DEVELOPMENT AND CHARACTERIZATION OF TEXAS BLUEGRASS X KENTUCKY BLUEGRASS INTERSPECIFIC HYBRIDS FOR THE SOUTHERN UNITED STATES

A Dissertation

by

MEGHYN BRIANNE MEEKS

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

Chair of Committee,	Ambika Chandra
Co-Chair of Committee,	Benjamin G. Wherley
Committee Members,	Russell W. Jessup
	Kevin L. Ong
Head of Department,	David Baltensperger

May 2015

Major Subject: Plant Breeding

ABSTRACT

Kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* Schreb.) are commonly grown cool-season grasses in the United States for home lawns and various sports turf applications. However, environmental conditions such as prolonged periods of drought and heat as well as heavy shade often result in a loss of visual appeal, early dormancy, or death of the turf stand. Hybrid bluegrass (*Poa* spp.) cultivars derived from Texas bluegrass (*P. arachnifera* Torr.) x Kentucky bluegrass interspecific crosses may serve as viable alternatives for perennial turfgrass in the southern United States. Methods to aid in the breeding and selection of Texas x Kentucky bluegrasses for these environmental conditions would be useful. The goals of this research were to 1) develop new interspecific hybrid bluegrasses and optimize a seed germination technique, 2) use flow cytometry and molecular markers for the characterization of hybrid progeny, and 3) evaluate the growth response and performance of hybrid bluegrasses maintained in shade.

In 2012 and 2013, controlled pollinations were made between different *Poa* species. Seed from these crosses were germinated through two different techniques that resulted in 61 new interspecific hybrids. A higher percentage of individuals (89%) were recovered through germination on nutrient agar medium than soil. In addition to these hybrids, 52 Texas x Kentucky hybrids created in 2001 were tested for three years in multiple locations across the southern states and the transition zone. Experimental hybrid TAES 5653, registered as DALBG 1201, was the superior genotype.

Flow cytometry was used to estimate the DNA content of 19 Texas bluegrass genotypes, and four Texas x Kentucky bluegrass hybrids from two different pedigrees. Variability in DNA content suggested variability in the ploidy levels of Texas bluegrass. Texas x Kentucky bluegrass interspecific hybrids had an intermediate DNA content demonstrating flow cytometry can identify true hybrids between parents with vastly different DNA content. A thioredoxin-like (*trx*) nuclear gene was also useful in identifying true Texas x Kentucky bluegrass hybrids. A distinctive 851 bp *trx* allele with a 163 bp insertion site was also identified. The insertion is evidently a transposable element that may help to elucidate ancestors of Texas bluegrass.

Another study was conducted to develop a method of comparing Texas x Kentucky bluegrass hybrids to commercial cultivars for shade tolerance. It was determined that evaluations should be conducted in the spring to early summer months using moderate shade (50%) levels, and high turfgrass quality and slower leaf elongation rates as selective traits. Trinexapac-ethyl was not necessary to reduce the leaf elongation rate or increase quality of dwarf hybrids under shade.

In summary, several methods have been tested to generate and evaluate new Texas x Kentucky bluegrass hybrids. Seed germination conducted under controlled conditions using agar medium, and flow cytometry and the *trx* nuclear gene can be employed in bluegrass breeding programs to identify true interspecific hybrids. Finally, improved dwarf-type hybrid bluegrasses, such as DALBG 1201 and TAES 5654, seem to have enhanced shade tolerance and potential for use as perennial turfgrass alternatives in the southern and transition zone regions of the United States.

DEDICATION

To my husband

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. Ambika Chandra, and my committee members Dr. Russell Jessup, Dr. Benjamin Wherley, and Dr. Kevin Ong, for their guidance and support throughout the course of this research.

Had it not been for the financial support of Monsanto, Inc., Texas A&M AgriLife Research, and NGTurf this research would not have been possible.

Thanks should also be extended to the faculty and staff at the Texas A&M Agrilife Research Center-Dallas, for welcoming me and treating me as a respected member of the research team. My success was also dependent on the wonderful support of the research, greenhouse, and field staff along the way. I would like to specially thank Ying Wu for performing crosses in 2013 during my maternity leave, and Dr. Anthony D. Genovesi for his guidance in laboratory experiments.

Finally, I would like to acknowledge Ninglin Yin at the Utah State University, Center for Integrated Biosystems in Logan, UT for the DNA sequencing service, and Dr. Arumuganathan and Mason Ma at the Flow Cytometry and Imaging Core Laboratory in Seattle, WA for the flow cytometry service. In addition, we are grateful for the contribution of Dr. Jason Goldman at the USDA-ARS in Woodward, OK by providing young leaf tissue samples of 20 *P. arachnifera* genotypes from his collection as well as two anonymous reviewers for their useful suggestions regarding the 163 bp insertion site.

I could not have completed this research without everyone's support. Thank you for everything!

NOMENCLATURE

AFLP	Amplified fragment length polymorphisms
DLI	Daily light integral
EST	Expressed sequence-based targets
F_1	First generation hybrid
ISSR	Inter-simple sequence repeats
LSD	Least significant difference
MITE	Miniature inverted transposable element
MLE	Mariner-like element
NPGS	National Plant Germplasm System
NTEP	National Turfgrass Evaluation Program
PCR	Polymerase chain-reaction
PPF	Photosynthetic photon flux
RAPD	Randomly amplified polymorphic DNA
SCAR	Sequence characterized amplified regions
SSR	Simple-sequence repeats
STS	Sequence-tagged sites
TAES	Texas Agriculture Experiment Station
trx	Thioredoxin-like gene
TXKY	Texas bluegrass x Kentucky bluegrass

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Turfgrasses are classified as either warm-season (C_4) or cool-season (C_3) based upon their respective photosynthetic reactions. The warm temperatures, high light intensities, and long day lengths of summer are ideal for warm-season grasses due to their high light saturation points, lower evapotranspiration (ET) rates, and lack of photorespiration. These factors contribute to warm-season grasses' adaptation to the southern United States, and the associated extreme conditions such as heat and drought. In contrast, cool-season species perform optimally at lower temperatures and also possess much lower light compensation points, relative to their warm-season counterparts, making them better adapted to the cooler temperatures and shorter photoperiods of fall and spring as well as to shaded conditions. Unlike warm-season grasses, cool-season grasses generally possess relatively high ET rates and undergo photorespiration under elevated light intensity and temperatures of summer. As a result, cool-season grasses are generally more sensitive to heat and drought than warm-season turfgrasses.

In recent years, record breaking periods of heat and drought have been most problematic in the southern United States. For home owners and turfgrass managers, there is great difficulty in maintaining acceptable turfgrass quality under these conditions especially when tightened water supplies and growing urban demands for water lead municipalities to impose water restrictions. These factors highlight the growing need for turfgrass breeders to develop improved heat and drought-adapted varieties.

Bluegrasses (*Poa* spp.) are cool-season turfgrasses that have potential for enhanced adaptability to the southern climate due to the revelation that heat and drought tolerances can be obtained from native species through interspecific hybridization. Currently, there are 575 *Poa* species known to exist, including both annual and perennial types (Gillespie and Soreng, 2005), and among them is a wide range of genetic and morphological diversity. Many of these species have the potential to serve as niche grass on golf courses, lawns, and forage pastures. The current issues that affect the growth and performance of bluegrasses are the interactions between various environmental factors such as shortages of water resources, high temperatures during the summer months, poor water quality, abiotic (heat, drought, and shade) and biotic (pests and diseases) stresses. Therefore, there is a need to breed for resistance and/or tolerance in bluegrass species to improve their performance and minimize the negative responses to environmental effects.

The primary objectives for this research were to generate and identify new hybrid bluegrasses that perform well in the southern region. These objectives were accomplished through the development of an improved seed germination method, a multiple environment performance trial, flow cytometry and marker-assisted selection, as well as greenhouse shade tolerance evaluations.

Interspecific *Poa* hybridization

Interspecific hybridization is a common practice used to introgress turfgrass quality, agronomic, and environmentally important traits from one species to another. Due to its native adaptation to the southern climate, Texas bluegrass (*Poa arachnifera*

Torr.) possesses important heat and drought tolerant characteristics that have become important for *Poa* breeders in the last half century (Williams, 1916; Hitchcock, 1950). Traditionally, it has served as an uncultivated forage grass, with approximately 40,000 acres in Texas (Aggie Horticulture, 2014). Unfortunately, it does not have desirable turf grass quality or seed trait characteristics. Unlike other forage grasses, its dioecious nature requires close proximity of male and female plants for seed production, but is sometimes compatible with other species. As a result, frequent genetic recombination produces heterogeneous progeny and heterogeneity in the population.

Kentucky bluegrass (*Poa pratensis* L.) is the most widely grown bluegrass species in the northern United States for turf and forage (Funk et al., 1981). It is not native to the United States, but has adapted to states within the transition zone as well as western states when irrigated. Its adaptation almost completely excludes Texas except for the panhandle region. The most distinguishable characteristics are its green to dark green colored leaves with boat-shaped tips, and pyramid-shaped inflorescence. Seed are produced via apomixis, a clonal form of reproduction that maintains homogeneity in populations. However, small percentages of out-crossing and facultative apomixis have created genetic and morphological diversity (Shortell, 2009; Huff, 2010). Different genotypes respond favorably or unfavorably to the same environmental conditions which portrays their advantageous or disadvantageous traits for breeding purposes and adaptability to specific regions.

Successful interspecific hybridizations between Texas and Kentucky bluegrasses rely heavily on environmental influences, reproductive, and chromosome compatibility.

In general, bluegrasses require cold temperature vernalization (Huff, 2003a), and 16 to 18 hr day lengths to flower in the spring (Hintzen and van Wijk, 1985; Read, 2001). Variability in vernalization requirements creates a short window of opportunity to make crosses. Due to its dioecious nature, Texas bluegrass is the most feasible female parent in hybridizations with Kentucky bluegrass. However, seed cannot be germinated immediately due to internal dormancy mechanisms and sometimes seed are not viable due to a lack of chromosome homology between parents (Clausen, 1961). Mating species with similar chromosome numbers improves the chances of wide-cross hybridization (Savidan et al., 2001). Thus, it would be beneficial for breeders to know the ploidy of projected parents to make the best pair-wise selections. Texas and Kentucky bluegrasses are polyploids with varying degrees of ploidy within each species ((Brown, 1939; Gould, 1958; Love and Love, 1975; Kelley et al., 2009). Methods used to count chromosome numbers have previously included crystal violet staining (Brown, 1939; Hartung, 1946; Grun, 1954), and pollen smearing (Gould, 1958), but these methods are laborious and time consuming compared to modern technology.

Flow cytometry is a more efficient tool at estimating chromosomes by measuring diploid (2C) nuclear DNA content from somatic leaf cell nuclei (Arumuganathan and Earle, 1991). One of the first documented uses in plants was from de Laat et al. (1987), who stated it was useful for both single plants and populations. The utility of flow cytometry in identifying interspecific hybrids between Texas and Kentucky bluegrasses has been hypothesized, but not tested. This would be useful for eliminating false hybrids from a pool of first generation (F_1) progeny. Alternatively, morphological markers have

been used over the past century, but can be time consuming since plants have to first complete a full life cycle to visually observe many morphological traits. DNA based, or molecular markers, offer the ability to determine genetic heritage from plant parents at a reasonable cost and time.

Marker-assisted characterization of interspecific hybrids

With the progression of modern molecular genetics, many marker systems are now available for use in breeding programs, but each marker system serves a different purpose. The majority of DNA based marker systems use polymerase-chain-reaction (PCR) to amplify genetic sequences. Those that have been used for bluegrasses include random amplified polymorphic DNA (RAPD) primers (Huff and Bara, 1993; Barcaccia et al., 1997), sequence characterized amplified region (SCAR) markers (Albertini et al., 2001; Abraham et al., 2005), amplified fragment length polymorphisms (AFLP) (Renganayaki et al., 2001), inter simple sequence repeats (ISSR) (Carson et al., 2007; Goldman, 2008), and microsatellites also known as simple sequence repeats (SSR) (Honig et al., 2010; Kindiger et al., 2011; Kindiger et al., 2013). Less costly but equally useful alternatives are expressed sequence tags (ESTs) (Zeid et al., 2010) or sequence-tagged site (STS) markers (Patterson et al., 2005). Both types use sequenced-based information to compare gene homologs between species. Genetic sequence alignments from diverse genomes can identify ancestral regions or highly conserved regions as potential markers. Nuclear genes can be exploited for this purpose if they occur in low-copy numbers, or when the frequency of alleles with a particular gene is limited. The *trx* gene (thioredoxin-like) was one such nuclear gene investigated to understand the

relatedness of different *Poa* species, including the evolution of *P. annua* from *P. infirma* x *P. supina* (Patterson et al., 2005; Mao and Huff, 2012). It was inferred from the *trx* gene research that this gene could be used to identify Texas x Kentucky bluegrass hybrids. The implications of this application are that only true hybrids would be selected for performance trials.

Abiotic and biotic stress evaluations

The warm-humid region that borders the southern edge of the transition zone and stretches towards the Gulf Coast is prone to very extreme summer weather conditions. Temperatures can easily exceed 100^{0}F (approximately 38^{0}C), and are accompanied by high humidity and periodically severe to extreme drought, as was the case in the summer of 2011 for the majority of the southern states (National Climatic Data Center, 2011). When heat and drought intensities increase over an extended period of time, grasses are needed to provide soil stabilization and a cooling effect (Morris, 2003). Since extreme events such as heat waves and drought are problems that are expected to persist, heat and drought tolerances are primary traits of interest for acceptability of turfgrasses in southern urban areas. Secondary traits include, but are not limited to, shade and disease tolerances. Evaluations over multiple years and environments identify the potential of each genotype for commercial potential.

The majority of ground cover in the southern region is currently planted with warm-season turfgrasses. Warm-season turfgrasses generally possess good water use efficiency and drought tolerance, but in some instances may require higher maintenance than cool-season turfgrasses, and undergo an extensive winter dormancy period due to

sub-optimal temperatures. Furthermore, few warm-season grasses are tolerant to low light conditions (Bell, 2011). Therefore, it has been proposed that breeding efforts should be focused on improving these stress tolerance traits, thereby extending the reach of adaptability into the warmer climates of the southern United States so that their benefits can be exploited for urban areas.

St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntz) is a popular southern lawn grass and is the predominant turf species in Texas due to its aggressive growth and shade adaptability. On golf courses and home lawns, tall fescue (*Festuca arundinacea* Schreb.) is a common cool-season turfgrass that is sometimes used for moderately shaded areas. Both of these species are often disliked for their coarse texture, laborious maintenance needs, and susceptibility to diseases. Bluegrasses could offer desirable visual appeal with finer-textured, green to dark green leaves, and lower mowing needs, but have historically not performed well under shaded conditions. In recent years, cultivar improvements and advances for shade tolerance have been achieved for *P. pratensis* through morphological characterization and intraspecific breeding (Bashaw and Funk, 1987; Brilman, 2009), but the likelihood of acceptability in the south is low because of the sensitivity to heat and drought relative to tall fescue. Hybrid bluegrasses could offer a competitive advantage to tall fescue for heat tolerance (Su et al., 2007), but their shade tolerance has not yet been explored. Therefore, data on the species' shade tolerance would be important for breeders to garnish more widespread acceptance of the species by homeowners and turfgrass managers.

Disease resistance is another characteristic to consider for cool-season grasses grown in shaded environments. The warm-humid environments found in the southern United States can place particularly high disease pressure on turfgrass. One of the most plaguing and detrimental of these diseases brown patch (*Rhizoctonia solani* Kühn). Different races of this pathogen are also pathogenic to warm-season grasses causing large patch. Although this pathogen does not produce spores, it grows by mycelium, which can spread the disease quite rapidly, especially under high maintenance regimes with frequent mowing and heavy nitrogen fertilization. In addition, the sclerotia bodies that overwinter beneath the turf surface, are resistant to cold, drought, and heat making it a difficult disease to control (Stowell and Gelernter, 1997). Cultural management practices to control brown patch include early morning irrigation to reduce leaf wetness duration, air circulation, and a reduction in nitrogen rates. While there are commercially available fungicides to control the disease, these are not recommended for residential use because of their high cost, need for re-application, and potential impacts on the environment. For these reasons, brown patch resistance is another important characteristic to consider in development of bluegrasses for the southern U.S.

CHAPTER II

BREEDING INTERSPECIFIC HYBRID BLUEGRASSES

Overview

Consumers in the southern United States desire improved turfgrass species that can tolerate extreme changes in climate, but also provide year-round color. For bluegrasses, environmental tolerances can be introgressed from native species into elite breeding material. However, different species are not always compatible for various reasons, and results in embryo abortion or few hybrids. Furthermore, methods to germinate new hybrid bluegrass generations quickly are not available through the literature. Therefore, the objectives of this research were to develop new interspecific hybrid bluegrasses and optimize a seed germination technique.

Introduction

Turfgrasses are one of the largest and versatile agricultural crops in the United States. Comprised of both cool- and warm-season species, the greatest benefits to humans, animals, and the environment can be observed in urban areas where they are used to control soil erosion alongside roadways, reduce pollution, provide recreational playing surfaces, and create a cooling-effect in an otherwise concrete infrastructure (Morris, 2003). However, turfgrasses are also the single largest irrigated crop covering between 40 and 50 million acres of land, an area that is approximately four times larger than that of cultivated corn (*Zea mays*) (Milesi et al., 2005; Morris et al., 2005). Consequently, it has become evident that many grasses are not sustainable during extended periods of high heat and drought. The alternative is to develop tolerant grasses

through breeding, so that water can continue to be conserved and the many benefits of turfgrasses can be exploited.

Bluegrasses (*Poa* spp.) are highly desirable for their green to dark-green color and fine leaf texture that can be observed year-round, but they are particularly sensitive to heat, drought, shade, disease, and insect pressures. Consumer desires to have bluegrasses that are both aesthetically pleasing and tolerant to heat and drought have encouraged breeders to identify sources of heat and drought tolerances. Some native *Poa* species found in uncultivated grasslands of the United States are potential sources of these traits (Gillespie and Soreng, 2005; Brittingham, 1943; Hitchcock, 1950; Huff, 2010). Wide-cross or interspecific hybridization is routinely used to introgress environmentally important traits from native species into breeding material. However, interspecific hybridization presents some challenges with differences in flowering time, reproductive mode, and ploidy.

A vernalization requirement of short days and cold temperatures is necessary to induce flowering in the spring (Huff, 2003a), but the duration of vernalization is dependent on the species. Thus, the window of opportunity to make crosses can be narrow. Additionally, reproductive morphology is not always conducive for cross-species pollination. Apomictic reproduction is common in either obligate or facultative forms which prevents or significantly reduces the probability of hybridization. This type of reproduction is most common in wild species (Mazzucato et al., 1996). Kentucky bluegrass is a popular seeded species with 25 to 96% apomixis levels, but its facultative nature allows for a low frequency of interspecific hybridization (Grazi et al., 1961; Han,

1969; Wieners et al., 2006; Huff, 2003a). Dioecious species, on the other hand, are often obligate sexual types that can be utilized as maternal parents without the need for emasculation. Texas bluegrass (*P. arachnifera* Torr.), Sandberg bluegrass (*P. secunda* J. Presl), and *P. ligularis* Nees ex. Steud. are examples of native dioecious species (Read, 1988).

Extreme differences in ploidy, or lack of homologous chromosomes, are other reasons why many hybridization attempts have failed (Clausen, 1961). Evolutionary events such as genome duplication and intragenic pollination have given rise to polyploidization, and created a large amount of chromosome number variation between and within bluegrass species (Acquaah, 2012). Most bluegrass species are polyploids, with sets of chromosomes in multiples of seven, but irregular meiotic events have resulted in aneuploids (Myers, 1947; Carnahan and Hill, 1961; Clausen, 1961). Although commonly accepted as an octoploid ($2n = 8x = 56$) (Hartung, 1946; Patterson et al., 2005), the chromosome numbers in Texas bluegrass have been observed to range from $2n = 42 – 91$ (Brown, 1939; Gould, 1958; Kelley et al., 2009). A much higher variation has been observed in Kentucky bluegrass, an aneuploid that exhibits a range from $2n = 24 – 124$ (Love and Love, 1975). Therefore, variability between these two species presents some challenges to making successful crosses.

Vinall and Hein (1937) reported that the first Texas x Kentucky bluegrass hybrid was made by George Oliver in 1908, but commercial varieties had not developed until the last 15 years. The bluegrass breeding program at the Texas A&M AgriLife Research Center – Dallas, formerly known as the Texas Agriculture Experiment Station (TAES),

was led by Dr. James Read who focused on improving forage grass quality and it was Read who recognized the potential of Texas x Kentucky hybrid bluegrasses for turfgrass applications (Read, 1994). The cultivar 'Reveille' was produced from this program and was the first commercial hybrid (Read et al., 1999). The improved visual quality combined with heat and drought tolerances in Reveille encouraged other universities and the commercial sector to start their own bluegrass breeding programs (Abraham et al., 2004; Su et al., 2007). The cultivars 'Bandera' (Smith et al., 2008) and 'Thermal Blue' (Hardison et al., 2008) are examples of hybrid bluegrasses produced from industry programs. Other successful interspecific crosses have been made between Kentucky bluegrass x alpine meadowgrass (*P. alpine* L.) (Akerberg and Bingefors, 1953), big bluegrass (*P. ampla* Merr.) x Kentucky bluegrass (Clausen, 1961), supine bluegrass x early meadowgrass (Huff, 2003b), and Texas bluegrass x Sandberg bluegrass and *P. ligularis* (Kindiger and Wipff, 2009).

Furthermore, internal dormancy mechanisms in *Poa* that are intended to maintain seed viability until favorable environmental conditions are present prevent immediate germination after harvesting, thus limiting plant development time for the next breeding cycle. Cold temperature stratification is required to break this dormancy and initiate germination, but the environmental conditions for seed to germinate vary by species; including differences in photoperiod, moisture, and cold-temperature durations (Froud-Williams et al., 1986; Simpson, 1990; Kaye, 1997; Vance, 2010; Mondoni et al., 2012). The first objective of this research was to develop new hybrid bluegrasses using a genetically diverse set of germplasm available at the Texas A&M AgriLife Research

Center in Dallas, TX. Secondly, an efficient seed germination technique was needed to process and develop new generations of hybrids.

Materials and methods

Breeding

Plant materials consisted of 10 Texas bluegrass genotypes collected by J.C. Read, 11 Kentucky bluegrass cultivars and accessions, six roughstalk bluegrass (*P. trivialis* L.) cultivars, two male *P. ligularis* (PI264407 and PI269650), one male *P. ligularis* (PI269650), and one male Sandberg bluegrass (PI232350) (Table 2-1). Four F_1 Texas x Kentucky (TXKY) hybrid bluegrasses were also included for potential backcrosses. Each genotype was established in one gallon pots with three replicates. Potting medium was composed of Sunshine VP mix (Sun Gro Horticulture, Inc.) and 5% sand (v:v) with an incorporated 7 kg/ cubic meter of osmocote (14-14-14) (Everris NA, Inc.).

