J pilon_pipeline         # job name
#BSUB -n 20                     # assigns 20 cores for execution
#BSUB -R "span[ptile=20]"       # assigns 20 cores per node
#BSUB -R "rusage[mem=2700]"     # reserves 2700MB memory per core
#BSUB -M 2700                   # sets to 2700MB process enforceable memory limit. (M * n)
#BSUB -W 48:00                  # sets to 48 hour the job's runtime wall-clock limit.
#BSUB -o stdout.%J              # directs the job's standard output to stdout.jobid
#BSUB -e stderr.%J              # directs the job's standard error to stderr.jobid

module load BWA/0.7.12-intel-2015B
module load SAMtools/0.1.19-intel-2015B
module load picard/1.119-Java-1.7.0_80
module load Pilon/1.20-Java-1.8.0_92

###################################### VARIABLES ######################
# TODO Edit these variables as needed:
threads=20                      # make sure this is <= your BSUB -n value

pe1_1="/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo_1P.fastq.gz"
pe1_2="/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/M19_06_trimmo_2P.fastq.gz"

read_group_id='M19_06'
library='pe'
sample='M19_06'
platform='ILLUMINA'

assembly='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/RhizoctoniaM1906onlyAssembly/ru
n_short-scaffolding-closing/sspace_M1906_500/sspace_M1906_500.final.scaff\
olds.fasta'
output_bam="${sample}_sorted_dedup.bam"
###################################### COMMANDS ###############
/scratch/datasets/genome_indexes/ucsc/
if [ ! -f ${assembly}.bwt ]; then
  bwa index $assembly
fi
bwa aln -t $threads $assembly $pe1_1 > pe1_1.aln.sai
bwa aln -t $threads $assembly $pe1_2 > pe1_2.aln.sai
bwa sampe -r "@RG\tID:$read_group_id\tLB:$library\tSM:$sample\tPL:$platform" $assembly
pe1_1.aln.sai pe1_2.aln.sai $pe1_1 $pe1_2 | samtools view -h -Sb - | samtools so\
rt -o -m 2G -@ 20 - sorted > ${sample}_sorted.bam
```

```
java -jar $EBROOTPICARD/MarkDuplicates.jar TMP_DIR=$TMPDIR I=${sample}_sorted.bam
O=$output_bam METRICS_FILE=${sample}.dup.metrics VALIDATION_STRINGENCY=LENIENT
samtools index $output_bam
java -Xmx50g -jar $EBROOTPILON/pilon-1.20.jar --genome $assembly --frags $output_bam  --output
$sample --outdir out_pilon --vcf --tracks --threads $threads
```

e. Genome completeness using BUSCO

```
#BSUB -L /bin/bash              # uses the bash login shell to initialize the job's execution
#BSUB -J busco                  # job name
#BSUB -n 20                     # assigns 20 cores for execution
#BSUB -R "span[ptile=20]"       # assigns 20 cores per node
#BSUB -R "rusage[mem=2700]"     # reserves 2700MB memory per core
#BSUB -M 2700                   # sets to 2700MB per process enforceable memory limit.
#BSUB -W 24:00                  # sets to 24 hours the job's runtime wall-clock limit.
#BSUB -o stdoutsrDNA.%J         # directs the job's standard output to stdout.jobid
#BSUB -e stderrsDNA.%J          # directs the job's standard error to stderr.jobid

module load BUSCO/3.0.2-intel-2017b-Python-3.6.3
#############################################################################
threads=20                      # make sure this is <= your BSUB -n value
genome_file='/scratch/user/spgaire/rhizoctonia/AG_11/M19_07_06_20/RhizoctoniaM1906onlyAssembly
/run_short-scaffolding-closing/sspace_M1906_500/out_pilon/M19_06.fasta'
# see available BUSCO lineages in this directory: /scratch/datasets/BUSCO/v3.0.2/
busco_lineage='/scratch/datasets/BUSCO/v3.0.2/basidiomycota_odb9'
busco_mode='genome'             # genome, transcriptome, protein
# --species:  see available species here: /software/easybuild/software/AUGUSTUS/3.3.2-intel-
2019a/config/species/
augustus_species='ustilago_maydis'
augustus_model='complete'     # partial (default), intronless, complete, atleastone, exactlyone
output_prefix="out_Busco_RhizoctDNA_${augustus_species}"