Around November 1st of each 2011 and 2012 years, plant materials were relocated outside to sandboxes built to 121.92cm x 731.52cm dimensions. Replicate pots were buried to a depth of 20.32 cm and spaced approximately 45.72 cm apart. Texas bluegrass and Kentucky bluegrass genotypes were planted in separate sand beds. Plants were watered as needed and fertilized monthly (20-20-20). Beginning around February 25th replicates were brought into the greenhouse every two weeks. Texas bluegrasses were not brought into the greenhouse until after inflorescences were heading. Photoperiods in the greenhouses were increased to 18 hr using overhanging cool-white fluorescent lights connected to a 24 hr timer. Bamboo stakes were used to support five-day old inflorescences covered with a Lawson pollination bag. The breeding method was

modified from Read (1994). The ideal crossing time was between 8 AM and 11AM daily before pollen shed. Seven-day old inflorescences of each species were tagged, paired for crossing, and covered with a labeled Lawson bag. Crosses were set in test tubes filled with nutrient solution (19.3g sucrose and 0.0195g 8-hydroxyquinoline citrate per 1-L of distilled water), and supported in test tube racks with vertical bamboo stakes. An oscillating fan connected to a 30-minute timer agitated the pollination bags for one minute every quarter hour. Pollen parents were removed from the pollination bags after anthers had desiccated. Female inflorescences were harvested 21 days after pollination. Weather data for each of the vernalization periods was collected from the National Climatic Data Center weather station in Richardson, TX (GHCND:USC00417588) (2012).

Table 2-1. Species and genotypes used for the 2012 and 2013 breeding seasons.

Genotype number	Common name (species)	Cultivar name or genotype ID	Parent	Year
1	Kentucky bluegrass (*P. pratensis* L.)	Absolute	Male	2012
2	Kentucky bluegrass (*P. pratensis* L.)	America	Male	2012
3	Kentucky bluegrass (*P. pratensis* L.)	Baron	Male	2012, 2013
4	Kentucky bluegrass (*P. pratensis* L.)	CS#2	Male	2012
5	Kentucky bluegrass (*P. pratensis* L.)	CS#4	Male	2012
6	Kentucky bluegrass (*P. pratensis* L.)	Kenblue[†]	Male	2013
7	Kentucky bluegrass (*P. pratensis* L.)	Limousine	Male	2012, 2013
8	Kentucky bluegrass (*P. pratensis* L.)	Midnight	Male	2012
9	Kentucky bluegrass (*P. pratensis* L.)	Pick MP07[†]	Male	2013
10	Kentucky bluegrass (*P. pratensis* L.)	RAM I	Male	2012

Table 2-1. Continued.

Genotype number	Common name (species)	Cultivar name or genotype ID	Parent	Year
11	Kentucky bluegrass (*P. pratensis* L.)	Touchdown[†]	Male	2012, 2013
12	Roughstalk bluegrass (*P. trivialis* L.)[‡]	Colt	Male	2013
13	Roughstalk bluegrass (*P. trivialis* L.)	Havana	Male	2012, 2013
14	Roughstalk bluegrass (*P. trivialis* L.)	Laser	Male	2013
15	Roughstalk bluegrass (*P. trivialis* L.)	Po-Lis	Male	2013
16	Roughstalk bluegrass (*P. trivialis* L.)	ProAm	Male	2013
17	Roughstalk bluegrass (*P. trivialis* L.)	Saber	Male	2012, 2013
18	Sandberg bluegrass (*P. secunda* J. Presl.)	PI232350	Male	2012, 2013
19	*P. ligularis* Nees ex Steud.	PI264407	Male	2012
20	*P. ligularis* Nees ex Steud.	PI269650[§]	Male	2012, 2013
21	*P. ligularis* Nees ex Steud.	PI269650	Female	2013
22	Texas bluegrass (*P. arachnifera* Torr.)	TAES 5987	Female	2012
23	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6012	Female	2012
24	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6019	Female	2012
25	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6020	Female	2012
26	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6021	Female	2012
27	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6022	Female	2012
28	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6024	Female	2012
29	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6025	Female	2012
30	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6027	Female	2012
31	Texas bluegrass (*P. arachnifera* Torr.)	TAES 6029	Female	2012
32	TXKY (6012 x CS#4)	DALBG 1201	Female	2012, 2013
33	TXKY (6012 x CS#4)	TAES 5655	Female	2013

Table 2-1. Continued.

Genotype number	Common name (species)	Cultivar name or genotype ID	Parent	Year
34	TXKY (TX 3-86 x Limousine)	TAES 5701	Female	2012
35	TXKY (6012 x Absolute)	TAES 5708	Female	2012

[†]Vegetative plant material for Kenblue, Pick MP07, and Touchdown was provided by Dr. Leah Brilman in 2013. This material endured vernalization temperatures in Corvallis, OR that was conducive for flower production.
[‡]All cultivars of roughstalk bluegrass, genotypes of Sandberg bluegrass, and *P. ligularis* were derived from seed obtained from GRIN (NPGS) in Pullman, WA.
[§] Seed obtained for PI269650 were collected from a population; male and female genotypes were generated from the same accession.

Seed stratification

Growth chamber conditions A diurnal growth chamber was set for a 12 hr photoperiod using fluorescent lights. The initial temperature started at 5°C for nine days, followed by 10°C for five days, and one day at each 15°C and 20°C.

Germination in soil (2012) Approximately 30 seed (~0.0104g) per cross were sown in 72-well trays filled with moistened potting medium, covered with plastic wrap, and placed in the growth chamber for stratification. Upon conclusion of the 16 day cycle, trays were moved to the greenhouse and placed under misting benches to observe the percentage of germination.

Germination on agar medium (2013) Seed were manually extracted from inflorescences under a dissecting microscope with tweezers. Seed from each cross were wrapped in individual nylon packets, surfaced sterilized in 500 ml 70% ethanol for two minutes, followed by one 30 min. wash in a solution of 5 g/L Trichloro-s-Triazinetrione (dual-action pool shock chlorination tablets; 93.5% Trichloro-etc. + 1.5% copper (II) sulfate pentahydrate + 5% inert with 84% available chlorine) and 1% Tween 20, and three 5 min. washes in 800 ml sterile distilled water (A.D. Genovesi, pers. comm.). All

sterilization was conducted using a stir plate. In a sterile environment, individual seeds (groups of 10) were sown on ½ x MS medium (1/2 MS salts, 0.5 mg/L B1, 50 g/L sucrose, 15mg/L asparagine, 3.5 g/L phytagel, pH 5.75) amended with 1.67g sucrose, 0.02% B1, and 0.05% asparagine on 100 mm x 25 mm petri plates (A.D. Genovesi, pers. comm.). Plates were placed in the growth chamber for stratification, and remained in the chamber upon conclusion of the 16-day cycle for an additional 28 days at 20°C. Percent germination was calculated from the total number of seeds plated for each cross.

Results

Vernalization was completed by March 21st each year when the last replicates were relocated to the greenhouse. For the 2012 and 2013 breeding seasons, vernalization lasted 142 and 140 days, respectively. The temperatures ranged from -5.6°C to 27.2°C in season 1, and -6.7°C to 30°C in season 2. The average low and high temperatures for these years were 6.2°C/5.1°C and 17.1°C/17.3°C, respectively.

Season 1

Fifty-six interspecific crosses were made in 2012 (Table 2-2). Texas bluegrass was the female parent for 82% of the crosses. The greatest percent germination was obtained from *P. ligularis* x Kentucky bluegrass (2.2%) followed by Texas bluegrass x *P. ligularis* (1.3%). Fifteen plants were generated using the potting soil as a germination medium for a total of 0.1% germination from the 1650 seeds sown.

Table 2-2. Percent germination achieved in 2012 from using the soil stratification method.

Female parent		Male parent	Selected cross #	Seed planted	Germination (%)
Texas bluegrass	x	Kentucky bluegrass	22	660	0
	x	Sandberg bluegrass	3	90	0
	x	Roughstalk bluegrass	10	300	0.3
	x	*P. ligularis*	10	300	1.3
P. ligularis	x	Kentucky bluegrass	3	90	2.2
	x	Sandberg bluegrass	1	30	0
	x	Roughstalk bluegrass	2	60	0
	x	TXKY F_1	1	30	0
TXKY F_1	x	Kentucky bluegrass	2	60	0
	x	Roughstalk bluegrass	1	30	0
	Total		**55**	**1650**	**0.1**

Season 2

One hundred thirty-four crosses were made in the 2013 breeding season (Table 2-3). Texas x Kentucky bluegrass crosses only accounted for 24% of the total number of crosses, and 75% of the remaining crosses were TXKY F_1 hybrids x Kentucky bluegrass. Other interspecific crosses with Sandberg bluegrass and *P. ligularis* were not performed. Only 11 of the crosses produced a total of 261 seed that were subjected to the agar stratification method. A single cross of Texas x Roughstalk bluegrass had the highest percentage of germination (36.1%). The overall average percent germination was 21.1%.

Table 2-3. Percent germination achieved in 2013 using agar stratification method.

Female parent		Male parent	Cross #	Selected cross #	Seed plated	Germination (%)
Texas bluegrass	x	Kentucky bluegrass	31	1	30	10
	x	Roughstalk bluegrass	3	1	61	36.1
TXKY F_1	x	Kentucky bluegrass	100	9	170	17.1
Total			**134**	**11**	**261**	**21.1**

Discussion

In both years, genotypes of Sandberg bluegrass, roughstalk bluegrass, *P. ligularis,* and female genotypes of Texas bluegrass began flowering 2 – 3 weeks before Kentucky bluegrasses despite having brought the Kentucky bluegrasses into the greenhouse earlier for flower induction. This indicated that the duration and temperature requirements for vernalization of Kentucky bluegrasses were longer and colder than the other species, and is in agreement to results from Canode and Perkins (1977). Thus, many types of crosses were prevented.

An additional struggle was that the Kentucky bluegrasses produced very few inflorescences from which to collect pollen or make pair-wise crosses. Consequently, Texas x Kentucky bluegrass crossing was limited using the available materials, and

depicts the difficulty in making crosses between these cool-season grasses in a southern environment where vernalization conditions may not be ideal.

It is suspected that genetic incompatibility and/or a combination of stigma or pollen inviability resulted in no fertilization or embryo abortion, and were the most likely reasons explaining the low number of crosses with seed and development of interspecific hybrids in both breeding seasons. The weight-based method for soil germination did not account for the actual number of live seed to obtain accurate percent germination values, and may have also been responsible for the low germination percentage in 2012. Although this method seemed most feasible for the large number of crosses that were attempted to germinate, the duration of the stratification process and waiting time to observe germination in the greenhouse was not efficient or successful. For these reasons, a germination method was adopted using 1/2x MS agar medium amended with nutrients, similar to what is used for embryo rescue techniques.

Although only 8% of the crosses from 2013 produced seed, a higher percentage of germination was achieved on agar. This can be attributed to eliminating inviable seeds during the extraction process, as well as the plating medium that provided sufficient moisture and nutrients for short term seedling development. It also suggests that the growth chamber conditions were appropriate for proper stratification. The longer incubation at 20°C may have improved this method (Larsen and Bibby, 2005), which has been optimized to an acceptable level of hybrid generation.

Ultimately, very few hybrid bluegrasses were generated in total. For future hybrid bluegrass breeding efforts, determining the actual number of chromosomes of individual accessions and cultivars would be helpful, as well as optimizing the vernalization conditions for Kentucky bluegrasses for simultaneous flowering with Texas bluegrass and greater pollen production. This would reduce the number of crosses and allow for greater feasibility in extracting seed manually and germinating hybrids through the agar stratification method.

CHAPTER III

REGISTRATION OF DALBG 1201 HYBRID BLUEGRASS*

Overview

DALBG 1201 (Reg. No. CV-101, PI 671854) is a turf-type interspecific F_1 hybrid derived from a cross between Texas bluegrass (*Poa arachnifera* Torr.) 20-11 (3-88) (PI 655088) as the female parent and a Kentucky bluegrass (*P. pratensis* L.) ecotype, CS#4, as the pollen parent. DALBG 1201 was evaluated under the designation 01-59-5 and TAES 5653. A total of 47 experimental hybrid bluegrass lines and three commercial checks, 'Rebel Exeda' tall fescue (*Festuca arundinacea* Schreb.) and 'Reveille' and 'Thermal Blue Blaze' hybrid bluegrass (*P. arachnifera* x *P. pratensis*) were evaluated at five locations (Auburn, AL; Starkville, MS; Raleigh, NC; Knoxville, TN and Dallas, TX) from 2009 to 2012. DALBG 1201 was characterized by its superior turfgrass quality, darker green color, and higher shoot density than Reveille and Thermal Blue Blaze. Its leaf texture was finer than Rebel Exeda and similar to the hybrid bluegrass checks. Linear regression analysis for turfgrass quality indicated that DALBG 1201 was more stress tolerant compared to the three commercial checks and was highly stable across varying environmental conditions. Its superior performance over a wide range of southern test locations suggested that DALBG 1201, cool-season turf-type hybrid

*Reprinted with permission from "Registration of DALBG 1201 hybrid bluegrass" by Meeks, M., A. Chandra, S.P. Metz, A.D. Genovesi, J.C. Read, et al., 2015. Journal of Plant Registrations, doi:10.3198/jpr2014.03.0015crc, Copyright [2015] by the Crop Science Society of America, Inc.

bluegrass, is well-suited for use on lawns, landscapes, and other recreational sites across the southern United States.

Introduction

The genus *Poa* (family: *Poaceae*) includes approximately 575 species, both annual and perennial types, that are native to temperate regions of northern and southern hemispheres (Brittingham, 1943; Hitchcock, 1950; Gillespie and Soreng, 2005; Huff, 2010). Of these, Kentucky bluegrass (*Poa pratensis* L.) is the predominant perennial species of *Poa* used in the United States for turf and forage applications (Funk et al., 1981). Although it displays excellent turfgrass quality, its use in southern climates is limited because of its sensitivity to heat and drought stress. The first successful attempt to genetically improve stress tolerance in the species was made in 1908 by George Oliver (Vinall and Hein, 1937) by hybridizing Kentucky bluegrass with Texas bluegrass (*P. arachnifera* Torr.). Texas bluegrass is well known for its heat and drought tolerance (Hitchcock, 1950) and is native to the southern region of the United States spanning from New Mexico to South Carolina. It was not until 1998, ninety years later that the first commercially available interspecific hybrid between Texas bluegrass and Kentucky bluegrass, 'Reveille', was developed (Read et al., 1999). This large span of time can be attributed to the limited amount of knowledge available for the two species as it pertains to their morphological and agronomic characteristics as well as the innate complexity of their genetic compositions (Rhoads et al., 1992; Renganayaki et al., 2001).

Members of the genus *Poa* exhibit different ploidy levels, diploid, polyploid, or aneuploid, with a basic chromosome number of x = 7. There is great variation in

chromosome number both within and among species of *Poa* (Patterson et al., 2005). Kentucky bluegrass is documented to have chromosome numbers ranging from 2n= 24 to 124 (Love and Love, 1975), and Texas bluegrass chromosome numbers range from 2x = 42 to 91 (Hartung, 1946; Kindiger et al., 2011). Flow cytometry has proven to be a rapid and useful method for estimating ploidy levels (Arumuganathan and Earle, 1991; Eaton et al., 2004). Even though greater success in hybridization may be achieved when high polyploid parents such as Kentucky bluegrass are used as pollinators (Pepin and Funk, 1974), extreme differences in ploidy levels is still one of the greatest barriers to successful interspecific hybridization in *Poa* (Kelley et al., 2009).

Kentucky bluegrass primarily produces seed asexually through apomixis, although it does produce sexual seed at a low frequency (Grazi et al., 1961; Han, 1969, Wieners et al., 2006), and is referred to as a facultative apomict. Reveille has been shown to produce 90% of its seed apomictically (Read et al., 1999). Texas bluegrass, in contrast to Kentucky bluegrass, is a sexually obligate dioecious species with separate female and male plants present in the breeding population. Since the release of Reveille other hybrid bluegrass cultivars with specific improvements in heat and drought tolerance, and disease resistance, have been developed including 'Bandera' (Smith et al., 2008) and 'Thermal Blue' (Hardison et al., 2008).

The overall objective of the current study was to develop turf-type hybrid bluegrass cultivars adapted to the southern United States. Following interspecific hybridization, a field study was conducted in five locations over a three year period to test the performance of 47 experimental hybrid bluegrass lines, including DALBG 1201

(Reg. No. CV-101, PI 671854), and two commercial hybrid bluegrass cultivars, Reveille and 'Thermal Blue Blaze' as checks. An elite tall fescue (*Festuca arundinacea* Schreb.), 'Rebel Exeda' was also included in the test because tall fescue is one of the most widely used cool-season grass species in the southern United States. DALBG 1201 outperformed other experimental and commercial hybrid bluegrass cultivars for turfgrass quality, color, and shoot density. DALBG 1201 was superior to Rebel Exeda tall fescue for leaf texture. A genotypes x environment stability indicated that DALBG 1201 was highly stable and more stress tolerant than the commercial hybrid bluegrass cultivars tested for turf quality, color and shoot density. Overall, DALBG 1201 exhibited desirable turfgrass quality traits making it well-suited for use in home lawns and recreational facilities in the southern United States.

Materials and methods

Interspecific hybridization

DALBG 1201 was one of 47 experimental Texas bluegrass x Kentucky bluegrass interspecific hybrids developed at the Texas A&M AgriLife Research and Extension Center at Dallas, TX between 1996 and 2002. Hybridization was performed according to the methods described by Read (1994). Progeny were assigned an identification number representing the cross year-cross number-progeny number; for example, DALBG 1201 was identified as 01-59-05. Selected progeny were later assigned a Texas Agriculture Experiment Station (TAES) number and DALBG 1201 was designated as TAES 5653.

Field study

These 47 experimental hybrids, derived from a range of diverse pedigrees and genetic backgrounds, along with five Texas bluegrass ecotypes (TAES 5679, 5681-5684), six Kentucky bluegrass genotypes (H8G-J86, PTDF-22-B-2, H86-712, TAES 5701, 5706, and 5709), two commercial hybrid bluegrass checks (Reveille, Thermal Blue Blaze), and Rebel Exeda tall fescue were assembled for a multi-location test. A total of 61 entries were planted under full sunlight in a randomized complete block (RCB) experimental design with three replications in each of five test locations and were evaluated over a period of three years from 2010 to 2012. Test sites were located in Auburn, AL; Dallas, TX; Starkville, MS; Raleigh, NC, and Knoxville, TN. For experimental hybrids, a 7.62 cm x 7.62 cm plug was planted in a 0.61 m x 0.61 m plot during September-October, 2009. Commercial hybrid bluegrass cultivars were established by seed planted at a rate of 14.65 g m^{-2}, and Rebel Exeda was seeded at 29.29 g m^{-2}. Plots were irrigated to promote establishment in year 1 of the trial, with at least 2.54 cm of irrigation applied weekly to supplement rainfall. Thereafter, irrigation was provided to prevent dormancy or stress. After initial establishment, plots were mowed at a 5.08 cm to 6.35 cm height. Nitrogen (N) was applied at rate of 19.53 to 29.29 g m^{-2} each year in split doses once in each fall, winter, and spring growing seasons. A weed management protocol was implemented according to local weed pressure at each test location. No preventative/curative pesticides were used during the course of the study to control insect or disease problems. Data for average monthly air

temperature and precipitation from 2010-2012 were collected from a local weather station at each test location and provided in supplementary tables (Table A-1 and A-2).

Data collection and analysis

Turfgrass performance data were collected on a monthly basis from 2010 to 2012, but the frequency of data collection varied by location. Traits included overall turfgrass quality (1 = poor; 9 = ideal), seasonal color (1 = straw brown; 9 = dark green), shoot density (1 = poor; 9 = maximum density), and leaf texture (1 = coarse; 9= fine). Summer turfgrass quality data were derived from the turfgrass quality ratings for the months of June to September (2010 - 2012) for each test location. For the purpose of this publication, only data for DALBG 1201 and the three commercial checks will be presented and discussed. All of the data-sets were analyzed using SAS 9.3 (SAS Inc., 2009). Means were compared using Fisher's LSD at the $\alpha = 0.05$ probability level. For each trait x location analysis, entries with means in the top statistical group were followed by the letter 'a.'

Genotype x environment stability analysis

The five test locations were ranked from poorest to best environment by calculating the mean performance of 46 experimental hybrid bluegrasses out of 50 test entries (excluding DALBG 1201, Reveille, Thermal Blue Blaze, and Rebel Exeda) at each location for each trait over three years of evaluation. All five environments were ranked lowest to highest based on visually scored parameters. For this stability analysis, differences in scale and variability of the qualitative turfgrass ratings at each location by individual evaluator were considered as environment effects. The poorest environment

was defined as the location that exhibited the lowest means for 46 entries for a particular trait tested over three years. Similarly, the best environment was defined as the location with the highest means for the tested traits. Trait performance of DALBG 1201 and the three check cultivars were then independently regressed against the location means so that the stability of each genotype for each trait could be evaluated using linear regression analysis across varying environmental conditions.

Results

Origin

DALBG 1201, tested as TAES 5653 and 01-59-5, is an interspecific F_1 hybrid developed in 2001 from a cross between Texas bluegrass ecotype 20-11 (3-88) (PI 655088) as the female parent and Kentucky bluegrass ecotype CS#4 as the pollen parent. Texas bluegrass ecotype 20-11 (3-88) was collected by J. C. Read in Dallas County, TX and was donated to GRIN (USDA-ARS National Genetic Resources Program, 1997) as TBPC 3-88 which was changed to PI 655088 in 2008 (Read et al., 1999). Kentucky bluegrass ecotype CS#4 was collected by J. C. Read and R.H. White in College Station, TX (Brazos County). DALBG 1201 represented a single genotype from a family of 16 progeny.

Traits and characteristics

Turfgrass quality – The turfgrass quality for DALBG 1201 was statistically superior to Reveille and Thermal Blue Blaze in four test locations (Auburn, AL; Starkville, MS; Raleigh, NC; Dallas, TX), and was in the same statistical group in Knoxville, TN (Table 3-1). The turfgrass quality of DALBG 1201 was statistically

similar to Rebel Exeda at Auburn, AL; Starkville, MS and Knoxville, TN and was superior in Dallas, TX. When averaged across all locations, the turfgrass quality of DALBG 1201 was statistically superior to commercial checks, Thermal Blue Blaze, Reveille and Rebel Exeda.

Table 3-1. Mean turfgrass quality for DALBG 1201 and three commercial checks for five test locations.

	Mean Turfgrass Quality at Test Locations†					
Entry	Auburn, AL	Starkville, MS	Raleigh, NC	Knoxville, TN	Dallas, TX	AVG‡
DALBG 1201	6.8a*	5.6a	6.3b	8.0a	5.6a	6.5a
Rebel Exeda§	6.7a	5.4ab	6.8a	8.0a	4.2b	6.2b
Reveille§	5.7b	5.0bc	5.2c	7.9a	4.5b	5.7c
Thermal Blue Blaze§	6.0b	4.6c	4.9c	8.1a	3.4c	5.4d
LSD¶0.05	0.6	0.5	0.5	0.3	0.7	0.2
CV#, %	9.6	9.9	8.1	3.8	16.4	9.0

* Significant at a probability level of 0.05.
† Turfgrass quality ratings were on a 1-9 scale; 1=poor, 9=ideal, 5 was the minimum acceptable. Mean represents turfgrass quality data for years 2010, 2011, and 2012 at each location.
‡ Average turfgrass quality of each entry across all locations from 2010 to 2012.
§ Commercial checks included are Rebel Exeda tall fescue, and Reveille and Thermal Blue Blaze hybrid bluegrasses.
¶ Fisher's LSD values were calculated at a probability level of 0.05.
# CV (Coefficient of Variation) indicates the percent variation of the mean in each column.

Summer turfgrass quality – Summer turfgrass quality for DALBG 1201 was superior to Reveille in Auburn, AL; Raleigh, NC; Knoxville, TN and was statistically similar in Starkville, MS and Dallas, TX (Table 3-2). DALBG 1201 outperformed Thermal Blue Blaze in Raleigh, NC; Knoxville, TN; Dallas, TX, and was statistically similar in Auburn, AL and Starkville, MS. DALBG 1201 outperformed Rebel Exeda in

Knoxville, TN and Dallas, TX, and exhibited statistically similar summer turfgrass quality in Auburn, AL; Starkville, MS and Raleigh, NC. The three-year average across all locations showed that DALBG 1201 performed significantly better than the commercial checks.

Based on the average air temperature and precipitation data for the months of June through September each year (2010 to 2012), Dallas, TX had the warmest average temperature, followed by Starkville, MS; Auburn, AL; Raleigh, NC and Knoxville, TN (Table A-1). The Dallas, TX location also had the least amount of precipitation over the three years, followed by Auburn, AL; Raleigh, NC; Knoxville, TN and Starkville, MS (Table A-2). Significantly superior performance in Auburn, AL and Dallas, TX, two of the warmest and driest locations, suggested that DALBG 1201 has improved summer stress tolerance. Improved summer performance of DALBG 1201 is likely due to it being an F_1 hybrid resulting from a cross between locally adapted ecotypes of Texas bluegrass and Kentucky bluegrass collected from Texas.

Table 3-2. Mean summer turfgrass quality ratings for DALBG 1201 and three commercial checks for each test location.

	Mean Summer Turfgrass Quality at Test Locations†					
Entry	Auburn, AL	Starkville, MS	Raleigh, NC	Knoxville, TN	Dallas, TX	AVG‡
DALBG 1201	6.7a*	5.0a	6.5a	8.6a	5.1a	6.1a
Rebel Exeda§	6.4a	5.0a	7.1a	8.0b	4.0b	5.9a
Reveille§	5.7b	4.7a	5.1b	8.2b	4.5ab	5.3b
Thermal Blue Blaze§	6.1a	4.3a	5.1b	8.1b	3.1c	5.0b
Fisher's LSD¶0.05	0.6	0.7	0.7	0.3	0.8	0.3
CV#, %	10.1	12.4	11.8	2.1	19.4	10.8

* Significant at a probability level of 0.05.
† Summer turfgrass quality ratings were on a 1-9 scale; 1=poor, 9=ideal, 5 was the minimum acceptable. Mean represented turfgrass quality data for Auburn, AL (2010, 2011, 2012), Dallas, TX (2010, 2011, 2012), Starkville, MS (2010, 2011), Raleigh, NC (2010, 2011, 2012), and Knoxville, TN (2010, 2011) from the months of June through September. Auburn, AL data from 2010 and 2012 only included the months of June through August. Dallas, TX data from 2010 only included the months of August and September.
‡ Average turfgrass quality of DALBG 1201 and three commercial checks across all locations from 2010 to 2012.
§ Commercial checks included were Rebel Exeda tall fescue, and Reveille and Thermal Blue Blaze hybrid bluegrasses.
¶ Fisher's LSD values were calculated at a probability level of 0.05.
# CV (Coefficient of Variation) indicated the percent variation of the mean in each column.

Seasonal color – DALBG 1201 was in the top statistical group across all five test locations for seasonal color (Table 3-3). With an average color rating of 7.3, DALBG 1201 was statistically darker green than Reveille (average rating of 6.2) and Thermal Blue Blaze (average rating of 6.1) across all five test locations. In comparison to Rebel Exeda, the color rating of DALBG 1201 was in the same statistical group at four out of five test locations except at Auburn, AL, where DALBG 1201 was statistically darker green.

Shoot density – Shoot density data were collected at four locations (Table 3-3). Mean shoot density of DALBG 1201 was significantly higher than Reveille and Thermal Blue Blaze in Raleigh, NC and Dallas, TX. DALBG 1201 was statistically similar to Rebel Exeda in all locations. On average, the shoot density rating of DALBG 1201 (7.1) was significantly higher than that of Reveille (6.1) and Thermal Blue Blaze (5.6), and comparable to Rebel Exeda (6.8).

Leaf texture – With an average leaf texture rating of 6.5, DALBG 1201 had a significantly finer leaf texture than Rebel Exeda (4.0) across all test locations (Table 3-4). The leaf texture rating for DALBG 1201 was in the same statistical group as Reveille at all five test locations. In comparison to Thermal Blue Blaze, the leaf texture rating for DALBG 1201 was in the same statistical group at four locations except Dallas, TX; where, DALBG 1201 had a significantly finer leaf texture (5.7). In conclusion, the leaf texture of DALBG 1201 is comparable to the commercially available hybrid bluegrass varieties included in the study, but significantly finer than the tall fescue check.

Table 3-3. Mean seasonal color and shoot density for DALBG 1201 and three commercial checks for each test location.

	Mean Seasonal Color at Test Locations[†]						Mean Shoot Density at Test Locations[‡]				
Entry	Auburn, AL	Starkville, MS	Raleigh, NC	Knoxville, TN	Dallas, TX	AVG[§]	Auburn, AL	Starkville, MS	Raleigh, NC	Dallas, TX	AVG[§]
DALBG 1201	8.1a[*]	6.5a	7.2a	7.5a	7.0a	7.3a	3.7a	8.5a	8.2a	6.7a	7.1a
Rebel Exeda[¶]	7.2b	6.8a	7.3a	7.3ab	7.0a	7.1a	3.0a	8.5a	8.2a	5.9ab	6.8a
Reveille[¶]	6.4c	6.0b	6.1b	6.5c	6.1b	6.2b	2.7a	8.3a	6.2b	5.7b	6.1b
Thermal Blue Blaze[¶]	6.2c	5.9b	6.0b	6.8bc	5.7b	6.1b	2.3a	8.5a	5.8b	4.6c	5.6c
Fisher's LSD[#] 0.05	0.6	0.4	0.5	0.5	0.5	0.3	1.3	0.9	1.1	0.8	0.5
CV††, %	11.3	6.6	9.2	7.5	10.5	9.3	25.0	9.6	13.9	13.9	13.5

* Significant at the 0.05 probability level.
† Seasonal color was taken on a scale of 1-9; 1=straw brown, 9=dark green. Mean represented color data from 2010 to 2012 at each location. Starkville, MS data was presented for 2010 and 2011.
‡ Shoot density was taken on a scale of 1-9; 9=maximum density. Mean represented density data from Auburn, AL (2010), Dallas, TX (2010, 2011, 2012), Starkville, MS (2010, 2011), and Raleigh, NC (2011, 2012).
§ Average seasonal color and shoot density across all locations from 2010 to 2012.
¶ Commercial checks included are Rebel Exeda tall fescue, and Reveille and Thermal Blue Blaze hybrid bluegrasses.
# Fisher's LSD values were calculated at a probability level of 0.05.
†† CV (Coefficient of Variation) indicated the percent variation of the mean in each column.

Table 3-4. Mean leaf texture for DALBG 1201 and three commercial checks at each test location.

	Mean Leaf Texture at Test Locations[†]					
Entry	**Auburn, AL**	**Starkville, MS**	**Raleigh, NC**	**Knoxville, TN**	**Dallas, TX**	**AVG[‡]**
DALBG 1201	6.2a[*]	5.8a	7.0a	8.7a	5.7a	6.5a
Rebel Exeda[§]	3.0b	4.3b	5.0b	7.0b	2.3c	4.0b
Reveille[§]	6.0a	5.5a	7.0a	8.3a	5.7a	6.3a
Thermal Blue Blaze[§]	6.6a	5.8a	7.0a	8.3a	4.0b	6.3a
Fisher's LSD[¶]0.05	0.9	0.6	0.3	1.3	0.9	0.4
CV[#], %	16.2	9.3	13.0	8.8	11.3	11.6

* Significant at the 0.05 probability level.

† Leaf texture was taken on a scale of 1-9; 1=coarse, 9= fine. Means represented data from Auburn, AL (2010, 2011, 2012), Dallas, TX (2012), Starkville, MS (2010, 2011), Raleigh, NC (2012), and Knoxville, TN (2011).

‡ Average leaf texture across all locations from 2010 to 2012.

§ Commercial checks included are Rebel Exeda tall fescue, and Reveille and Thermal Blue Blaze hybrid bluegrasses.

¶ Fisher's LSD values were calculated at a probability level of 0.05.

CV (Coefficient of Variation) indicated the percent variation of the mean in each column.

Disease damage – Rust (*Puccinia* spp.) was only observed in Auburn, AL during the months of May (2010) and April (2011), and Raleigh, NC during the months of April and June (2012). DALBG 1201 and commercial checks exhibited no disease damage in 2010 and 2011 in Auburn, AL. In Raleigh, NC, no rust was observed on Rebel Exeda. DALBG 1201 (7%), Thermal Blue Blaze (7%), and Reveille (5%) showed minimal rust damage in Raleigh, NC (data not shown).

Genotype x environment stability analysis – Stability was defined as consistently superior performance of a genotype across varying environments (poorest to best) based on the performance of traits under consideration. Superior performance in the poorest environments and higher levels of stability (lower slope coefficient) indicates high stress tolerance and wide adaption of a genotype across environments, traits that are highly desirable when breeding improved stress tolerant cultivars.

Stability analysis of turfgrass quality indicated that Dallas, TX represented the poorest environment and Knoxville, TN was the best. Turfgrass quality ratings for DALBG 1201 were not only higher in Dallas, TX relative to all three commercial checks (Fig. 3-1), but it also had the lowest slope coefficient of 0.698, indicating the highest stability across locations.

For shoot density, DALBG 1201 outperformed all three commercial checks under poorest environments (Auburn, AL) indicating its potential for stress tolerance, and had the second highest stability across locations next to Rebel Exeda as indicated by the slope coefficient of 1.064 (Fig. 3-2). For seasonal color, although DALBG 1201 had highest cultivar mean across all five locations and its slope indicated reasonable stability

(Fig. 3-3) its coefficient of determination (R^2) was low suggesting that the linear regression model did not fit the data. Non linearity has been shown to be an important tool in analyzing genotype x environment interactions in other crops and systems (Yang, 2014) and it appears that DALBG 1201 is following a non-linear regression for the color response. For leaf texture, all three hybrid bluegrasses (DALBG 1201, Reveille and Thermal Blue Blaze) out-performed Rebel Exeda when evaluated at the location with the poorest environmental conditions (Dallas, TX). DALBG 1201 had the second lowest slope coefficient of 0.967 next to Reveille (b = 0.957) and was more stable across locations relative to Thermal Blue Blaze and Rebel Exeda (Fig. 3-4).

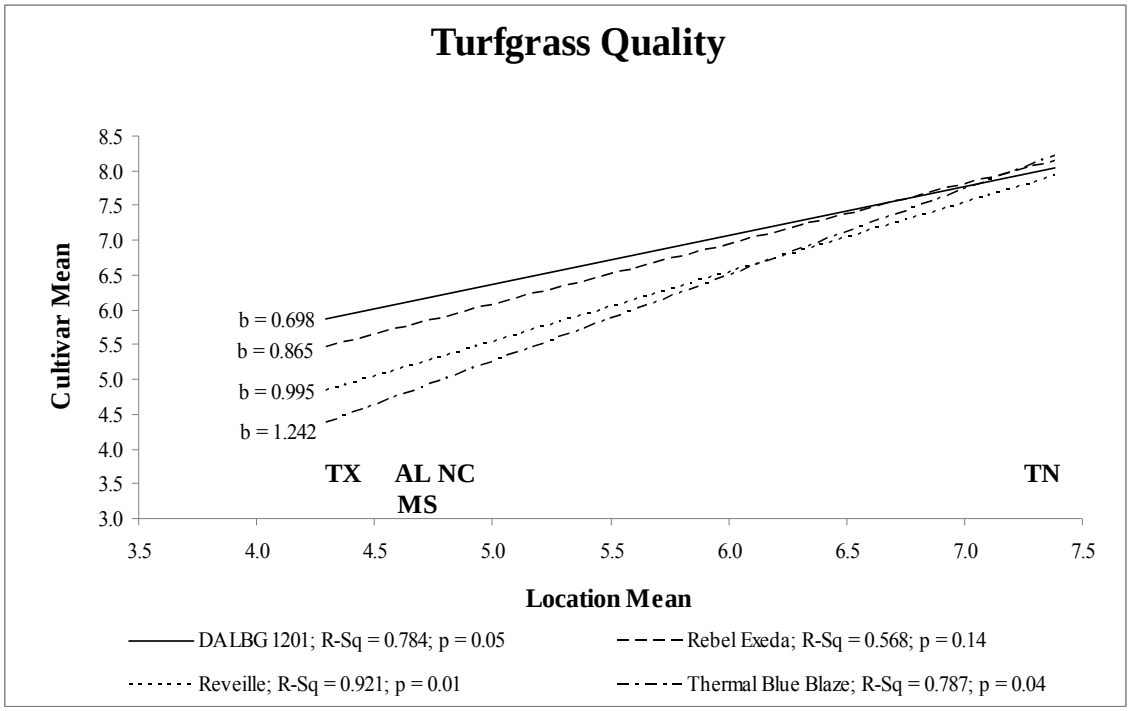


Fig. 3-1. Genotype x environment stability analysis of DALBG 1201 and the three commercial checks Rebel Exeda, Reveille, and Thermal Blue Blaze for turfgrass quality.

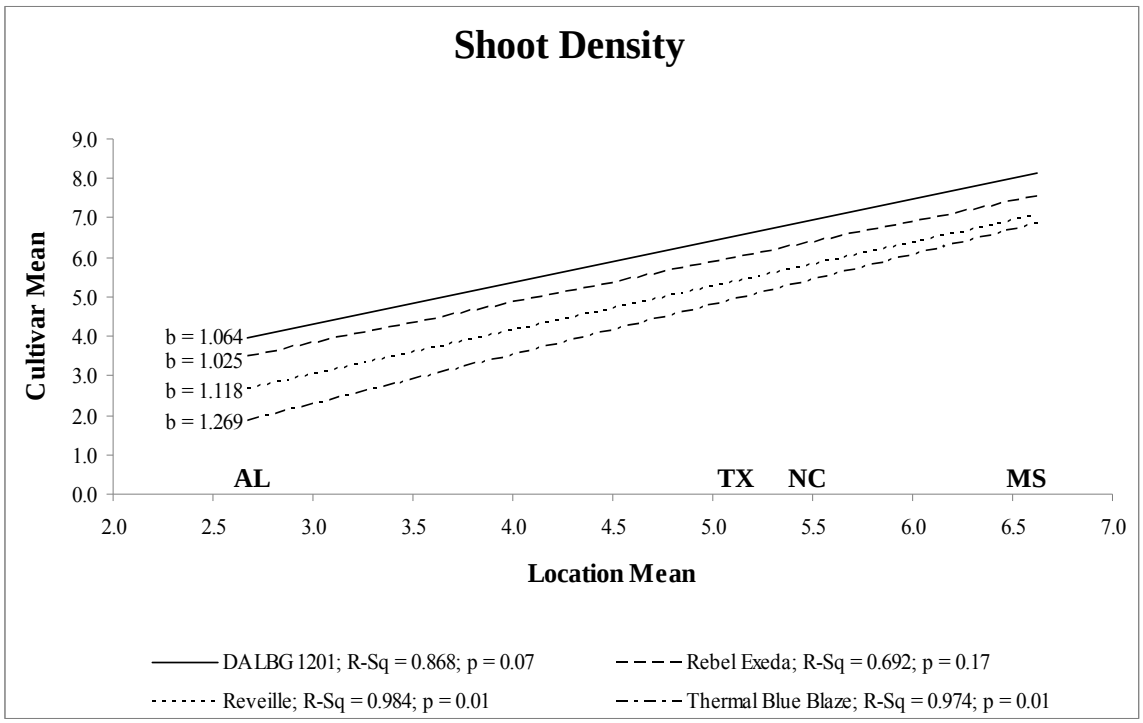


Fig. 3-2. Genotype x environment stability analysis of DALBG 1201 and the three commercial checks Rebel Exeda, Reveille, and Thermal Blue Blaze for shoot density.

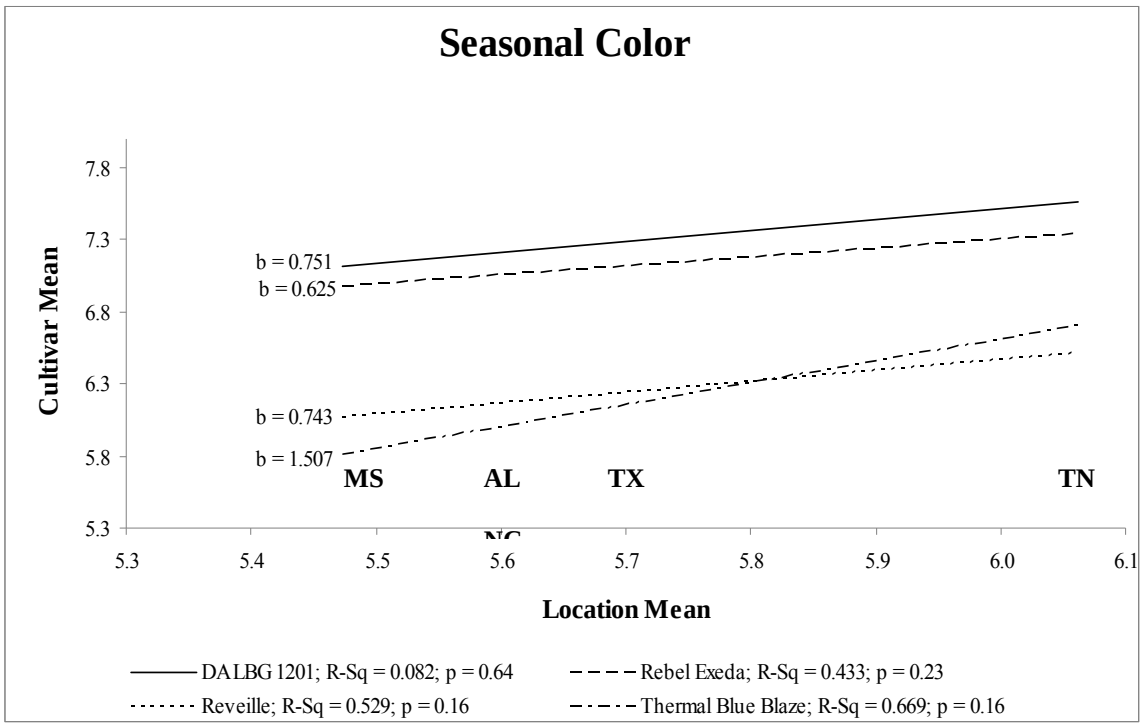


Fig. 3-3. Genotype x environment stability analysis of DALBG 1201 and the three commercial checks Rebel Exeda, Reveille, and Thermal Blue Blaze for seasonal color.

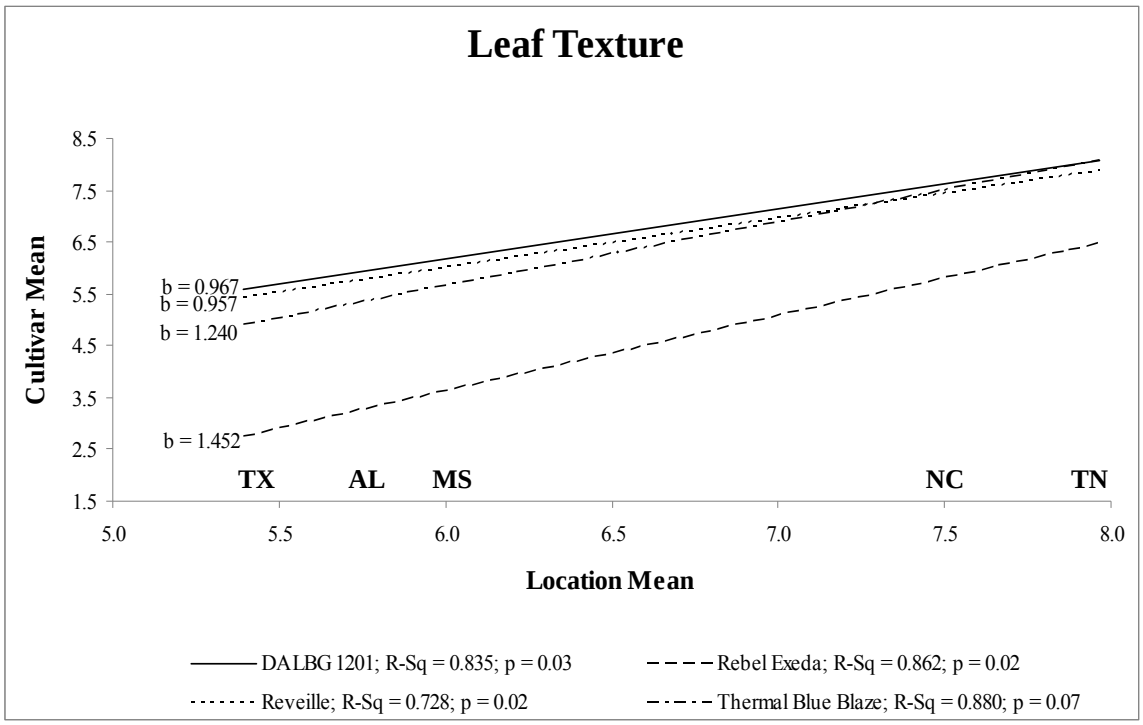


Fig. 3-4. Genotype x environment stability analysis of DALBG 1201 and the three commercial checks Rebel Exeda, Reveille, and Thermal Blue Blaze for leaf texture.

Conclusions

DALBG 1201 is a cool-season grass recommended for use in home lawns, landscape, and other recreational sites across the southern United States. It is an F_1 hybrid bluegrass with exceptional turfgrass quality, dark green color, high shoot density, medium-fine leaf texture and ability to persist under a range of environmental stresses typically encountered in the southern United States. Regressed stability analysis for turfgrass quality indicated that DALBG 1201 is more stable than all three commercial cultivars it was compared to across the five test locations.

CHAPTER IV

THE APPLICATION OF FLOW CYTOMETRY AND A THIOREDOXIN-LIKE NUCLEAR GENE FOR BREEDING TEXAS X KENTUCKY BLUEGRASS HYBRIDS

Overview

Interspecific hybridization between *Poa arachnifera* and *Poa pratensis* has shown to be a proven method to develop turf-type hybrid bluegrass cultivars for the southern United States. Unlike in *P. pratensis*, the use of DNA markers for molecular characterization and flow cytometry to determine nuclear DNA contents have not been extensively utilized in *P. arachnifera*. In the present study, 2C nuclear DNA content of 19 different genotypes of *P. arachnifera* (both males and females) were determined to range from 8.02 to 13.24 pg/2C using flow cytometry. The DNA content of the interspecific hybrids between *P. arachnifera* and *P. pratensis* from two different pedigrees were found to be intermediate between their two parents. Furthermore, we demonstrate the utility of the *trx* (thioredoxin-like) nuclear gene in the identification of interspecific hybrids between *P. arachnifera* and *P. pratensis* using sequence and phylogenetic analyses. A newly discovered 851 bp *trx* allele might serve as a useful marker to differentiate *P. arachnifera* from *P. pratensis*, and provide insight into the evolutionary origin of *P. arachnifera*.

Introduction

Poa pratensis L. (Kentucky bluegrass) is a popular cool-season turfgrass species that exhibits high turfgrass quality characteristics but generally lacks the heat and

drought tolerance limiting its widespread use for residential lawns and sports turf in the southern United States (Hall, 1996). *Poa arachnifera* Torr. (Texas bluegrass) is a native grass exhibiting heat and drought tolerance, but lacks the density, color and texture desirable for turfgrass applications. Interspecific hybridization between *P. arachnifera* and *P. pratensis* has shown to be a proven method to develop heat and drought tolerant turf-type cultivars of hybrid bluegrass ['Reveille' (Read et al. 1999), 'Boutique' (Meyer et al., 2005), 'Longhorn' (Rose-Fricker et al., 2007), 'Thermal Blue' (Hardison et al., 2008), 'Bandera' (Smith et al., 2008), 'Spitfire' (Smith and Meyer, 2009)].