# copy augustus config to $SCRATCH
if [ ! -d "$SCRATCH/my_augustus_config/config" ]; then
  echo "Copying AUGUSTUS config directories to $SCRATCH/my_augustus_config"
  mkdir $SCRATCH/my_augustus_config
    if [ "-$EBROOTAUGUSTUS" == "-" ]; then
        echo "Augustus module not loaded"; exit 1
    fi
  rsync -r $EBROOTAUGUSTUS/ $SCRATCH/my_augustus_config
  chmod -R 755 $SCRATCH/my_augustus_config
fi
export AUGUSTUS_CONFIG_PATH="$SCRATCH/my_augustus_config/config"

python $EBROOTBUSCO/scripts/run_BUSCO.py -f --in $genome_file --mode $busco_mode --species
$augustus_species --cpu $threads --lineage $busco_lineage --out $output_pref\
ix --tmp_path $TMPDIR --augustus_parameters="--genemodel=$augustus_model"
```

f. Sequence alignments using Bowtie2

```
#BSUB -L /bin/bash              # uses the bash login shell for job environment
#BSUB -J bowtie2_pe             # job name
#BSUB -n 20                     # assigns 4 cores for execution
#BSUB -R "span[ptile=20]"       # assigns 4 cores per node
#BSUB -R "rusage[mem=2000]"     # reserves 2500MB memory per core
#BSUB -M 2000                   # sets to 2500MB per process enforceable memory limit.
#BSUB -W 10:00                  # sets to 24 hour the job's runtime wall-clock limit.
#BSUB -o stdout.%J              # directs the job's standard output to stdout.jobid
#BSUB -e stderr.%J              # directs the job's standard error to stderr.jobid
module load Bowtie2/2.2.5-intel-2015B
module load SAMtools/1.2-intel-2015B-HTSlib-1.2.1-r2
module load picard/2.18.27-Java-1.8.0
########## INPUTS ##########
pe1_1='/scratch/user/spgaire/rhizoctonia/AG_11/mapping/T18_732_trimmo_1P.fastq.gz'
pe1_2='/scratch/user/spgaire/rhizoctonia/AG_11/mapping/T18_732_trimmo_2P.fastq.gz'
genome_index_prefix='/scratch/user/spgaire/rhizoctonia/AG_11/Bowtie2/M19_06'
######## PARAMETERS ########
threads=20                      # make sure this is <= your BSUB -n value
# rg = read group
rg_id='T18_732'
rg_platform='ILLUMINA'
rg_sample='T18_732'
rg_library='ILLUMINA'
##################################### COMMANDS ##########################
# add read group id and other RG values
bowtie2 -p $threads --rg-id "$rg_id" --rg "LB:$rg_library" --rg "SM:$rg_sample" --rg "PL:$rg_platform"
-x $genome_index_prefix -1 $pe1_1 -2 $pe1_2 | samtools view -bS \
- > $TMPDIR/${rg_sample}_bwa_sampe_out.sam
# sort sam picard
java -Xmx48g -jar $EBROOTPICARD/picard.jar SortSam TMP_DIR=$TMPDIR
I=$TMPDIR/${rg_sample}_bwa_sampe_out.sam O=$TMPDIR/${rg_sample}_sorted.bam
SO=coordinate VALIDATION_\
STRINGENCY=LENIENT
# mark duplicates with picard
java -jar $EBROOTPICARD/picard.jar MarkDuplicates TMP_DIR=$TMPDIR
I=$TMPDIR/${rg_sample}_sorted.bam O=${rg_sample}_sorted_dedup.bam
METRICS_FILE=${rg_sample}_dup.metri\
cs VALIDATION_STRINGENCY=LENIENT
# index bam files
samtools index ${rg_sample}_sorted_dedup.bam
```

g. Variant calling using FreeBayes

```
#BSUB -L /bin/bash              # uses the bash login shell to initialize the job's execution
#BSUB -J freebayes              # job name
#BSUB -n 20                     # assigns 4 cores for execution
#BSUB -R "span[ptile=20]"       # assigns 4 cores per node
#BSUB -R "rusage[mem=5000]"     # reserves 2500MB memory per core
#BSUB -M 5000                   # sets to 2500MB per process enforceable memory limit. (M * n)
#BSUB -W 70:00                  # sets to 4 hour the job's runtime wall-clock limit.
#BSUB -o stdout.%J              # directs the job's standard output to stdout.jobid
#BSUB -e stderr.%J              # directs the job's standard error to stderr.jobid
#BSUB -P 082824742376

module load FreeBayes/2015-12-15-intel-2015B

########## INPUTS ##########
fasta_reference='/scratch/user/spgaire/rhizoctonia/AG_11/Bowtie2/denovo_assembly/M19_06.fasta'

# bam file must be sorted by reference position
bam_file='/scratch/user/spgaire/rhizoctonia/AG_11/Bowtie2/*.bam'


########## OUTPUTS #########
vcf_out_file='Rhizoctonia_solani.vcf'

##################################### COMMANDS ########################
freebayes --fasta-reference $fasta_reference $bam_file > $vcf_out_file
```