The genus *Poa* is a member of the grass family *Poaceae* and contains both polyploids and aneuploids which reproduce either sexually or via apomixis (Clausen, 1961). *P. arachnifera* is a sexually reproducing species that is primarily dioecious (Read, 1988). *P. pratensis*, on the other hand, is a facultative apomict (Huff and Bara, 1993) which requires pollination to produce endosperm but the unreduced apospory embryo sac is developed from somatic tissue (nucellar region). Fertilization of the meiotically derived embryo sac with a reduced egg is known to occur in *P. pratensis* but at a low frequency (Akerberg, 1939; Kiellander, 1942; Barcaccia et al., 1997). Therefore, the likelihood of generating hybrids between these two species is higher when *P. arachnifera* is used as the maternal parent.

The base chromosome number for the genus *Poa* is seven. *P. arachnifera* is most commonly known as an octoploid (2n = 8x=56) (Hartung, 1946; Patterson et al., 2005), although chromosome numbers can range from 2n = 42 - 91 (Brown, 1939; Gould, 1958; Kelley et al., 2009). *P. pratensis* is an aneuploid exhibiting a wide range of

chromosomes (2n = 24 – 124) (Love and Love, 1975). Flow cytometry is a relatively rapid method used to estimate ploidy by measuring the 2C nuclear DNA content of leaf cell nuclei (Arumuganathan and Earle, 1991; Eaton et al., 2004). To our knowledge, information on the DNA content for *P. arachnifera* and interspecific hybrids between *P. arachnifera* and *P. pratensis* have not been reported in the literature.

Molecular markers serve as a useful tool to characterize species and identify interspecific hybrids. In *P. pratensis*, the utility of Random Amplified Polymorphic DNA (RAPD) (Huff and Bara, 1993; Barcaccia et al., 1997), Sequence Characterized Amplified Region (SCAR) (Albertini et al., 2001) and Simple Sequence Repeat (SSR) (Honig et al., 2010) have been demonstrated. Published marker systems for *P. arachnifera* include Amplified Fragment Length Polymorphisms (AFLP) (Renganayaki et al., 2001) and SSR markers (Kindiger et al., 2011; Kindiger et al., 2013). Inter Simple Sequence Repeats (ISSR) (Goldman, 2008) and SCARs (Abraham et al., 2005) have been reported for the identification of *P. arachnifera* x *P. pratensis* hybrids.

Low-copy number nuclear genes with polymorphic intron regions possessing evolutionary changes such as point mutations and insertions/deletions ("indels") have shown to demonstrate genetic relatedness among species (Small et al., 1998). One such nuclear gene investigated to understand the relatedness of different *Poa* species is the *h* class thioredoxin-like (*trx*) gene (Patterson et al., 2005). Although, thioredoxins within the *h* class have diverged over time to serve multiple plant functions including oxidative stress avoidance during germination (Besse and Buchanan, 1997; Wong et al., 2002; Serrato and Cejudo, 2003; Vieira Dos Santos and Rey, 2006), cellular development

(Juttner et al., 2000), and self-incompatibility (Zhang et al., 2004), but for the most part, their true functions remain unclear. The role of thioredoxins in higher plants such as *Arabidopsis thaliana* and *Populus* spp. has already been extensively reviewed (Gelhaye et al., 2004), but has not been fully investigated in *Poa*. Patterson et al. (2005) reported the *trx* nuclear gene sequences from one accession of *P. arachnifera* and two *P. pratensis* cultivars, however, the usefulness of *trx* as a genetic marker for the identification and verification of *P. arachnifera* x *P. pratensis* hybrids was not tested. The goal of the present study was to 1) determine the nuclear DNA content of male and female genotypes of *P. arachnifera*, and 2) evaluate the usefulness of flow cytometry and the *trx* nuclear gene in the identification of *P. arachnifera* x *P. pratensis* interspecific hybrids.

Materials and Methods

Flow cytometry analysis

Two independent flow cytometry experiments (November 27, 2013 and January 8, 2014) were conducted to determine the 2C DNA content of nine male (TAES 5679, 5680, 5682, 5683, 6032, 6033, 6036, 6037, 6038) and 10 female (TAES 5987, 5990, 6012, 6019, 6020, 6021, 6027, 6029, 6030, 6031) ecotypes of *P. arachnifera* from the Texas A&M AgriLife Research germplasm collection, three *P. pratensis* ecotypes or cultivars (CS#4, 'Huntsville,' and 'Kenblue'), and five *P. arachnifera* x *P. pratenis* hybrids (Reveille, 'DALBG 1201', TAES 5654, 5655, and 5656). These hybrids belonged to two different pedigrees; pedigree #1 comprised of Reveille, a hybrid between TAES 6012 and Huntsville, and pedigree #2 comprised of DALBG 1201

(Meeks et al., 2015), TAES 5654, 5655, and 5656 which are hybrids between TAES 6012 and CS#4. Each entry was replicated four times in both experiment except, TAES 6012, 5990, DALBG 1201, Reveille and Huntsville which were replicated eight times in experiment 2. Tukey's honestly significant difference (HSD) test was used for genotype means separation at a significance level of $P < 0.05$.

Approximately 50mg of young leaf tissue was collected from plants maintained in greenhouse at 24°C/15°C, and analyzed according to a modified method of Arumuganathan and Earle (1991) at the Flow Cytometry and Imaging Core Laboratory (Virginia Mason Research Center, Seattle, WA). Nuclei suspensions were prepared by chopping plant tissue in a 0.5 mL solution containing 10 mM $MgSO_4.7H_2O$, 50mM KCl, 5 mM Hepes, pH 8.0, 3 mM dithiothreitol, 0.1 mg / mL propidium iodide, 1.5 mg / mL DNAse free RNAse (Rhoche, Indianapolis, IN) and 0.25% Triton X-100. The suspended nuclei were withdrawn using a pipette, filtered through 30-µm nylon mesh, and incubated at 37°C for 30 min. A suspension of chicken red blood cells (CRBC) was added to each sample as an internal standard (2.33 pg/2C). Fluorescence intensities of the stained nuclei were measured by a FACScalibur flow cytometer (Becton-Dickinson, San Jose, CA). The propidium iodide fluorescence area signals (FL2-A) from 1000 nuclei were collected and analyzed by CellQuest software (Becton-Dickinson, San Jose, CA) on a Macintosh computer. The sample nuclear DNA content was determined by dividing the mean position of the G0/G1 (nuclei) sample peak by the mean position of the CRBC and multiplying by 2.33 pg/2C (CRBC DNA content).

trx gene amplification and cloning

Genomic DNA was extracted from young leaf tissue of 51 *P. arachnifera* genotypes using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA.). These 51 *P. arachnifera* genotypes include, 11 plants of PI 655089 (W6 17724; Germplasm Resources Information Network) used in Patterson et al. (2005), and 40 *P. arachnifera* ecotypes: 20 (10 female and 10 male) from Texas A&M AgriLife Research germplasm collection in Dallas, TX and 20 (including 3 known females and 1 male) from the USDA-ARS germplasm collection in Woodward, OK. Genomic DNA was also extracted from 25 *P. arachnifera* x *P. pratensis* experimental hybrids, and 26 *P. pratensis* genotypes belonging to 11 different morphological classes (Aggressive, Bellevue, BVMG, Compact, Compact America, Compact Midnight, CELA, Cheri, Common, Julia, and Mid-Atlantic) (Brilman, 2009).

The primer pair sequence for the *trx* gene and the thermal cycling conditions were obtained from Patterson et al. (2005). PCR reactions were in 25 ul volumes of distilled water, 1x GoTaq green buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.15 uM of each primer, 1 unit/ul GoTaq Flexi DNA polymerase (Promega, Madison, WI). At least 60 ng of template DNA was used in each PCR reaction. PCR amplification was visualized on 1.5% superfine agarose gels (Amresco, Solon, OH) stained with ethidium bromide.

Cloning and sequencing of *trx* amplicons was performed on six *P. arachnifera* (TAES 6012, 5684, 5990, 6027, 6029, and D4 Isolate #8), two *P. pratensis* (Huntsville, CS#4) and three *P. arachnifera* x *P. pratensis* hybrids (Reveille, DALBG 1201 and TAES 5654). A 1:10 dilution was made in distilled water for each PCR reaction. A total

of 2 µl of the diluted PCR reaction was added to cloning reaction mixtures using the Strataclone PCR cloning kit (Agilent Technologies, Santa Clara, CA), and 1 µl of the PCR reaction was added to Strataclone SoloPak Competent Cells (Agilent Technologies, Santa Clara, CA). Cells were incubated for 2 h prior to plating 105 µl on LB agar amended with 10 µl/ml ampicillin (Teknova, Hollister, CA) and 10 µl/ml x-gal (Amresco, Solon, OH). Colonies for each genotype were streak plated and incubated overnight at 37°C. Singular colonies of cells collected with toothpicks were directly dabbed into new 25 µl *trx* PCR reactions. Each colony containing the desired fragment was incubated in LB broth with 1 µl/ml ampicillin overnight at 37°C for 16-18 h. Samples were prepared using a StrataPrep Plasmid Miniprep Kit (Agilent Technologies, Santa Clara, CA) and digested with EcoR1 (New England Biolabs, Ipswich, MA.) for 2 h. Positive clones were sequenced with the T3 primer by Utah State University Center for Integrated Biosystems (Logan, UT) with a 3730 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA).

Sequence and phylogenetic analyses

A total of 77 new sequences were generated from the present study. Thirty-two of these sequences belonging to *P. arachnifera, P. pratensis* and *P. arachnifera* x *P. pratensis* hybrids (Table 4-1) were analyzed with 60 *Poa* sequences from Patterson et al. (2005), excluding those from *P. alpina, P. annua*, *P. bulbosa*, and *P. supina*. The phylogenetic analysis of the remaining 45 new sequences belonging to TAES 5684 and D4 Isolate #8 (both with questionable identity based on our PCR and amplification results) was performed with 32 sequences from Table 4-1 as well as the *P. arachnifera*

and *P. pratensis* sequences from Patterson et al. (2005). *Phalaris arundinacea* was used an outgroup. *MEGA* 6 (Tamura et al., 2013) was used to align the sequences with CLUSTALW default settings (Thompson et al., 1994). Maximum Parsimony (MP) analysis was conducted using the nucleotide substitution model, all sites (gaps/missing data), Tree-Bisection-Reconnection (TBR) branch swapping, and 1,000 bootstrap replications. Overall and pairwise genetic distances were computed using the Tamura-Nei model (Tamura and Nei, 1993) with gamma distribution = 1.93, pairwise deletion of gaps/missing data, uniform rates of substitution, and 1,000 bootstrap replications. The dissimilarity genetic distance matrix computed in *MEGA 6* was transformed by NTSYS 2.2 (Rohlf, 2005) with the DCenter module, and used to compute Principal Components Analysis (PCA) with the Eigen module. Eigenvectors with square root (lambda) scaling were plotted by columns with the Mod3D module to generate 3D plot images. The sequence analysis of the 851bp PCR product was conducted using NCBI (Geer et al., 2010), RNAFold webserver to develop a heat map (Hofacker et al., 1994; Zuker and Stiegler, 1981; and McCaskill, 1990) and microRNA database, miRBase (Griffith-Jones et al., 2006).

Table 4-1. Source and GenBank accession numbers for genotypes of P. arachnifera, P. pratensis and their hybrids.

Species	Genotype or cultivar	Source	GenBank accession number (bp)
P. arachnifera			
	PI 655089	Patterson et al (2005)	1.a[†]: AY589248 (667)
	(W6 17724)		1.b: AY589286 (554)
	TAES 6012	Texas A&M AgriLife Research	2.a: KF896992 (686)
			2.b: KF896990 (572)
			2.c: KF896989 (851)
	TAES 5990	Texas A&M AgriLife Research	3.a: KJ511248 (572)
			3.b: KJ511249 (850)
	TAES 6027	Texas A&M AgriLife Research	4.a: KJ511251 (572)
			4.b: KJ511250 (846)
	TAES 6029	Texas A&M AgriLife Research	5.a: KJ511252 (572)
			5.b: KJ511253 (853)
P. pratensis			
	cv. Kenblue	Patterson et al (2005)	1.a[†]: AY589237 (563)
			1.b: AY589252 (672)
			1.c: AY589261 (570)
			1.d: AY589280 (563)
	cv. Coventry	Patterson et al (2005)	2.a: AY589238 (563)
			2.b: AY589250 (652)
			2.c: AY589243 (671)
			2.d: AY589263 (571)
			2.e: AY589279 (563)

Table 4-1. Continued.

Species	Genotype or cultivar	Source	GenBank accession number (bp)
P. pratensis			
	cv. Huntsville	Texas A&M AgriLife Research	3.a: KJ511255 (669)
			3.b: KF896997 (688)
			3.c: KF896996 (582)
	CS#4	Texas A&M AgriLife Research	4.a: KF896993 (669)
			4.b: KJ511254 (688)
			4.c: KF896994 (581)
P. arachnifera x P. pratensis			
	cv. Reveille	Texas A&M AgriLife Research	1.a‡: KF897001 (851)
			1.b: KF897000 (688)
			1.c: KJ511256 (686)
			1.d: KF896998 (669)
			1.e: KJ511257 (581)
			1.f: KF896999 (572)
	cv. DALBG 1201	Texas A&M AgriLife Research	2.a: KF897002 (852)
			2.b: KJ511259 (688)
			2.c: KF897005 (686)
			2.d: KF897003 (669)
			2.e: KF897004 (581)
			2.f: KJ511258 (572)

Table 4-1. Continued.

Species	Genotype or cultivar	Source	GenBank accession number (bp)
P. arachnifera x P. pratensis			
	TAES 5654	Texas A&M AgriLife Research	3.a: KF897006 (851)
			3.b: KJ511261 (688)
			3.c: KJ511260 (686)
			3.d: KF897008 (581)
			3.e: KF897009 (572)

[†]*trx* sequences from *P. arachnifera* and *P. pratensis* are designated following Patterson et al (2005) categorical scheme.
[‡]*P. arachnifera* x *P. pratensis* hybrids are designated in order of their sequence lengths (largest to smallest).

Results

2C nuclear DNA content

Analysis of variance showed no significant differences between the two flow cytometry experiments. Therefore, the data from both experiments were pooled, and mean 2C DNA content across both experiments is presented in Figure 4-1a and 4-1b. The mean nuclear DNA content measured for 19 *P. arachnifera* ecotypes exhibited a wide variation ranging from 8.55 to13.56 pg/2C, including nine male ecotypes exhibiting DNA content from 8.55 to 13.13 pg/2C, and 10 female ecotypes ranging from 8.82 to 13.56 pg/2C (Fig. 4-1a). This observed variation in mean DNA content of *P. arachnifera* separated into three statistical groups. Of the three *P. pratensis* genotypes examined, the DNA content for Kenblue (7.28 pg/2C; Fig. 4-1b) had also been previously reported by Eaton et al. (2004) to be 7.38 ±0.61 pg/2C and Wieners et al. (2006) to be 6.36 pg/2C.

P. arachnifera x *P. pratensis* hybrids exhibited an intermediate DNA content to its parents for each of the two pedigrees; pedigree # 1 = TAES 6012 x Huntsville and pedigree #2 = TAES 6012 x CS#4 (Fig. 4-1b). Pedigree # 1 hybrid, Reveille, had a mean DNA content of 10.62 pg/2C, statistically intermediate to TAES 6012 (12.84 pg/2C) and Huntsville (8.26 pg/2C). Similarly, pedigree # 2 hybrids, 5656, 5655, 5654 and DALBG 1201 showed a range from 9.39 to 10.91 pg/2C which is statistically intermediate to its two parents, TAES 6012 and CS#4 (7.91 pg/2C).

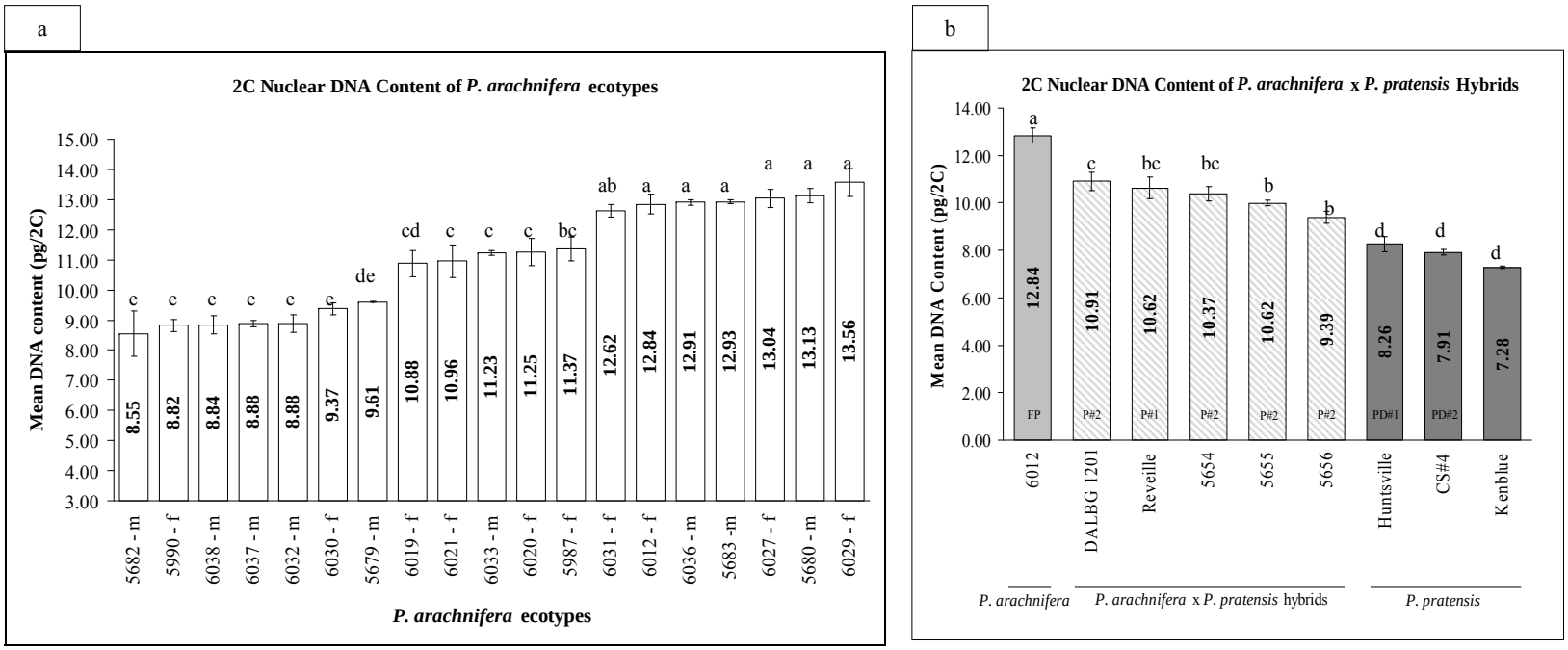


Fig. 4-1. Nuclear DNA content (pg/2C) of 19 *P. arachnifera* ecotypes, and *P. arachnifera* x *P. pratensis* hybrids. (a) Nuclear DNA content of male (m) and female (f) genotypes of *P. arachnifera*. (b) Five *P. arachnifera* x *P. pratensis* hybrids (diagonal patterns) from two different pedigrees (P#1 and P#2) exhibited intermediate DNA content between the female parent (FP), and *P. pratensis* pollen donors Huntsville (PD#1) and CS#4 (PD#2). P#1: TAES 6012 x *P. pratensis* cv. Huntsville = Reveille; P#2: TAES 6012 x *P. pratensis* ecotype CS#4 = DALBG 1201, 5654, 5655, and 5656; *P. pratensis* cv. Kenblue was an internal check. Samples were replicated across two dates. Means were averages of eight replicated samples for each genotype except 6012, DALBG 1201, Reveille, and Huntsville which represented an average of twelve replicated samples. Bars represent ± 1 standard deviation. Tukey's HSD was used for means separation.

trx gene amplification

PCR amplification of the *trx* gene was performed on 51 genotypes of *P. arachnifera* including 11 individuals of PI 655089 (W6 17724), the *P. arachnifera* accession used in Patterson et al. (2005). All 11 individuals of PI 655089 were observed to generate the 572 bp product described by Patterson et al. (2005), but the 686 bp product could not be uniformly visualized (1.5% agarose gel) in all 11 individuals (Fig. 4-2). There were instances when the 686 bp product would be faintly visualized on an agarose gel from a genotype, but later be visually undetectable from the same genotype from another PCR reaction. Therefore, we cannot state that it is not present in all examined *P. arachnifera*, but rather amplification and visualization of the 686 bp product on agarose gels was inconsistent. In addition to these two products (572 bp and 686 bp), a 851 bp product not previously reported was observed in all 11 individuals of PI 655089. PCR amplification of the *trx* gene on the remaining 40 *P. arachnifera* genotypes showed similar banding patterns and the presence of the 851 bp product in 38 out of 40 genotypes. Of the 20 genotypes from the Texas A&M AgriLife germplasm collection, the 851 bp band was present in all 10 females (Fig. 4-3a) and 9 out of 10 male genotypes (Fig. 4-3b). TAES 5684 (lane 5; Fig 4-3b), labeled as a male plant of *P. arachnifera* in the germplasm collection, exhibited a banding pattern similar to *P. pratensis*, and further sequence analysis confirmed it to be a genotype of *P. pratensis* (Fig. C-1). Of the 20 genotypes from the USDA-ARS germplasm collection, the 851 bp band was present in 19 genotypes (Fig. 4-4a and 4-4b). Genotype D4 Isolate # 8 (lane 8; Fig. 4-4a) of *P. arachnifera* showed the absence of the 851 bp band, and further

sequence analysis of *trx* from isolate #8 confirmed it to be a genotype of *P. arachnifera* (Fig. C-2). Furthermore, the PCR amplification of the *trx* gene in 25 cultivars of *P. pratensis* from 11 different morphological classes showed the absence of the 851 bp product (Fig. 4-5a, b, and c). Based on our results we conclude that the newly discovered 851 bp band is present at high frequency (98%; 49 out of 50) in the *P. arachnifera* germplasm examined, and is absent in 100% of the morphologically diverse *P. pratensis* cultivars tested.

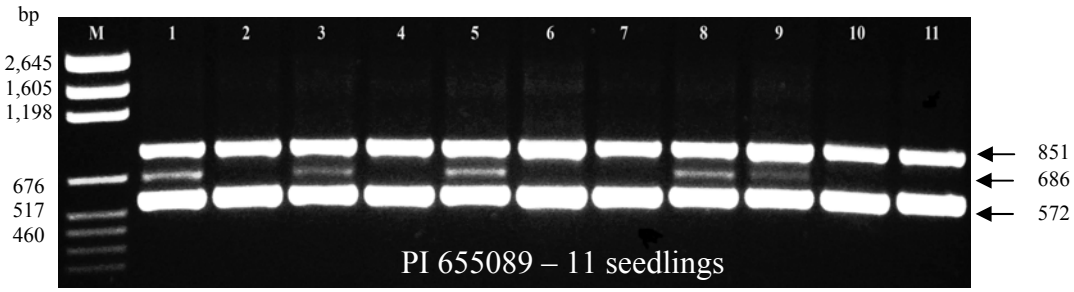


Fig. 4-2. PCR amplification of the trx gene in eleven individuals of PI655089. Arrows point to the three bands observed in *P. arachnifera*. Note: 686bp band was not detected in all *P. arachnifera* genotypes under our PCR conditions. Lane M is a 2.645Kb PGEM DNA marker.

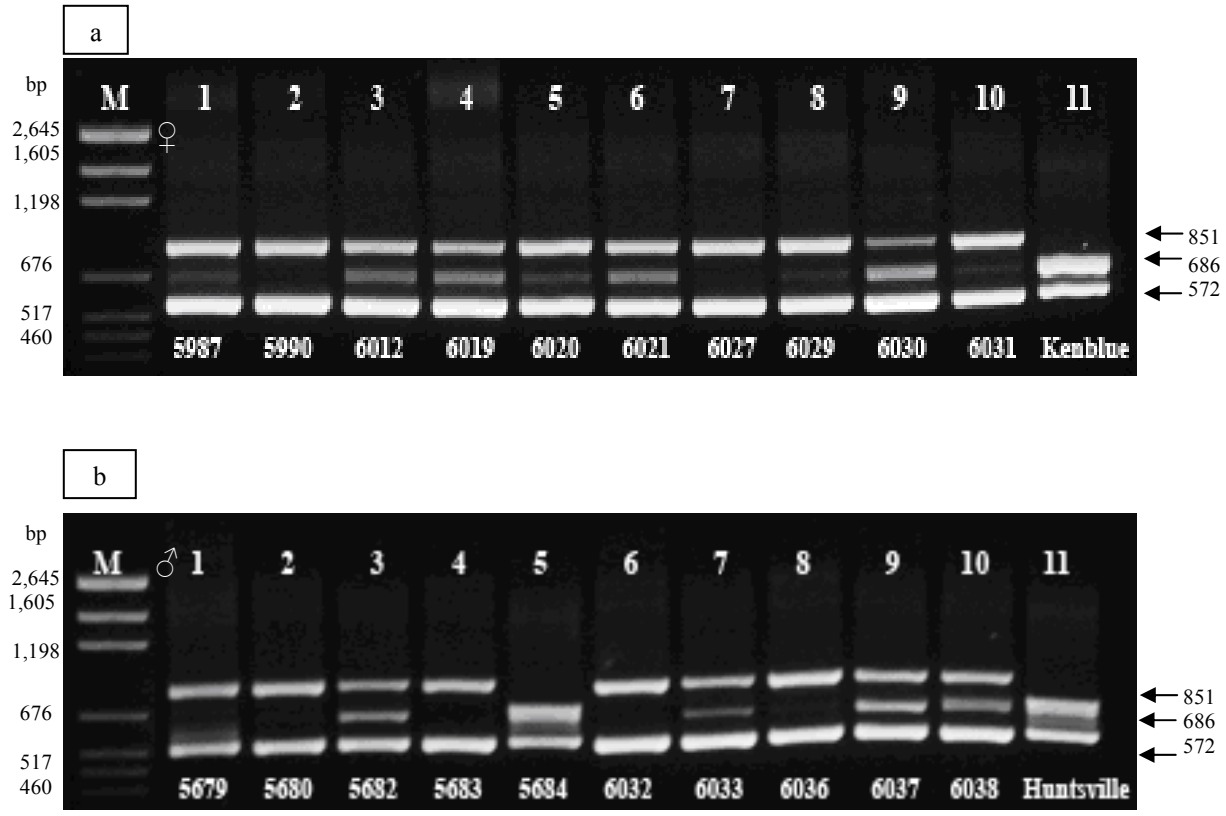


Fig. 4-3. PCR amplification of the *trx* gene on 10 female (a) and 10 male (b) ecotypes of *P. arachnifera* (lanes 1-10) in the germplasm collection at Texas A&M AgriLife Research in Dallas, TX. *P. pratensis* cv. Kenblue and Huntsville were included in the last lanes. Arrows point to the three bands observed in *P. arachnifera*. Note: 686bp band was not detected in all *P. arachnifera* genotypes under our PCR conditions. *Trx* sequence analysis has confirmed 5684 to be a genotype of *P. pratensis.* Lane M is a 2.645Kb PGEM DNA marker.

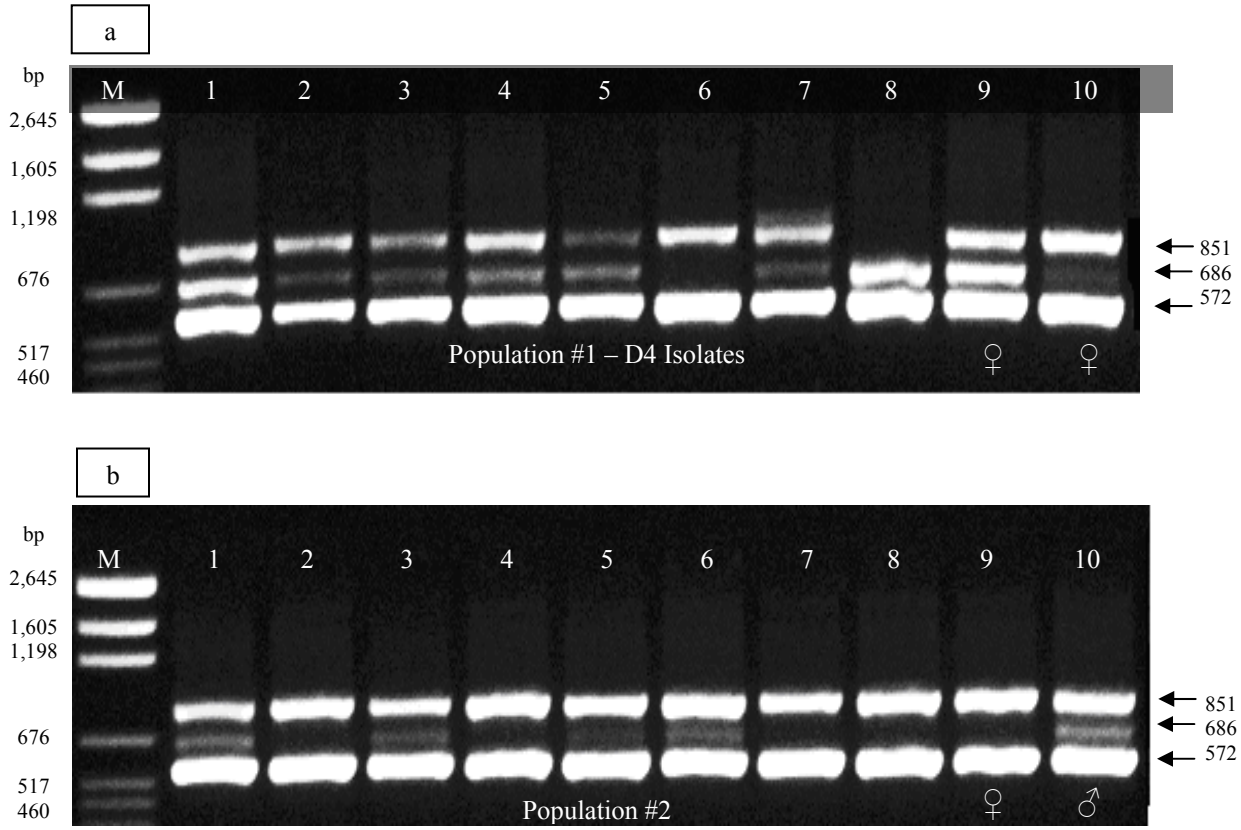


Fig. 4-4. PCR amplification of the *trx* gene on 20 isolates derived by seed from two open-pollinated female ecotypes, of which 3 are known females and 1 is a known male, in the USDA-ARS germplasm collection in Woodward, OK. Arrows point to the three bands observed in *P. arachnifera*. Note: 686bp band was not detected in all *P. arachnifera* genotypes under our PCR conditions. *Trx* sequence analysis has confirmed isolate #8 (a) to be *P. arachnifera*. Lane M is a 2.645Kb PGEM DNA marker.

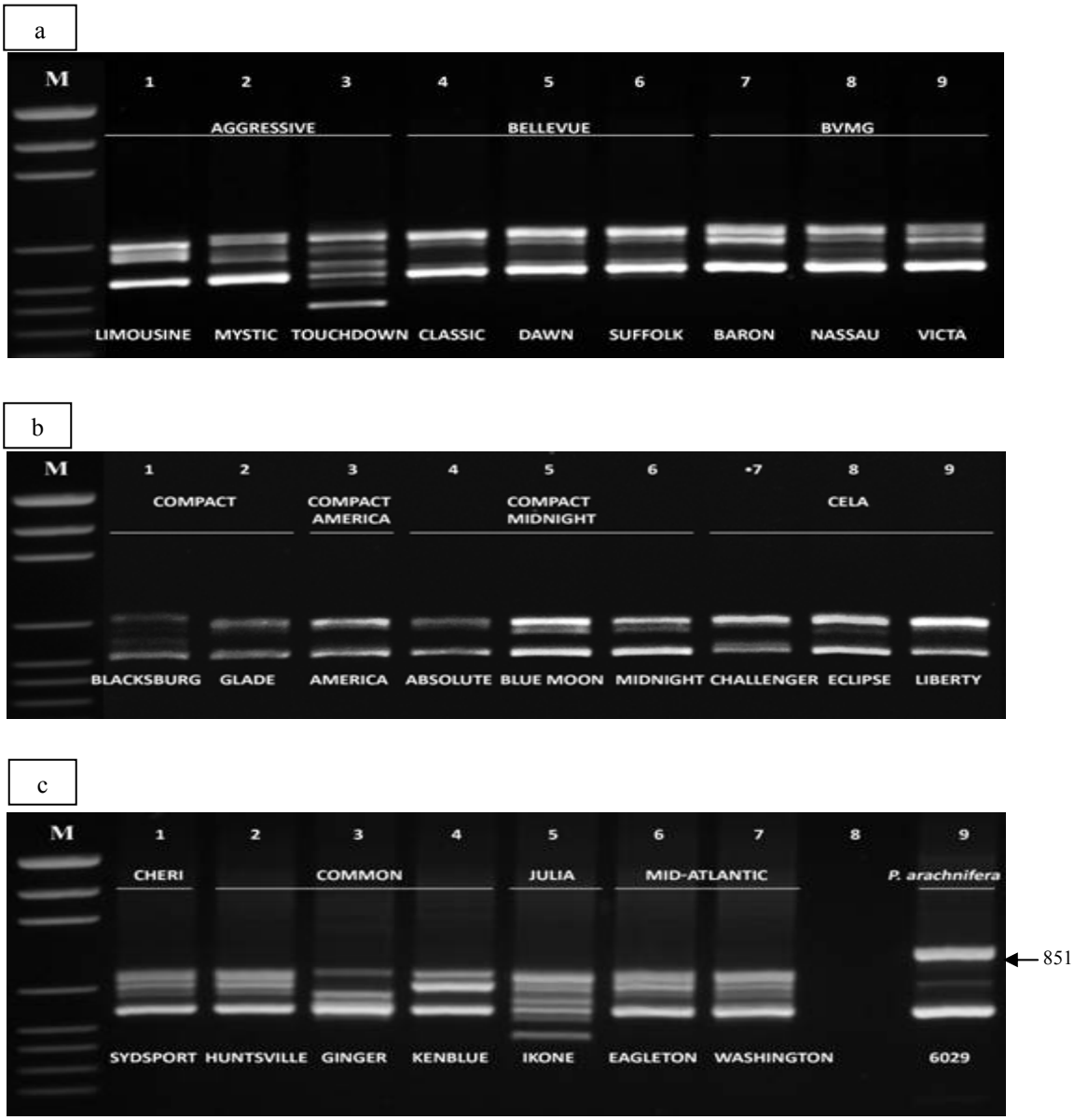


Fig. 4-5. PCR amplification of the trx gene in twenty-five cultivars of *P. pratensis* from eleven morphological classes. a) Nine cultivars for the Aggressive, Bellevue, and BVMG classes. b) Nine cultivars for the Compact, Compact America, Compact Midnight, and CELA classes. c) Seven cultivars for the Cheri, Common, Julia, and Mid-Atlantic classes as well as an unloaded lane (lane 8) and *P. arachnifera* genotype 6029 for comparison. Lane M is a PGEM DNA marker with bands (bp) in a descending order of 2,645, 1,605, 1,198, 676, 517, and 460. The 851 bp allele present in 6029 is absent from all twenty-five *P. pratensis* cultivars.

In order to test the utility of the *trx* gene for the identification of interspecific hybrids between *P. arachnifera* and *P. pratensis*, the gene was amplified from five hybrids belonging to two pedigrees, #1 and #2 (Fig. 4-6). PCR banding patterns were consistent among hybrids from both pedigrees. The unique 851 bp product was found to be present in all hybrids [lane 2 (pedigree #1), and lanes 5-8 (pedigree #2)], and the *P. arachnifera* parent, TAES 6012 (lanes 1 and 4) but was absent in both *P. pratensis* parents (lanes 3 and 9). In addition to these five hybrids, the *trx* gene was also amplified from 20 additional interspecific hybrids from seven different pedigrees and the 851 bp products was found to be present in all 20 hybrids (Fig. 4-7).

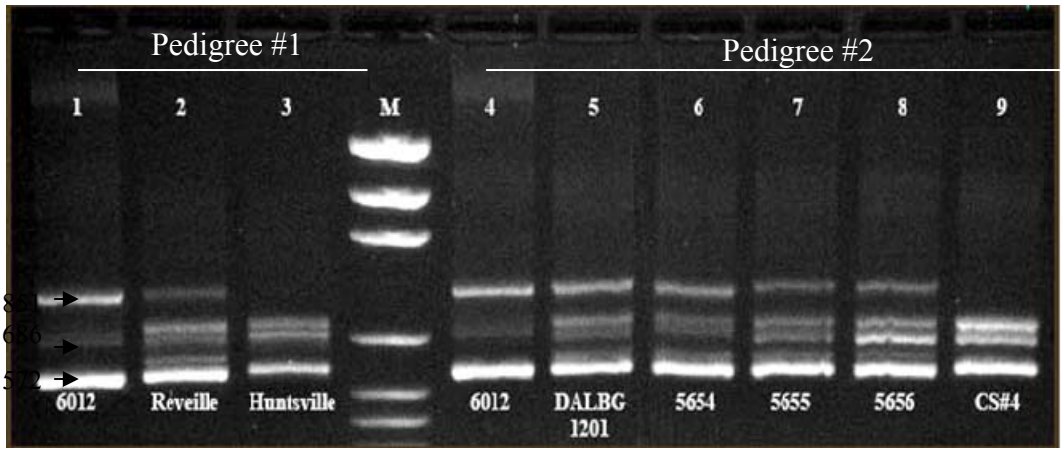


Fig. 4-6. Agarose gel image showing amplification of *trx* genes from two *P. arachnifera* x *P. pratensis* pedigrees. In pedigrees #1 and #2, 6012 (lanes 1 and 4) is the *P. arachnifera* parent (maternal), Huntsville (lane 3) and CS#4 (lane 9) are the *P. pratensis* parents (pollen donors) in pedigree #1 and pedigree #2, respectively; Lanes 2, 5, 6, 7 and 8 represent interspecific hybrids within each respective pedigree.

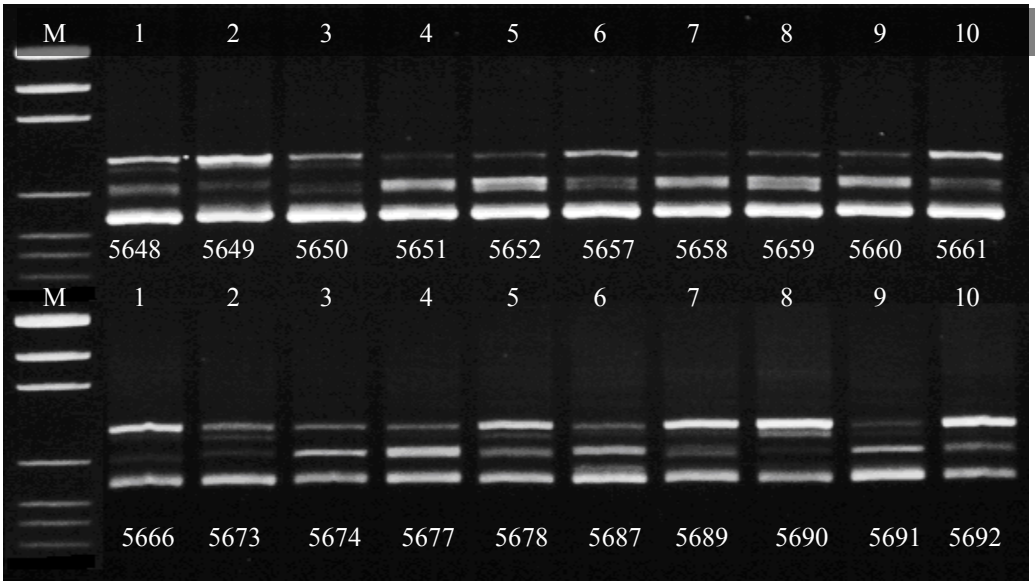


Fig. 4-7. Agarose gel image showing amplification of *trx* genes in 20 additional *P. arachnifera* x *P. pratensis* hybrids (pedigrees not provided). Lane M is a 2.645Kb PGEM DNA marker.

Sequence and phylogenetic analyses

A total of 77 new *trx* sequences were generated. To compare our sequences to those previously reported by Patterson et al. (2005), we performed a multiple sequence alignment with the 32 new sequences (nine from *P. arachnifera*, six from *P. pratensis* and 17 from *P. arachnifera* x *P. pratensis* hybrids) and other *Poa trx* sequences (Patterson et al., 2005) obtained from GenBank to re-construct the most parsimonious phylogenetic tree. The nucleotide sequences were aligned for 938 positions, of which 540 were conserved, 366 were variable with 137 singleton sites and 228 parsimony informative sites. The phylogenetic analysis showed the placement of all 32 new sequences within the well-supported sequence class A and class C (Fig. 4-8), as defined by Patterson et al. (2005). Furthermore, class B and class D *Poa* sequences maintained their topology in our phylogenetic analysis (compressed nodes ■ in Fig. 4-8).

A total of 22 new sequences from TAES 5684 and 23 new sequences from D4 Isolate #8 were aligned separately with 32 above mentioned sequences from this study and 11 sequences belonging to *P. arachnifera* and *P. pratensis* from Patterson et al. (2005). Based on the phylogenetic analysis, TAES 5684 (labelled as *P. arachnifera*) was determined to be a genotype of *P. pratensis* (Fig. C-1) with all 22 sequences clustering with *P. pratensis*. Although the 851 bp band was absent in the amplification of D4 Isolate #8, the phylogenetic analysis confirmed it to be a genetic variant of *P. arachnifera,* and not a genotype of *P. pratensis* (Fig. C-2).

Sequences obtained from the hybrids clustered with the *trx* sequences obtained from *P. pratensis* (denoted by ●) in three distinct groups; one in class A and two in class C (Fig. 4-8; taxa in green). Similarly, other sequences obtained from the same three hybrids clustered with the *trx* sequences obtained from *P. arachnifera* (approximately 572, 686 and 851 bp in length) in three separate clusters; one in class A and two in class C. Class A contained a grouping of *P. arachnifera* sequences approximately 572 bp in length (shown in blue), and class C contained a grouping of sequences approximately 686 bp in length (shown in blue). The new and unique 851 bp sequences obtained from *P. arachnifera* and the hybrids clustered within class C and are shown in red. Sequence similarity and the phylogenetic grouping of the *trx* sequences obtained from three hybrids with sequences obtained from both parental species demonstrate the utility of *trx* gene as a marker for the identification of hybrids between *P. arachnifera* and *P. pratensis*.

Principal components analysis of class A and C sequences showed that 99.37% of the genetic variation was explained by three eigenvectors, the majority of which was explained by eigenvector 1 (88.05%). A three dimensional PCA representation from the genetic distance matrix (Fig. 4-9) revealed that within class A, the two distinct groups (one containing *P. arachnifera* and the hybrid sequences and the other with *P. pratensis* and hybrid sequences, Fig. 4-8) were genetically dissimilar resulting in a larger circumscription of class A as compared to the class C. Furthermore, the new 851 bp sequences (indicated as solid red square symbol, Fig. 4-9) fell well within the circumscription of class C. The placement of 851 bp sequences within class C supports the extension of class C in the phylogenetic analysis (Fig. 4-8) to encompass the new 851 bp sequence as indicated by the dotted bracket.

Characterization of the new 851 bp sequence showed that this intronic region contains a unique 163 bp insertion that is absent in other *Poa* sequences reported by Patterson et al (2005). Without the 163 bp insertion the remaining 688 bp sequence was found to be highly similar to other class C sequences. The BLAST search results for the 163 bp region indicated no sequence similarity with any *Poa* sequences in GenBank. However, we found sequence similarity between the terminal ends of the 163 bp region to a nonautonomous *Stowaway* MITE (miniature-inverted transposable element) (Bureau and Wessler, 1994) found in *Australopyrum velutinum* Nees (mountain wheat grass) (Fig. 4-10). *Stowaway* MITE and Tc1/*mariner*-like elements (MLE) have been shown to share 'TA' target site preference (shaded in Fig. 4-10) and a conserved 10-bp terminal repeat (5′-'CTCCCTCCGT-3′) (boxed region in Fig. 4-10) (Bureau and Wessler, 1994)

suggesting MLEs to be autonomous partners of the *Stowaway* MITEs (Feschotte et al., 2002; Feschotte et al., 2003). Further investigation of the 163 bp region showed that this insertion can fold on to itself to form a hairpin stem loop structure. A heat map of the 163 bp insertion region was developed using RNAFold showing a minimum free energy of -60.10 kcal/mol and high base-pair probabilities of the stem loop (Fig. 4-11a; red region). Hairpin stem loop structures found in RNAfold are a common MITE characteristic (Bureau and Wessler, 1994) which can be processed into smaller fragments, or microRNAs, known to regulate gene function (Piriyapongsa and Jordan, 2007 and 2008; Kuang et al., 2009). To further characterize this insertion region, miRBase detected sequence similarity of the stem loop structure of the insertion region with mi5568 (*Sorghum bicolor*), mi1120 and mi1130 (*Triticum aestivum* and *Hordeum vulgare*), mi5825 (*Oryzae sativa*), mi5174 (*Brachypodium distachyon*) (Fig. 4-11b), suggesting a potential role as a precursor to a micro RNA.

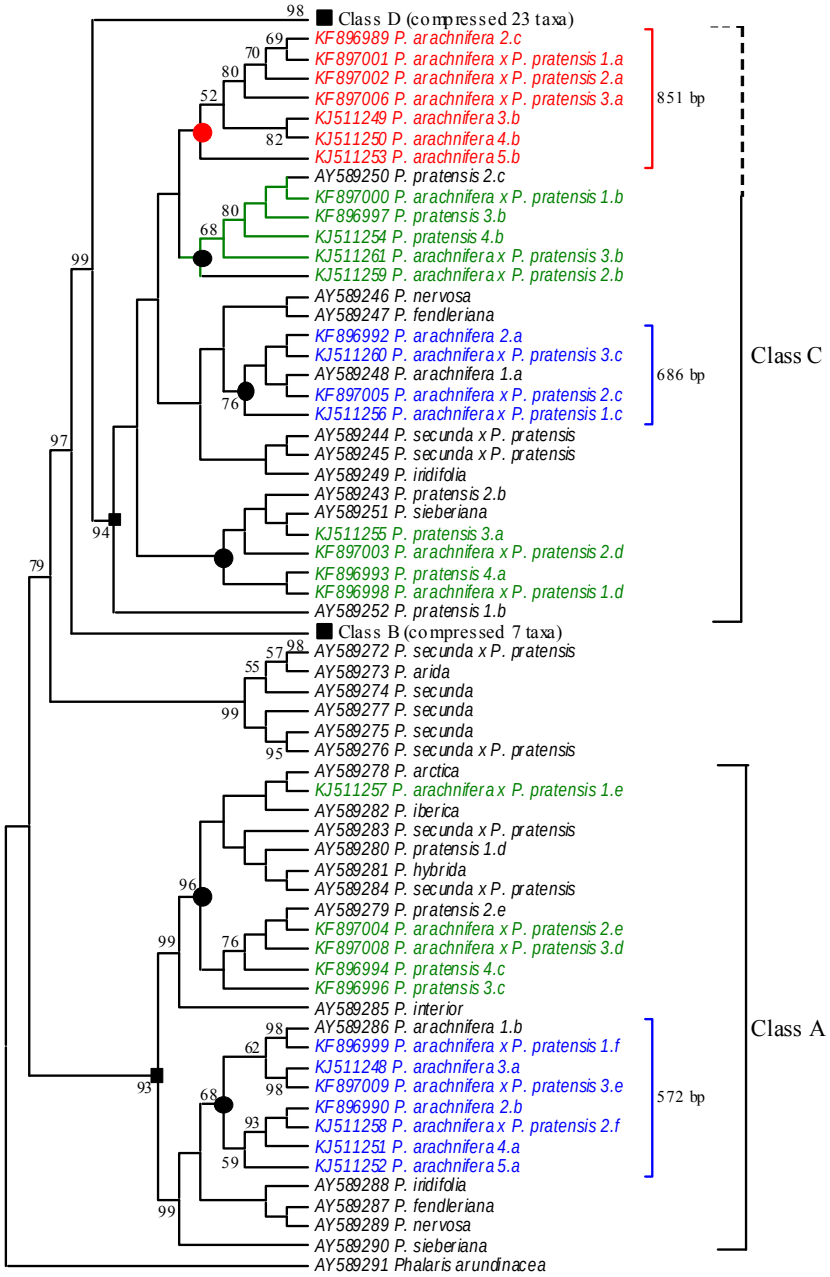


Fig. 4-8. The most parsimonious tree produced for the *trx* sequences using Mega 6.0. The tree is rooted to *Phalaris arundinacea*. Clusters (●) in blue indicate new sequences from *P. arachnifera* and the hybrids, and clusters in green indicate new sequences from *P. pratensis* and the hybrids. Red cluster (●) shows the new 851 bp *P. arachnifera* sequence group containing the 163 bp insertion. Nodes shown as ■ represent four classes as reported in Patterson et al. (2005). The numerical values followed by the lower case alphabetical letter correspond to GenBank accession numbers in Table 4-1. Numbers above or below the tree branch indicate the bootstrap values.

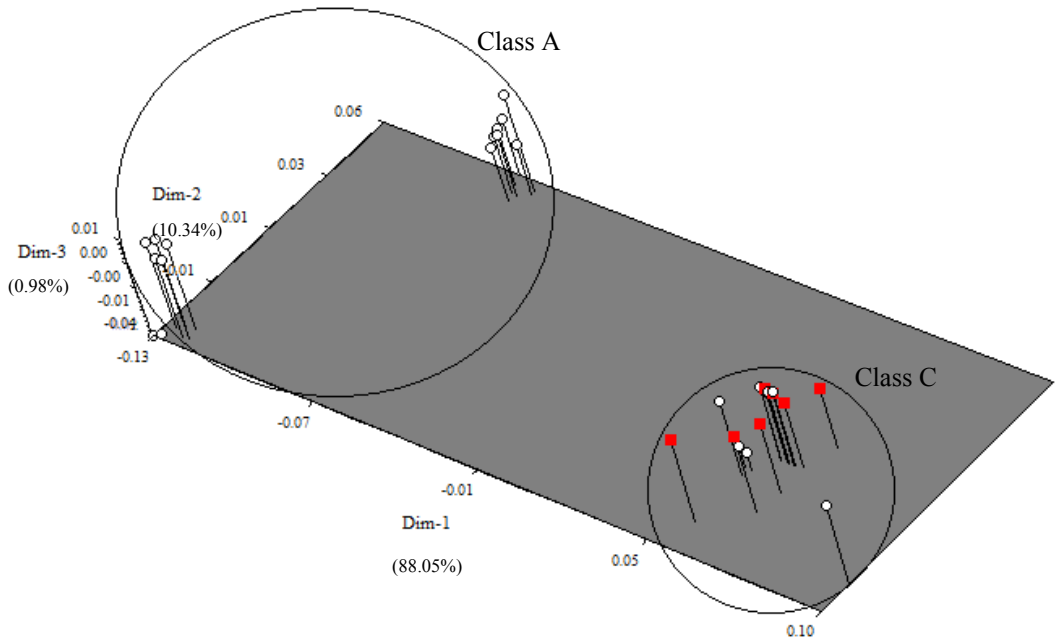


Fig. 4-9. Principal components analysis of class A and C sequences found in *P. arachnifera*, *P. pratensis* and their hybrids. Pairwise Jukes-Cantor genetic distances computed in Mega 6.0 were plotted using the three largest eigenvectors calculated by NTSYS 2.2. Red square symbol indicate the placement of 851bp sequence obtained from *P. arachnifera* and all hybrids within the circumscription of Class C.

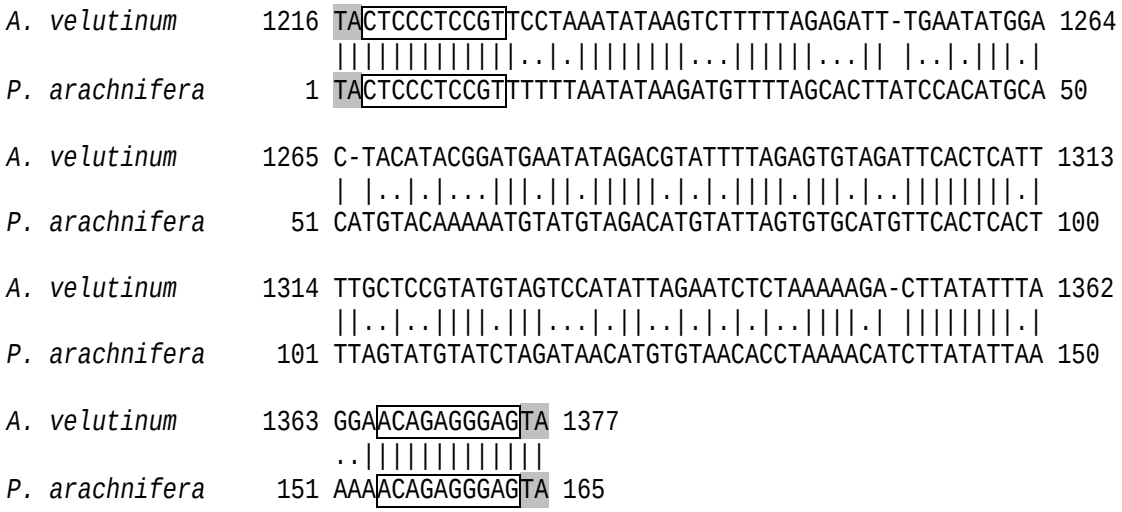
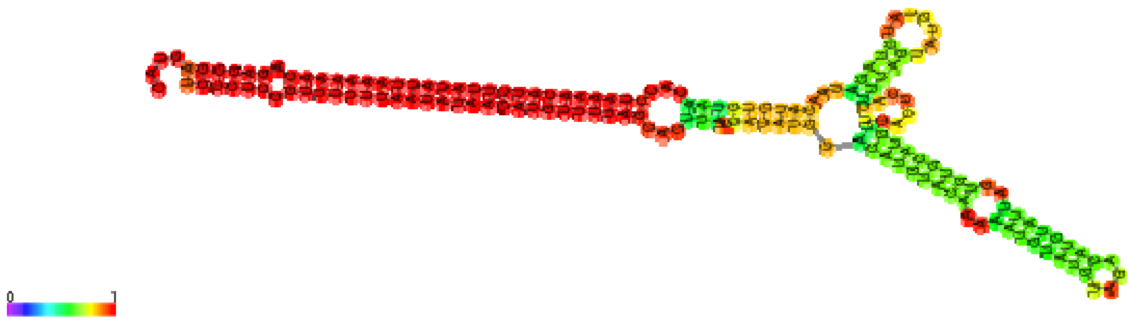


Fig. 4-10. BLAST alignment of the 163 bp insertion site from *P. arachnifera* (extended to include the first 'TA' duplication site) with a *Stowaway* MITE sequence from *Australopyrum velutinum* beta-amylase gene (AY821693.1). The 'TA' target site preference (shaded grey) and the following 10 bp conserved terminal sequences (boxed) are characteristic of MITEs shared between the two sequences.

a



b

Common Name	miRNA Sequence Name	Mismatches	Conserved Sequence Sites
Texas bluegrass	***P. arachnifera* 163bp**	**Query**	**UCCCUCCGUUUUUUAAUAUAAGAUGUUUUAGCAGUAUGUAUCUAGAUAACUAAAACAUCUUAUAUUAAAAAACAGA**
Common Wheat	tae-miR1130	5 mismatch/23	---CCUCCGUCUCGUAAUGUAAGACG--
Common Wheat	tae-miR1120	3 mismatch/20	----CUCCGUCUCAUAAUAUAAGAC---
Barley	hvu-miR1130	4 mismatch/21	---CGUCUUAUAUUAAGUUACAGA
Barley	hvu-miR1120	4 mismatch/20	---CUCCGUCCCAUAAUAUAAGA---
Sorghum	sbi-miR5568g-5p	2 mismatch/18	--------------AUUAUAAGAUGUUUUGGC-----------------CCAAAACAUCUUAUAAU----------
Sorghum	sbi-miR5568b-3p	2 mismatch/17	--------------------------------ACUAUGUAUCUAGAAAA---------------------------
Sorghum	sbi-miR5568d-5p	1 mismatch/15	----------------------------------UAUGUAUCUAGAAAA---------------------------
Rice	osa-miR5825	6 mismatch/24	-----CCGUUUCAGGUUAUCAGAUGUUUU---
Purple False Bromegrass	bdi-miR5174d-5p	2 mismatch/16	UCCCUCCGUUUCAUAA--

Fig. 4-11. Minimum free energy stem loop structure of the 163 bp insertion site in *P. arachnifera* (8a); conserved sequence alignment (shaded grey) of 163 bp *P. arachnifera* insertion site with annotated mature miRNA sequence using miRBase (8b).

Discussion

First generation hybrids within the *Poaceae* family have been shown to have an intermediate genome size to its parents (Laurie and Bennett, 1985). Results of our flow cytometry analysis show that Reveille and all other tested hybrids had an intermediate mean DNA content between *P. arachnifera* and *P. pratensis* parental genotypes. The variation in the nuclear DNA content in four hybrids of pedigree #2 (TAES 6012 x CS#4) ranging from 9.21 to 11.14 pg/2C could possibly be originating from some sort of chromosomal re-arrangements (duplications/deletions), loss of non-homeologous chromosomes or due to random variation in the DNA content of the apomictic pollen parent. Future cytological investigation would help reveal the reasons for such observed differences.

As compared to several studies documenting genome size variation in *P. pratensis* (Huff and Bara, 1993; Barcaccia et al., 1997; Eaton et al. 2004; and Murovec et al., 2009) and other turfgrasses (Arumuganathan et al., 1999), very little has been shown for *P. arachnifera*. The current study documents a wide variation in the nuclear 2C DNA content in 19 different genotypes of *P. arachnifera*, possibly suggesting variation in ploidy levels. Previous cytological research has documented *P. arachnifera* having ploidy levels of 6x (2n=42) (Brown, 1939; Gould, 1958), 8x (Hartung, 1946; Patterson et al., 2005) and higher (Kelley et al., 2009). Future cytological investigation of these genotypes of *P. arachnifera* will help explain their observed 2C DNA content variation.

In addition to other *Poa* species, Patterson et al. (2005) reported the *trx* nuclear gene sequences from one accession of *P. arachnifera* (PI 655089) and two cultivars of *P. pratensis*, but the usefulness of *trx* as a genetic marker for the identification and verification of *P. arachnifera* x *P. pratensis* hybrids was not tested. The current study describes the utility of the *trx* gene as a sequence-based tool for the confirmation of interspecific hybrids between the two species. Furthermore, the analysis of TAES 5684 and D4 Isolate #8 demonstrated how the *trx* gene can also be used to accurately characterize mislabeled or unknown individuals of *P. arachnifera* and *P. pratensis*. Furthermore, the unique 851 bp sequence, discovered in the present study, captivated our interest because it exhibited a clear presence in 98 % of the examined *P. arachnifera* genotypes (from a total of 50) and 100% of the tested hybrids (a total of 25) from seven different pedigrees. This 851 bp was found to be absent in 100% of *P. pratensis* genotypes tested (a total of 25).

Further investigation of the 851 bp sequence showed the presence of a 163 bp insertion which was absent from all other *Poa* sequences. Higher polyploids, such as *P. arachnifera*, most likely originated as a result of multiple polyploidization events (Soltis and Soltis, 1999). Doubling of a tetraploid genome, hybridization between two tetraploid parental genomes or a hexaploid and a diploid genome are likely pathways resulting in the formation of an octoploid *P. arachnifera*. We speculate that the 163 bp insertion is likely a result of chromosomal restructuring resulting from genomic shock and an associated increase in transposon activity (Matzke and Matzke, 1998) during the formation of tetraploid parental species from its diploid progenitors, or coming together

of the two parental tetraploid species in the formation of an octoploid *P. arachnifera*. Since the 163 bp insertion was not detected in any other *Poa* species in GenBank, this suggests that the parental species of *P. arachnifera* harboring the 851 bp sequence is either extinct or has not yet been discovered. Nonetheless, the presence of the 851 bp sequence in the majority of *P. arachnifera* genotypes examined suggests that such chromosomal rearrangements happened early on in the evolutionary process, probably right at the initiation of *P. arachnifera* speciation. Performing quantitative PCR to determine copy number and florescent *in situ* hybridization to determine genome location may shed more light on the evolutionary origin of this unique 851 bp sequence in *P. arachnifera*.

CHAPTER V

DEVELOPMENTAL RESPONSES OF HYBRID BLUEGRASS AND TALL FESCUE AS INFLUENCED BY LIGHT INTENSITY AND TRINEXAPAC-ETHYL

Overview

Interspecific hybrids between Texas bluegrass (*Poa arachnifera* Torr.) and Kentucky (*Poa pratensis* L.) bluegrass are known to exhibit good heat tolerance, which has aided in their adaptation to the warmer climates of the southern United States. The objectives of this study were to 1) evaluate growth responses of five interspecific hybrids (*P. arachnifera* x *P. pratensis*) (three experimental lines, 'Reveille', and 'Thermal Blue'), 'Rebel Exeda' tall fescue (*Festuca arundinacea* Schreb.) (shade tolerant standard), 'Kenblue' Kentucky bluegrass (shade sensitive standard), and 'CS#4 (a shade tolerant ecotype of Kentucky bluegrass persisting for multiple years in a shaded environment in College Station, TX) under light environments of full sun, moderate, and heavy shade, 2) to evaluate the relative growth and quality responses from application of gibberellic acid inhibitor, trinexepac ethyl, and 3) determine the daily light integral required to achieve acceptable quality in each genotype. Ten-week greenhouse experiments were conducted at Texas A&M AgriLife Research-Dallas. Within each of the shade environments, a randomized complete block design with 3 replications was used to accommodate a factorial arrangement with 8 genotype x 2 trinexapac-ethyl (TE, 0 or 0.023 $kg \cdot ha^{-1}$ a.i.) treatments. Turfgrass quality, leaf elongation rates, and clipping dry weights were measured every two weeks. Percent green cover was also determined

using digital image analysis at the conclusion of each experiment. Leaf elongation rate was determined to be the best quantitative parameter under full sunlight to identify dwarf-type hybrid bluegrasses, and under moderate shade to identify shade tolerant genotypes. Only 4 weeks of shade exposure were necessary to make selections based on leaf elongation rate. Photosynthetic photon flux levels in heavy shade were too low to allow for meaningful comparisons. Hybrid bluegrass genotypes DALBG 1201 and TAES 5654 displayed acceptable performance under both moderate and heavy shade conditions, and were comparable to Rebel Exeda in turfgrass quality. Although TE reduced leaf elongation within all entries and light environments, it did not improve turf quality.

Introduction

Shade tolerance is an important factor for selecting turfgrasses for home lawns and recreational facilities. Trees and buildings reduce the quality and intensity of sunlight that can cause stand thinning and reduced turfgrass quality (Tegg and Lane, 2004). Beard (1973) estimated that 20 to 25% of established turfgrass stands are impacted by some type of light restriction, and indicated that most turfgrasses cannot survive at low light intensities.

Cool-season (C_3) turfgrass species generally have lower light compensation points than warm-season (C_4) turfgrass species. Greater photochemical efficiency at low light intensities contributes to the generally superior shade adaptability of C_3 grasses (Kephart and Buxton, 1996). Despite this, there is still considerable variation in shade tolerance among cool season turfgrass species. Tall fescue (*Festuca arundinacea*

Schreb.) is the predominant species for shaded areas in the transition zone with good to moderate tolerance (Wu et al., 1985); whereas Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) generally have very poor shade tolerance (Beard, 1973). Fine fescues (*Festuca* spp.), on the other hand, display the best shade tolerance of the cool-season turfgrass species (Gardner and Taylor, 2002). However, only a few of these species have shown potential for adaptability to the southern or transition zones of the United States. In recent years, there has been renewed interest in developing heat, drought, and shade-tolerant cool-season turfgrass species that may offer year-round growth in these climates.

Tall fescue is a cool-season grass that displays a wide range of genetic variability and greater adaptation to various environmental conditions including heat, drought, and shade relative to other cool-season species (Hurley, n.d.). Forage type varieties of tall fescue are used in low maintenance areas in the transition zone of the United States for their tolerance to summer heat and drought, but have a coarse leaf texture and a light-green color (Watkins and Meyer, 2004). Semi-dwarf and dwarf-types with finer leaf textures and high turfgrass quality have become popular for use in shaded conditions, but require greater maintenance and are highly susceptible to brown patch (*Rhizoctonia solani* Kühn) and other diseases (Watkins and Meyer, 2004; Wu et al., 1985). Thus, while tall fescue is often desired for its year-round green color, it often requires annual fall overseeding following summer stress due to its susceptibility to disease and its bunch-type growth habit. For these reasons, breeders have recently been investigating the potential for alternative cool-season species for southern environments.

Kentucky bluegrasses are well adapted to the northern United States and are popular for their appealing visual quality and finer leaf texture that is desirable for lush home lawns and playing fields. With twelve classes, this species is genetically and morphologically diverse, and although Compact America, Compact Midnight, and Mid-Atlantic types display greater tolerances to heat, drought, and shade, they are generally not well adapted to southern environments (Brilman, 2009; Hall, 1996; Morris, 2010).

Genes conferring heat and drought tolerance have been introgressed into the Kentucky bluegrass genome through interspecific hybridization with Texas bluegrass (*Poa arachnifera* Torr.), a native species adapted to the southern plains of the United States (Hitchcock, 1950). Some of these hybrids have demonstrated markedly better performance when compared to Kentucky bluegrass and tall fescue (Abraham et al., 2008; Su et al., 2007; Meeks et al., 2015), and have allowed for a greater range of adaptability into more southern environments. However, to date, there is limited information regarding the shade tolerance of these hybrid bluegrasses.

The performance of Kentucky bluegrasses and other cool-season species have been evaluated under various shade structures covered with shade cloth either in the glasshouse or outside in the field (Burner and West, 2010; Lin et al., 1999; Feldhake et al., 1985; Tan and Qian, 2003; Tegg and Lane, 2004; Watson et al., 1984), under tree canopies (Gardner and Taylor, 2002; Wherley et al., 2005), and in growth chambers (Wood, 1968). In most cases, full sun, moderate (≈ 50 %), or heavy (≈ 80 %) shade are used for comparisons, but establishment methods and experiment duration vary depending on the objectives and location. In general, tall fescue has been considered

highly shade tolerant, while Kentucky bluegrass has been shade intolerant (Gardner and Goss, 2012). The performance of tall fescues under shade in the southern United States has been evaluated for forest grazing lands (Watson et al., 1984), but limited published data are available for cultivars adapted for residential or recreational purposes.

Some useful parameters typically used to characterize turfgrass growth responses under shaded environments include clipping yield, turfgrass quality and color (Burner and West, 2010; Cockerham et al., 2002; Feldhake et al., 1985; Lin et al., 1999; Watson et al., 1984; Wood, 1968). One of the most documented shade avoidance mechanisms is an increase in leaf elongation in genotypes that are intolerant (Beard, 1965; Tan and Qian, 2003; Tegg and Lane, 2004) resulting in accelerated energy depletion in plant tissue (Qian and Engelke, 1999) and increased mowing frequency by the turfgrass manager or homeowner. High leaf elongation rates have also been correlated to significant reductions in percent green cover in St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), one of the most shade tolerant warm-season turfgrass species (Wherley et al., 2013). Reduction in canopy density or percent cover under long-term shaded conditions was also observed in Kentucky bluegrass (Gardner and Taylor 2002).

Gibberellins are acids involved in the regulation of plant growth and development (Graebe, 1987). Of the five forms of gibberellic acids (GA) found in Kentucky bluegrasses (Juntilla et al., 1997), shoot and leaf elongation are controlled by the biosynthesis of GA_1 from its precursor GA_{20} (Reid and Ross, 1991; Tan and Qian, 2003). Dwarf-type genotypes of Kentucky bluegrass display significantly lower levels

of GA_1 (Tan and Qian, 2003). Increased day lengths from spring to fall can promote natural GA synthesis and therefore increased shoot elongation (Junttila et al., 1997). During this time, turfgrass managers often apply plant growth regulators (PGRs) such as flurprimidol or trinexapac-ethyl (TE) to reduce mowing frequency. TE in particular interrupts the biosynthesis of GA_1 from GA_{20}, thereby decreasing shoot growth in grasses under full sunlight and shaded conditions (Lickfeldt et al., 2001; Stier et al., 1999; Tan and Qian, 2003). However, there have been inconsistent responses reported following application of TE to shaded turfgrasses. Qian et al. (1998) reported a significant improvement in turf quality for zoysiagrass due to TE application under shade. Others have reported increases in turfgrass color (Steinke and Stier, 2003; Stier and Rogers, 2001; Wang et al., 2009), but still others report mixed or no benefits on overall turfgrass quality (Ervin and Koski, 2001; Gardner and Wherley, 2005).

The objectives of this study were to 1) compare shade performance and growth response of experimental hybrid bluegrasses to shade tolerant ‘Rebel Exeda’ tall fescue, CS#4 Kentucky bluegrass and ‘Thermal Blue’ hybrid bluegrass, as well as to shade sensitive ‘Kenblue’ Kentucky bluegrass and ‘Reveille’ hybrid bluegrass, 2) assess the effects of TE on the growth response of the grass species under three light environments, and 3) determine the daily light integral required to achieve acceptable quality in each genotype.

Materials and methods

Plant materials

Five hybrid bluegrasses (*P. arachnifera* x *P. pratensis*) ['Reveille', 'Thermal Blue', 'DALBG 1201' (PI 671854), TAES 5654, and TAES 5655] were evaluated in comparison to two Kentucky bluegrasses ('Kenblue' and CS#4, an ecotype and male parent to DALBG 1201, TAES 5654 and TAES 5655), and one tall fescue ('Rebel Exeda'). Rebel Exeda is an improved turf-type tall fescue that has good tolerance to shaded conditions (Wallace et al., 2013). Kenblue is classified as a common type Kentucky bluegrass with sensitivity to low light conditions because it expresses increased shoot elongation as a shade avoidance mechanism that reduces stand density and overall turfgrass quality (Morris, 2010; Richardson et al., 2010; Tan and Qian, 2003). Reveille and Thermal Blue are shade sensitive and shade tolerant hybrids, respectively (Morris, 2010 and 2013).

Plant material was established in 10 cm diameter round pots for six months prior to the spring and late summer studies, during winter and spring 2013-2014. One week prior to each experiment, these fully established, 10 cm plugs were transplanted into 20 cm diameter pots to initiate the shade experiment. Soil medium was composed of Sunshine VP mix (Sun Gro Horticulture, Inc.) and 5% (v:v) sand. Osmocote (14-14-14) (Everris NA, Inc.) fertilizer was incorporated into potting soil at a rate of 7.2 kg m^{-3}. All plants were treated uniformly and were trimmed to 5 cm on the start date of each experiment. Pots were watered 2-3 times weekly for full sunlight treatments, and 1-2

times weekly for the shade treatments. Irrigation was supplied to fully saturate the soil at each irrigation event.

Experimental design

Two independent experiments were performed for 10 weeks under a glasshouse at the Texas A&M AgriLife Research Center in Dallas, TX in 2014. The first experiment occurred from May-July and the second from August-October. Daytime greenhouse temperatures from May-July ranged from 24°- 29°C, with minimum nighttime temperatures of 18°C. From August-October, daytime temperatures ranged from 21°-33°C, with a minimum nighttime temperature of 12°C.

The experiments were set up as a randomized complete block design with treatments arranged in a 2 x 3 factorial design with three replicates. Two TE treatments of 0 (untreated control, water) or 0.023 $kg \cdot ha^{-1}$ a.i. were applied to plants every four weeks within each of three light environments (full sunlight, moderate shade, and heavy shade). TE (Primo Maxx, Syngenta Co., Ltd., Switzerland) treatments were delivered using a hand-pumped tank sprayer and fan nozzle. For the shaded environments, two PVC shade structures were built to a scale of 274 cm L x 152 cm W x 91 cm H, and were covered on all sides with black polypropylene shade cloth. Shade cloth density was 40% (moderate) and 80% (heavy) to account for the filtered light through the glass roof. A 60 cm opening across the bottom of the north side of the frame allowed for air flow and ease-of-access. . Pots were placed 30.48 cm inside the north and south edges of the benches to prevent direct transmission of unfiltered light. Preventative fungicide applications were tank-mixed with the TE treatments using DaconilZn (Syngenta Co.,

Ltd., Switzerland) at 0.79 kg·ha^{-1} a.i. and Heritage (Syngenta Co., Ltd., Switzerland) at 0.003 kg·ha^{-1} a.i. Plant material and blocks (TE treatments) within each shade environment were randomized before each monthly chemical treatment.

Photosynthetically active radiation (PAR) quantum light sensors connected to a WatchDog 1425 Micro Station data logger (Spectrum Technologies, Inc., Aurora, IL) recorded photosynthetic photon flux (*PPF*) hourly at canopy height in each of the three light environments. In comparison to the outdoor ambient *PPF*, full sunlight transmission through the glasshouse at 1:00 PM on cloudless days was calculated from fourteen daily measurements. The average daily light integrals (DLI) were calculated across the duration of each experiment for each light environment using the Specware Software (Spectrum Technologies, Inc., Aurora, IL).

Measurements

Pots were trimmed every two weeks to the original 5 cm height using an 8.9 cm i.d. polyvinyl-chloride guide pipe cut to height. Clippings were collected and dried at 70°C for 48 hr. (Tan and Qian, 2003). Leaf elongation measurements were collected prior to trimming. Data for leaf elongation and clipping production were collected every two weeks for a total of five collection dates in each experiment. Leaf elongation (cm·d^{-1}) was measured from the three tallest leaves of each pot. Average daily leaf elongation rate was obtained by dividing total elongation over the growth period by the number of days since trimming.

Turfgrass quality was collected bi-weekly, but for the purpose of this publication only final turf quality data at week 10 are presented. Turfgrass quality was rated on a 1

to 9 scale with a completely brown turf canopy = 1, perfectly dense, uniform, and green canopy =9, and minimally acceptable quality = 5. The overall mean turfgrass quality for each genotype in the three light environments was regressed against the total calculated DLI averages for each light environment to determine minimum DLI for acceptable turfgrass quality (Bunnell et al., 2005).

Final green cover (%) was assessed at week 10 using digital images of each pot and SigmaScan Pro Version 5.0 (Systat, Inc., Richmond, CA) with the Turf Analysis 1.2 macro (Karcher and Richardson, 2005). Threshold hue (16 – 47) and saturation (38 – 56) settings were adjusted to account for yellow or dead tissue. Final percent green cover values were plotted against average leaf elongation rates for full sun, moderate shade, and heavy shade to better understand the relationship between the two parameters for shade screening evaluation.

Analysis of variance (ANOVA) and Pearson's correlation analyses were performed using JMP 10 (SAS Institute Inc., 2012), with shade environments analyzed independently. Fixed factors included experiment, TE treatment, and genotype. Replications were treated as random variables. Collection week was only considered a fixed effect for leaf elongation. Experimental differences were determined using Student's t-test at a significance level of $P \leq 0.05$. Mean separation was performed using Tukey's honestly significant difference test at a significance level of $P \leq 0.05$.

Results

Measured average photosynthetic photon flux (*PPF)* and average daily light integrals (DLI) levels differed between the two experiments, potentially due to changes

in photoperiod caused by the summer solstice, but could also be related to greater cloud cover during the second experiment (data not shown). Day lengths were longer before June 21st, and thereafter became shorter until the autumn equinox. Light transmission in the full sunlight environment was measured to be 89 ± 10.2% and 76 ± 4.5% of ambient *PPF* for experiments 1 and 2, respectively (Table B-1). The shade cloth used for moderate shade produced light levels of 47 ± 5.7% and 29 ± 12.4% of ambient *PPF* for experiments 1 and 2, respectively. The shade cloth used for heavy shade produced light levels of 20 ± 3.1% and 9 ± 3.2% of ambient *PPF* for experiments 1 and 2, respectively. For reference, it has been suggested that cool season grasses required a minimum of 2 - 5% of full sunlight to achieve light compensation levels (Beard, 1973).

Due to variation in light levels by season, daily light integral (DLI) was also calculated for each experiment. Kentucky bluegrass has been reported to require a DLI of at least 11.1 mol $m^{-2}d^{-1}$ for acceptable quality (Cockerham et al., 2002). Based on calculated mean DLIs for each light environment x experiment, mean DLI fell below this reported critical level of 11.1 mol $m^{-2}d^{-1}$ under both moderate and heavy shade for experiment 2 (Table B-1). Additionally, greenhouse temperatures were slightly higher during the August to October period, which could have contributed to higher rates of photorespiration in the latter experiment (Su et al., 2007). Changes in *PPF* and DLI influenced growth and performance of genotypes under shaded environments between experimental seasons. A similar observation was reported by Lin et al. (1999).

Turfgrass quality

Quality ratings were highest in full sunlight followed by moderate and heavy shade (Table 5-1). Within each environment, no significant differences were calculated between experiments, but significant experiment x genotype interactions indicated that some genotypes performed differently between the two experiments.

In experiment 1, hybrid bluegrass DALBG 1201 and TAES 5654 were consistently in the top statistical group in full sun and moderate shade as was their Kentucky bluegrass parent CS#4, and the shade tolerant check Rebel Exeda. Under heavy shade however, only DALBG 1201, TAES 5654, and Rebel Exeda maintained above acceptable turfgrass quality. The shade intolerant Kenblue and shade tolerant Thermal Blue did not display acceptable turfgrass quality under either shade environment. Similar trends were observed in experiment 2 where DALBG 1201, TAES 5654, CS#4, and Rebel Exeda placed in the top statistical group in full sun and moderate shade environments. Under heavy shade only DALBG 1201 and TAES 5654 exhibited above acceptable turfgrass quality. The quality ratings of Kenblue were not acceptable in any environment. Turfgrass quality of commercial hybrids, Thermal Blue and Reveille were only acceptable under full sunlight. In conclusion, hybrid bluegrasses DALBG 1201 and TAES 5654 exhibited turfgrass quality comparable to that of shade tolerant tall fescue, Rebel Exeda under full sunlight and both shade environments, and superior quality to the shade tolerant commercial hybrid bluegrass, Thermal Blue. Furthermore, Meeks et al. (2015) previously reported DALBG 1201 and TAES 5654 as having a finer leaf texture than Rebel Exeda, and highly stable performance across the southern U.S.

Table 5-1. Final turfgrass quality ratings in the control treatments under full sunlight, moderate shade, and heavy shade environments for each genotype.

	Final turfgrass quality (1-9 scale) [†]											
	Full sunlight				Moderate shade				Heavy shade			
Genotype	Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2	
Rebel Exeda	7.7	ab	7.0	ab	6.7	a	5.0	abc	5.3	ab	2.7	b
Kenblue	5.7	c	4.7	c	4.0	d	3.3	c	2.7	b	2.3	b
CS#4	7.7	ab	7.0	ab	5.7	abc	4.3	abc	4.7	ab	2.0	b
Thermal Blue	6.7	bc	6.0	bc	4.3	cd	3.7	bc	3.7	ab	2.7	b
Reveille	5.7	c	8.0	ab	5.0	bcd	4.0	abc	3.7	ab	4.3	ab
DALBG 1201	8.7	a	9.0	a	7.0	a	7.3	a	5.7	a	6.0	a
TAES 5654	9.0	a	9.0	a	6.3	ab	7.0	ab	5.3	ab	5.7	a
TAES 5655	6.3	bc	7.0	ab	5.0	bcd	6.0	abc	2.7	b	4.3	ab
ANOVA												
Experiment[‡]	NS				NS				NS			
Genotype[§]	****		****		****		**		**		***	
Experiment x Genotype	**				**				**			

NS, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.
ANOVA = analysis of variance.
[†] Turfgrass quality ratings were assessed on a 1-9 scale (1 = brown/tan or dead; 5 = minimum acceptable quality)
[‡] Differences between experiments were determined using Student's t-test at a significance level of $P \leq 0.05$.
[§] Genotype means were separated using Tukey's honestly significant difference test at a significance level of $P \leq 0.05$.

Final percent green cover

Final percent green cover, as determined through digital image analysis, was highest in full sunlight and decreased with higher shade densities (Table 5-2). It was also observed that green cover was statistically highest in experiment 1 across all environments, and genotype coverage varied between experiments.

In experiment 1, all genotypes covered greater than 96%, 87%, and 77% of the soil area in full sun, moderate and heavy shade, respectively. In experiment 2, final

percent green cover was greater than 85%, 74%, and 67% in full sun, moderate and heavy shade, respectively. Digital image analysis detected minor significant differences between genotypes in each environment. However, in most cases, four out the five hybrid bluegrass genotypes were not significantly different from each other or Rebel Exeda. Kenblue and Thermal Blue, in most cases, exhibited the lowest percent green cover across environments.

Table 5-2. Final percent green cover assessed in the control treatments under full sunlight, moderate shade, and heavy shade environments for each genotype.

	Final green cover (%)[†]											
	Full sunlight				Moderate shade				Heavy shade			
Genotype	Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2	
Rebel Exeda	99.8	a	95.0	ab	99.0	a	84.6	ab	96.2	a	67.2	c
Kenblue	96.3	b	85.6	bc	87.3	b	76.5	ab	87.4	b	82.5	ab
CS#4	99.2	a	96.6	a	88.3	b	68.9	b	77.5	b	69.8	c
Thermal Blue	96.3	b	85.4	c	88.3	b	79.5	ab	88.2	b	75.8	bc
Reveille	99.5	a	94.9	abc	94.6	a	74.6	ab	91.0	ab	82.9	ab
DALBG 1201	99.6	a	99.5	a	98.6	a	93.1	a	93.8	ab	90.5	a
TAES 5654	99.7	a	99.5	a	96.0	a	92.3	a	92.1	ab	89.2	a
TAES 5655	99.1	a	95.7	a	94.6	a	86.1	ab	89.2	b	85.3	ab
ANOVA												
Experiment[‡]	****				****				****			
Genotype[§]	****		***		****		**		****		****	
Experiment x Genotype	***				NS				****			

NS, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.
ANOVA = analysis of variance.
[†] Final green cover was assessed as a percentage using digital image analysis during the final week of each experiment.
[‡] Differences between experiments were determined using Student's t-test at a significance level of $P \leq 0.05$.
[§]Genotype means were separated using Tukey's honestly significant difference test at a significance level of $P \leq 0.05$.

Cumulative clipping production

Clipping production in shade has been extensively tested on warm-season grasses but has not been found to be a reliable shade tolerance indicator (Busey and Davis, 1991). Inconsistent shoot growth responses in shade have been attributed to shade avoidance responses whereby shade intolerant cultivars exhibit increased shoot growth relative to tolerant cultivars. In cool-season grasses, however, clipping biomass production can decrease with decreasing light intensity (Cockerham et al., 2002; Lin et al., 1999; Watson et al., 1984). This may result from increased leaf succulence and narrower, thinner leaves of cool-season turfgrasses. To our knowledge, clipping production has not been evaluated as a shade tolerance indicator for hybrid bluegrasses. In this study, experiment, genotype, and experiment x genotype effects were all significant factors in the model analysis, except under moderate shade in experiment 2 where all genotypes produced similar amounts of clippings (Table 5-3).

Clipping production for moderate and heavy shade was, on average, 50% and 80% less than full sunlight during experiment 1, and approximately 60% and 81% less than full sunlight in experiment 2, respectively. Under full sunlight and moderate shade environments, clipping production from four out of the five tested hybrids with an exception of Thermal Blue were not significantly different from each other or Rebel Exeda. In these environments, tested Kentucky bluegrasses and Thermal Blue exhibited the highest cumulative clipping production. Our results from heavy shade were consistent with Cockerham et al. (2005), who reported that heavy shade (DLI between 2.2 and 0.9 mol m^{-2} d^{-1}) nearly stopped growth completely. Additionally, the cumulative clipping production data under heavy shade showed that similar amounts of clippings were obtained between genotypes that were tolerant (Rebel Exeda) and intolerant (Kenblue). Ultimately, meaningful differences in regards to shade tolerance could not be inferred using this measurement.

Table 5-3. Cumulative clipping dry weight measured in the control treatments under full sunlight, moderate shade, and heavy shade environments for each genotype.

	Cumulative clipping production (g)[†]											
	Full sunlight				Moderate shade				Heavy shade			
Genotype	Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2	
Rebel Exeda	11.6	ab	4.3	b	3.8	cd	2.3	a	2.0	bc	1.4	ab
Kenblue	16.7	a	9.5	ab	5.6	bc	2.4	a	1.7	bc	0.6	b
CS#4	14.2	a	9.2	ab	10.0	a	2.8	a	4.6	a	1.4	ab
Thermal Blue	16.0	a	10.7	a	7.9	ab	2.5	a	2.4	b	1.6	ab
Reveille	5.9	b	5.4	b	3.6	cd	2.7	a	1.2	bc	1.0	ab
DALBG 1201	6.1	b	6.2	ab	4.6	cd	2.8	a	2.4	b	1.7	ab
TAES 5654	6.1	b	4.6	b	3.4	cd	3.1	a	1.7	bc	2.0	a
TAES 5655	7.1	b	5.6	ab	2.9	d	3.7	a	1.0	c	1.0	ab
ANOVA												
Experiment[‡]	****				****				****			
Genotype[§]	****		****		****		NS		****		*	
Experiment x Genotype	**				****				****			

NS, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.
ANOVA = analysis of variance.
[†] Clippings were collected every two weeks during each experiment. Genotypes were compared by their cumulative clipping production in each experiment.
[‡] Differences between experiments were determined using Student's t-test at a significance level of $P \leq 0.05$.
[§] Genotype means were separated using Tukey's honestly significant difference test at a significance level of $P \leq 0.05$.

Daily leaf elongation rate

Imposed light restrictions on turfgrasses cause leaf elongation rates to increase as a shade avoidance mechanism that ultimately depletes stored plant energy of shade intolerant genotypes (Beard, 1965; Tan and Qian, 2003; Tegg and Lane, 2004). In this study, significant differences due to experiment, genotype, experiment x genotype, collection week, and collection week x genotype were indicated in the analysis (Table 5-4). Under the conditions of experiment 1, measurements under full sunlight identified DALBG 1201 and TAES 5654 exhibiting statistically lower leaf elongation rates as

compared to Thermal Blue, Reveille, Kenblue and CS#4. Leaf elongation rate of the dwarf-type tall fescue, Rebel Exeda, was statistically similar to DALBG 1201 and TAES 5654 under full sunlight suggesting that these hybrid bluegrasses behave like dwarf phenotypes. Under moderate and heavy shade, four of five hybrid bluegrasses with an exception of Thermal Blue exhibited statistical similar leaf elongation rates to Rebel Exeda. Kenblue had the highest leaf elongations rates in all three environments followed by Thermal Blue and CS#4.

Under the conditions of experiment 2, DALBG 1201, TAES 5654 and Reveille had statistically similar leaf elongation rates to Rebel Exeda under full sunlight and moderate shade. Kenblue had highest leaf elongations rates in these two environments followed by Thermal blue, TAES 5655 and CS#4. Under heavy shade, four out of five hybrid bluegrasses had lowest leaf elongation rates comparable to Rebel Exeda whereas Thermal Blue had the highest leaf elongation rate followed by Kenblue.

Table 5-4. Daily leaf elongation rate measured in the control treatments under full sunlight, moderate shade, and heavy shade environments for each genotype.

	Daily leaf elongation rate ($cm \cdot d^{-1}$)[†]					
	Full sunlight		Moderate shade		Heavy shade	
Genotype	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Rebel Exeda	0.7 d	0.7 d	0.8 de	0.8 d	0.8 c	0.5 d
Kenblue	1.8 a	1.6 a	1.8 a	1.6 a	1.5 a	1.0 b
CS#4	1.0 c	1.1 c	1.1 c	1.0 c	1.1 b	0.8 bc
Thermal Blue	1.3 b	1.4 b	1.5 b	1.3 b	1.3 ab	1.3 a
Reveille	1.0 c	0.7 d	1.0 cd	0.8 d	0.7 c	0.6 cd
DALBG 1201	0.7 d	0.7 d	0.8 e	0.7 d	0.7 c	0.6 cd
TAES 5654	0.6 d	0.6 d	0.7 e	0.7 d	0.6 c	0.7 cd
TAES 5655	0.9 c	1.0 c	0.9 cde	1.0 c	0.7 c	0.8 bcd
ANOVA						
Experiment[‡]	*		****		****	
Genotype[§]	****	****	****	****	****	****
Experiment x Genotype	**		****		****	
Week	****		****		****	
Experiment x Week	NS		*		****	
Week x Genotype	NS	****	***	***	****	NS
Experiment x Genotype x Week	*		NS		*	

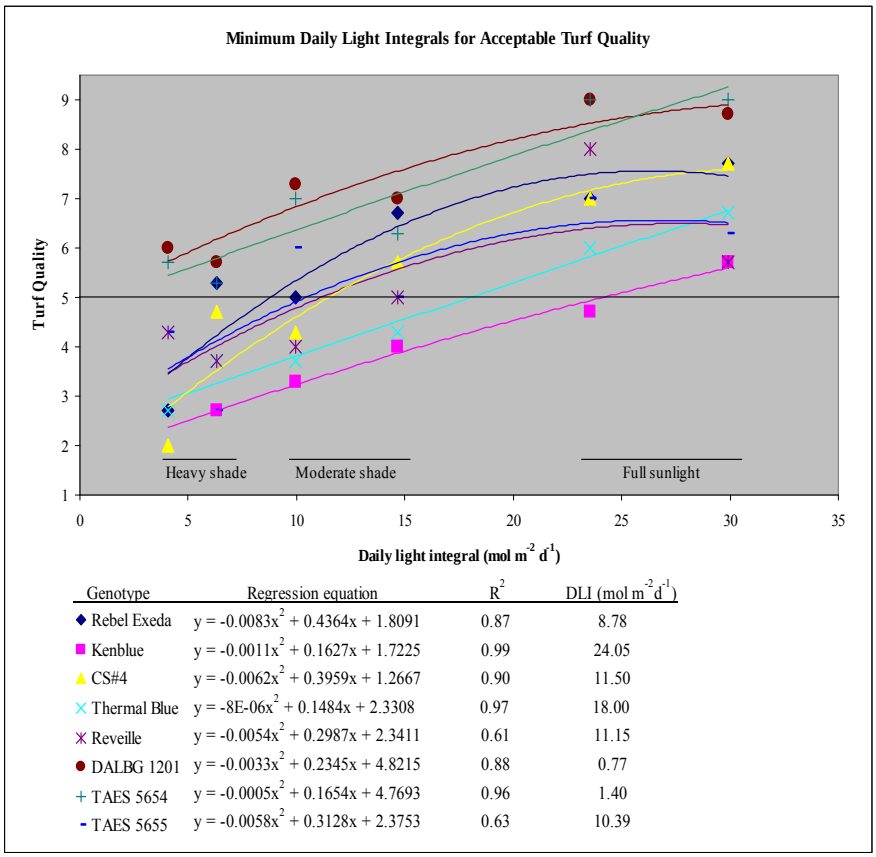
NS, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.
ANOVA = analysis of variance.
[†]Leaf elongation was measured from the three tallest leaves every two weeks in each experiment and divided by the number of days since trimming to determine the average daily leaf elongation rate. The experiment averages across all collection days are presented.
[‡]Differences between experiments were determined using Student's t-test at a significance level of $P \leq 0.05$.
[§]Genotype means were separated using Tukey's honestly significant difference test at a significance level of $P \leq 0.05$.

Regression analysis

Mean turfgrass quality ratings for each genotype in Table 5-1 were regressed against the calculated DLI for each light environment in Table B-1 (Fig. 5-1). All coefficients of determination (R^2) were ≥ 0.61. The minimum DLI for each genotype was determined using the polynomial regression equations where y = 5 (minimum

acceptable turfgrass quality) (Bunnell et al., 2005). These results were similar to the mean performance of each genotype in experiment 1 (Table 5-1). Rebel Exeda (8.78 mol m^{-2} d^{-1}), DALBG 1201 (0.77 mol m^{-2} d^{-1}) and TAES 5654 (1.40 mol m^{-2} d^{-1}) had the lowest DLI requirements to maintain acceptable quality that fell below the DLI range for heavy shade tolerance (6.33 ± 2.05 and 4.08 ± 1.63 Table B-1). Minimum DLI requirements for Kentucky bluegrass CS#4, and hybrid checks Thermal Blue and Reveille, suggested tolerance to moderate shade. The shade sensitivity of Kenblue (24.05 mol m^{-2} d^{-1}) was supported by a high light requirement equivalent to full sunlight.

Leaf elongation rate data collected from experiment 1 was chosen to perform the moderate shade x heavy shade regression analysis because light intensities in experiment 2 were too low to allow for meaningful comparisons. This linear regression showed a strong correlation value of $r = 0.95$ ($P = 0.0002$) suggesting that the tested genotypes performed similarly under both shaded environments, and that leaf elongation rate is a reliable indicator of genotype performance under shade (Fig. 5-2). Additionally, final percent green cover data was plotted against that of leaf elongation rate. All correlations were negative demonstrating a decline in final green cover as leaf elongation rate increased (Table 5-5). The strongest correlation was obtained under moderate shade in experiment 1 ($r = - 0.84$). These results suggest that moderate shade is the best shade environment to evaluate genotype performance, and leaf elongation rate is a reliable indicator to predict the final percent green cover of genotypes. These results were consistent with St. Augustinegrass shade response evaluations (Wherley et al., 2013).



Genotype	Regression equation	R^2	DLI (mol m^{-2} d^{-1})
Rebel Exeda	$y = -0.0083x^2 + 0.4364x + 1.8091$	0.87	8.78
Kenblue	$y = -0.0011x^2 + 0.1627x + 1.7225$	0.99	24.05
CS#4	$y = -0.0062x^2 + 0.3959x + 1.2667$	0.90	11.50
Thermal Blue	$y = -8E\text{-}06x^2 + 0.1484x + 2.3308$	0.97	18.00
Reveille	$y = -0.0054x^2 + 0.2987x + 2.3411$	0.61	11.15
DALBG 1201	$y = -0.0033x^2 + 0.2345x + 4.8215$	0.88	0.77
TAES 5654	$y = -0.0005x^2 + 0.1654x + 4.7693$	0.96	1.40
TAES 5655	$y = -0.0058x^2 + 0.3128x + 2.3753$	0.63	10.39

Fig. 5-1. Regression analysis determining the minimum required daily light integral (x) to achieve acceptable turfgrass quality (y = 5). Regressions were formed for each genotype using the overall turfgrass quality means from Table 5-1 and experimental DLI from each light environment in Table B-1. Regression equations, coefficients of determination (R^2), and the calculated minimum DLI for each genotype are presented.

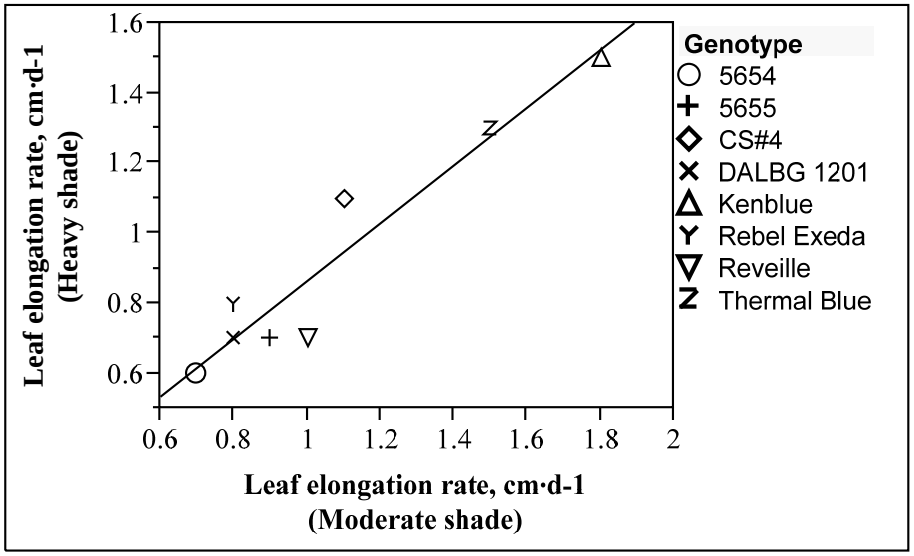


Fig. 5-2. Linear regression and correlation of leaf elongation rate between moderate shade and heavy shade produced in experiment 1. Genotypes are depicted by different symbols.

Table 5-5. Correlations between leaf elongation rate and final percent green cover in both experiments under all shade environments.

		Experiment 1			Experiment 2		
Trait comparison	Statistics	Full sunlight	Moderate shade	Heavy shade	Full sunlight	Moderate shade	Heavy shade
Leaf elongation rate x Final green cover (%)[†]	r[‡]	-0.55	-0.84	-0.61	-0.61	-0.41	-0.19
	P-value	0.0049	< 0.0001	0.0016	0.0016	0.0439	0.3757

[†]Leaf elongation rate is the overall average from weeks 2, 4, 6, 8, and 10; final percent green cover is the calculated amount from week 10.
[‡]Pearson's correlation coefficient.

An analysis was also performed to determine the minimum number of weeks in shade needed for accurately assessing leaf elongation rates. In the present study, data for leaf elongation was collected every two weeks (week 2, 4, 6, 8 and 10) (Table B-2). The mean leaf elongation rates for each collection week were regressed against the overall 10-week averages (where differences between hybrid bluegrasses were significant). Based on these results, collection week 4 had the highest correlation coefficient of $r = 0.99$ ($p < 0.0001$) (Table B-2). The data suggest that, in order to achieve the same results of 10 weeks of shade exposure, leaf elongation rate would only need to be measured at week 4 when trimming every two weeks. Hybrid bluegrasses DALBG 1201 and TAES 5654 were in the same statistical group as Rebel Exeda at 4 weeks, indicating superior shade tolerance in the form of reduced leaf elongation rate to other tested hybrids and Kentucky bluegrasses.

Effect of TE on turfgrass quality

The effect of TE on the turfgrass quality of individual genotypes differed by light environment, and no significant differences were observed in the majority of the tested genotypes (Table B-3). Under full sunlight, TE treatment did not affect turf quality in any genotypes except TAES 5654 where TE application resulted in 19% decline in turf quality. Under moderate shade, the final turfgrass quality of Thermal Blue was increased by 30% following TE treatment, thereby improving the quality above minimum acceptable level. In contrast, DALBG 1201 and TAES 5655 exhibited significant decline in turf quality under moderate shade following TE treatment. Under heavy shade, a significant reduction in turf quality was observed only for Reveille. ANOVA also

detected a main effect of experiment for heavy shade quality. This was likely due to the substantially low *PPF* levels during experiment 2, which likely approached light compensation points for many of the entries. Our results for effects of TE on turf quality in heavy shade are consistent with Gardner and Wherley (2005), who reported turfgrass quality of cool-season grasses including tall fescue was not enhanced with TE treatment under ≈ 90% shade levels.

Effect of TE on leaf elongation rate

The application of TE resulted in significant reductions in leaf elongation rate for all genotypes in all three environments and both experiments (Table B-4). The observed reductions were greatest under heavy shade (60% - 73%), followed by moderate shade (50% - 64%), and full sunlight (29% - 46%) in experiment 1. Overall, a greater percentage reduction was observed during experiment 2 when day lengths were shortest. Percent reduction during experiment 2 ranged from 75 – 90% under heavy shade, followed by 50 – 75% under moderate shade, and 29 – 60% in full sunlight. This more noticeable suppression of growth which occurred in late summer months is in agreement with Ervin and Koski (2001) who also reported greater growth reductions from TE in Kentucky bluegrass in July and August as compared to May.

Discussion

To our knowledge, this is the first documentation of the developmental response of Texas bluegrass x Kentucky bluegrass interspecific hybrids to reduced light environments, and in comparison to tall fescue. Daily leaf elongation rate was the best quantitative indicator to identify dwarf-type phenotypes, and shade tolerant lines under

moderate (50%) shade. Additionally, only four weeks was necessary to observe genotype differences using daily leaf elongation rate, making it feasible to rapidly and efficiently evaluate experimental lines. The moderate shade environment was the best selective environment for shade tolerance in our study. We also found that the spring-early summer months offered more favorable conditions for observing genotypic differences under 50% and 80% shade compared to early fall. Genotypes (Rebel Exeda, DALBG 1201 and TAES 5654) that were shade tolerant had slower leaf elongation rates, and lower DLI requirements to display acceptable turfgrass quality. Although TE effectively reduced leaf elongation rate in all environments, it did not improve turfgrass quality.

DALBG 1201 and TAES 5654 exhibited superior turfgrass quality, reduced leaf elongation rates, and high percent green cover under shaded environments, and performed similarly to the shade tolerant check, Rebel Exeda tall fescue. However, both hybrid bluegrasses exhibit a narrower leaf blade as compared to Rebel Exeda, a trait highly desired by homeowners and turf managers. Furthermore, DALBG 1201 is a stress tolerant and highly stable cultivar that is well adapted for use in the southern U.S. Collectively, these results support the use of DALBG 1201 and TAES 5654 as viable alternatives for use in landscapes of the southern U.S. where moderate and heavy shade can suppress turfgrass growth.

CHAPTER VI

CONCLUSIONS

This research focused on the development of new interspecific hybrids between Texas and Kentucky bluegrass for adaptation to the southern United States. This involved breeding work, strategies to optimize seed germination, performance trials in multiple locations and years, marker-assisted characterization, and shade tolerance evaluations.

The methods performed during the breeding work were conducive for controlled interspecific hybridization. However, inducing flowering in Kentucky bluegrasses was problematic, and suggests that breeding material for this species should either be obtained from other locations where environmental conditions are optimum for vernalization, or a controlled environment such as a growth chamber be used to vernalize Kentucky bluegrass in the southern United States. In addition, future hybridization attempts should be guided by the compatibility of different species by determining their ploidy levels. This would allow for more focused attempts that would significantly reduce the number of crosses and enable greater efficiency of the optimized seed germination protocol.

Multi-location testing at five different locations over a period of three years showed that DALBG 1201 was superior to all experimental hybrid bluegrass lines examined, commercial hybrid bluegrass checks, and Rebel Exeda tall fescue. For this reason, it was released and registered as a new hybrid bluegrass cultivar for use on home lawns and recreational facilities that can be established vegetatively.

One of the important finding of this research was the extent of nuclear DNA content variation depicted in Texas bluegrass. The 2C DNA content estimation using flow cytometry showed significant variation in the genome size in Texas bluegrass lines tested. Molecular markers are modern tools that can be used to eliminate genotypes that are not true hybrids before conducting field performance trials. We employed the use of a thioredoxin-like (*trx*) nuclear gene marker system for the identification of the tested experimental hybrids and cultivars. Additionally, it was determined that a 98% of the tested Texas bluegrass genotypes have a third *trx* allele copy (851 bp) that is absent in Kentucky bluegrass. Furthermore, a large 163 bp insertion region unique to Texas bluegrass was discovered within the 851 bp allele sequences, and was determined to be a miniature-inverted repeat transposable element or jumping gene. This new discovery may aid future investigations into the origin of Texas bluegrasses, or in particular the relationship of the 851 bp Texas bluegrass *trx* allele to other grass species.

Lastly, select hybrid bluegrasses were maintained under shaded environments to evaluate their response in comparison to commercial hybrid bluegrasses, a shade intolerant Kentucky bluegrass variety (Kenblue), and Rebel Exeda tall fescue which is well known for moderate shade tolerance. Our results show that daily leaf elongation rate was the best quantitative indicator of shade tolerance. A short time-span of only four weeks would be necessary for these evaluations that could allow for more genotypes or repetitions during the peak growth season. The application of TE resulted in both beneficial and detrimental effects on turfgrass quality depending on the genotype, although leaf elongation was significantly reduced for all tested genotypes however, our results suggests its use is not necessary for dwarf genotypes. The shade study analysis designated hybrid bluegrasses DALBG 1201 and TAES 5654 for use under moderate shade environments for their high turfgrass quality and reduced leaf elongation rate that would make management easier.

In conclusion, interspecific hybridization between Texas and Kentucky bluegrasses can be greatly assisted by proper vernalization conditions, ploidy determination in each species, and molecular marker technology that can result in high performing hybrids tolerant to environmental conditions on home lawns and low-maintenance areas in the southern United States.

REFERENCES

Abraham, E.M., B. Huang, S.A. Bonos, and W.A. Meyer. 2004. Evaluation of drought resistance for Texas bluegrass, Kentucky bluegrass, and their hybrids. Crop Sci. 44:1746-1753.

Abraham, E.M., M. Aa, J. Honig, C. Kubik, and S.A. Bonos. 2005. The use of SCAR markers to identify TBG x KBG hybrids. Int. Turfgrass Soc. Res. J. 10:495-500.

Acquaah, George. 2012. Polyploidy in plant breeding. In: Principles of plant genetics and breeding, 2nd ed. John Wiley and Sons Inc., Chichester, UK. p. 462-469. doi: 10.1002/9781118313718.ch24

Aggie Horticulture. 2014. Forage grasses. Texas A&M AgriLife Extension. http://aggie-horticulture.tamu.edu/vegetable/guides/the-crops-of-texas/forage-grasses/ (accessed 19 Jun 2014).

Albertini, E., G. Barcaccia, A. Perceddu, S. Sorbolini, and M. Falcinelli. 2001. Mode of reproduction is detected by Parth1 and Sex1 SCAR markers in a wide range of facultative apomictic Kentucky bluegrass varieties. Mol. Breed. 7:293-300.

Akerberg, E. 1939. Apomictic and sexual seed formation in *Poa pratensis* L. Hereditas 25: 359-370.

Akerberg, E., and S. Bingefors. 1953. Progeny studies in the hybrid *Poa pratensis* x *Poa alpina.* Hereditas. 39(1-2):125-136.

Arumuganathan, K., and E.D. Earle. 1991. Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Rep. 9(3):229-233.

Arumuganathan, K., S.P. Tallury, M.L. Fraser, A.H. Bruneau, and R. Qu. 1999. Nuclear DNA content of thirteen turfgrass species by flow cytometry. Crop Sci. 39:1518-1521.

Barcaccia,G., A. Mazzucato, A. Belardinelli, M. Pezzotti, S. Lucretti, and M. Falcinelli. 1997. Inheretance of parental genomes in progenies of *Poa pratensis* L. from sexual and apomictic genotypes as assessed by RAPD markers and flow cytometry. Theor. Appl. Genet. 95:516-524.

Bashaw, E.C. and C.R. Funk. 1987. Apomictic grasses. In: W.R. Fehr, editor, Principles of cultivar development, Vol 2. Crop species. Macmillian Publishing Co., New York. p. 40-82.

Beard, J.B. 1973. Turfgrass science and culture. Prentice Hall, Englewood Cliffs, NJ.

Beard, J.B. 1965. Factors in the adaptation of turfgrasses to shade. Agron. J. 57:457-459.

Bell, G.E. 2011. Turfgrass physiology and ecology: Advanced management principles. Cambridge University Press, UK.

Besse, I., and B.B. Buchanan. 1997. Thioredoxin-linked plant and animal processes: the new generation. Bot. Bull. Academia Sinica (Taipei). 38:1-11.

Brilman, L. 2009. Kentucky bluegrass classification. In: Ideas in Play. Seed Research of Oregon. http://www.sroseed.com/IdeasInPlay/PDF_articles/HeatTol_Blue.pdf (accessed 1 August 2014).

Brittingham, W.H. 1943. Type of seed formation as indicated by the nature and extent of variation in Kentucky bluegrass and its practical implications. J. Agric. Res. 76:225-264.

Brown, W.L. 1939. Chromosome complements of five species of *Poa* with an analysis of variation in *Poa pratensis*. Am. J. Bot. 26(9):717-723.

Bunnell, B.T., L.B. McCarty, J.E. Faust, W.C. Bridges, Jr., and N.C. Rajapakse. 2005. Quanitifying a daily light integral requirement of a 'TifEagle' Bermudagrass golf green. Crop Sci. 45(2):569-574.

Bureau, T.E., and S.R. Wessler. 1994. *Stowaway*: a new family of inverted-repeat elements associated with genes of both monocotyledonous and dicotyledonous plants. Plant Cell 6: 907-916.

Burner, D.M., and C.P. West. 2010. Improving tall fescue shade tolerance: identifying candidate genotypes. Agroforest. Syst. 79(1): 39-45.

Busey, P., and E.H. Davis. 1999. Turfgrass in the shade environment. Proc. Fla. State Hort. Soc. 104:353-358.

Canode, C.L., and M. Perkins. 1977. Floral induction and initiation in Kentucky bluegrass cultivars. Crop Sci. 17:278-282.

Carnahan, H.L., and H.D. Hill. 1961. Cytology and genetics of forage grasses. Bot. Rev. 27(1):1-162.

Carson, T.D., D.B. White, and A.G. Smith. 2007. Distinguishing creeping bentgrass (*Poa annua* var. *reptans*) genotypes using inter-simple sequence repeat markers. HortScience 42(2):373-377.

Clausen, J. 1961. Introgression facilitated by apomixis in polyploid *Poas*. Euphytica 10:87-94.

Cockerham, S.T., S.B. Ries, G.H. Riechers, and V.A. Gibeault. 2002. Turfgrass growth response under restricted light: growth chamber studies. In: California Turfgrass Culture. Univ. of Calif. Coop. Ext. Volume 52 (3-4): 13-20.

de Laat, A.M.M., W. Gohde, and M.J.D.C Vogelzang. 1987. Determination of ploidy of single plants and plant populations by flow cytometry. Plant Breed. 99(4):303-307.

Eaton, T.D., J. Curley, R.C. Williamson, and G. Jung. 2004. Determination of the level of variation in polyploidy among Kentucky bluegrass cultivars by means of flow cytometry. Crop Sci. 44:2168-2174.

Ervin, E.H., and A.J. Koski. 2001. Trinexapac-ethyl increases Kentucky bluegrass leaf cell density and chlorophyll concentration. HortScience. 36(4): 787-789.

Fei, S. 2003. Vernalization requirement of Texas bluegrass (*Poa arachnifera*). Poster presented at the 2003 ASA, CSSA, and SSSA International Annual Meeting, Denver, CO. 2-6 Nov. 2003.

Feldhake, C., J. Butler, and R. Danielson. 1985. Turfgrass evapotranspiration: Responses to shade preconditioning. Irr. Sci. 6(4):265-270.

Feschotte, C., X. Zhang, and S.R. Wessler. 2002. Miniature inverted-repeat transposable elements and their relationship to established DNA transposons. In: N.L. Craig et al., editors, Mobile DNA II. ASM Press, Washington, D.C. p. 1147-1158.

Feschotte, C., L. Swamy, and S.R. Wessler. 2003. Genome-wide analysis of mariner-like transposable elements in rice reveals complex relationships with Stowaway miniature inverted repeat transposable elements (MITEs). Genetics 163:747-758.

Froud-Williams, R.J., J.R. Hilton, and J. Dixon. 1986. Evidence for an endogenous cycle of dormancy in dry stored seeds of *Poa trivialis* L. New Phytol. 102:123-131.

Funk, C.R., R.E. Engel, and R.W. Duell. 1981. Kentucky bluegrasses and their culture in New Jersey lawns. Rutgers Turfgrass Conf. Proc., New Brunswick, New Jersey. p. 117-137.

Gardner, D.S., and J.A. Taylor. 2002. Change over time in quality and cover of various turfgrass species and cultivars maintained in shade. HortTech. 12(3):465-469.

Gardner, D.S., and B.G. Wherley. 2005. Growth response of three turfgrass species to nitrogen and trinexapac-ethyl in shade. HortScience 40(6):1911-1915.

Gardner, D.S., and R.M. Goss. 2012. Management of turfgrass in shade. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. American Soc. of Agron., Madison, WI. p. 219-247.

Geer, L.Y., A. Marchler-Bauer, R.C. Geer, L. Han, J. He, S. He, C. Liu, W. Shi, and S.H. Bryant. 2010. The NCBI BioSystems database. Nucleic Acids Res. 38: D492-6.

Gelhaye, E., N. Rouhier, and J.P. Jacquot. 2004. The thioredoxin *h* system of higher plants. Plant Physiol. Biochem. 42:265-271.

Gillespie, L.J., and R.J. Soreng. 2005. A phylogenetic analysis of the bluegrass genus *Poa* based on cpDNA restriction site data. Syst. Bot. 30(1):84-105.

Goldman, J.J. 2008. The use of ISSR markers to identify Texas bluegrass interspecific hybrids. Plant Breed. 127:644-646.

Gould, F.W. 1958. Chromosome numbers in southwestern grasses. Am. J. Bot. 45(10):757-767.

Graebe, J. E. 1987. Gibberellin biosynthesis and control. Annu. Rev. Plant Physiol. 38:419-465.

Grazi, F., M. Umaerus, and E. Akerberg. 1961. Observations on the mode of reproduction and the embryology of *Poa pratensis*. Hereditas 47:489-541.

Griffith-Jones, S., R.J. Grocock, S. van Dongen, A. Bateman, and A.J. Enright. 2006. miRBase: microRNA sequences, targets an gene nomenclature. Nucleic Acids Res. 34:D140-D144. doi: 10.1093/nar/gkj112

Grun, P. 1954. Cytogenetic studies of *Poa*. I. Chromosome numbers and morphology of interspecific hybrids. Am. J. Bot. 4:671-678.

Hall, M. 1996. Agronomy facts: Kentucky bluegrasses. Penn. State Univ. Press, University Park, PA.

Han, S.J. 1969. Effects of genetic and environmental factors on apomixes and the characteristics of non-maternal plants in Kentucky bluegrass (*Poa pratensis* L.). Ph.D. diss., Rutgers Univ., New Brunswick, NJ.

Hardison, J., J.B. Burr, J.R. Frelich, and G. Marquez. 2008. Hybrid variety of (Texas bluegrass X Kentucky bluegrass) X Kentucky bluegrass designated 'HB 329'. U.S. Plant Patent 18439. Date issued: 9 February.

Hartung, M.E. 1946. Chromosome numbers in *Poa, Agropyron*, and *Elymus*. Am. J. Bot. 33(6):516-531.

Hintzen, J.J., and A.J.P. van Wijk. 1985. Ecotype breeding and hybridization in Kentucky bluegrass (*Poa pratensis* L.). In: F. Lemarie, editor, 5th International Turfgrass Research Conference, Avignon, France.

Hitchcock, A.S. 1950. Manual of the grasses of the United States. 2nd ed. Revised by A. Chase. USDA Miscellaneous Publication 200. U.S. Govt. Print. Office, Washington, DC.

Hofacker, I.L., W. Fontana, P.F. Stadler, S. Bonhoeffer, M. Tacker, and P. Schuster. 1994. Fast folding and comparison of RNA secondary structures. Monatshefte f. Chemie. 125:167-188.

Honig, J.A., S.A. Bonos, and W.A. Meyer. 2010. Isolation and characterization of 88 polymorphic microsatellite markers in Kentucky bluegrass (*Poa pratensis* L.). HortScience 45(11):1759-1763.

Huff, D.R. 2003a. Kentucky bluegrass. In: M.D. Casler and R.R. Duncan, editors, Turfgrass biology, genetics, and breeding. John Wiley and Sons Inc., Hoboken, NJ. p. 27-37.

Huff, D.R. 2003b. Annual bluegrass. In: M.D. Casler and R.R. Duncan, editors, Turfgrass biology, genetics, and breeding. John Wiley and Sons Inc., Hoboken, NJ. p. 39-51.

Huff, D.R. 2010. Bluegrasses. In: B. Boller, U. K. Posselt and F. Veronesi, editors, Handbook of plant breeding: Fodder crops and amenity grasses. Vol. 5, Springer Publishing. New York, NY, p. 345-379. doi: 10.1007/978-1-4419-0760-8_15

Huff, D.R. and J. M. Bara. 1993. Determining genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow cytometry and silver-stained RAPD markers. Theor. Appl. Genet. 87:201-208.

Hurley, R. (n.d.). Turf-type tall fescues. 1 Jan. 2015. < http://archive.lib.msu.edu/tic/mitgc/article/1989141.pdf>.

Junttila, O., O.M. Heide, B. Lindgard, and A. Ernstsen. 1997. Gibberellins and photoperiodic control of leaf growth in *Poa pratensis*. Physiol. Plant. 65: 135-145.

Juttner, J., D. Olde, P. Landridge, and U. Baumann. 2000. Cloning and expression of a distinct subclass of plant thioredoxins. Eur. J. Biochem. 267:7109-7117.

Karcher, D. E., and M. D. Richardson. 2005. Batch analysis of digital images to evaluate turfgrass characteristics. Crop Sci. 45:1536-1539.

Kaye, T.N. 1997. Seed dormancy in high elevation plants: implications for ecology and restoration. In: T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke, and M.V. Wilson, editors, Conservation and management of native plants and fungi. Native Plant Society of Oregon, Corvallis, OR.

Kelley, A.M., P.G. Johnson, B.L. Waldron, and M.D. Peel. 2009. A survey of apomixes and ploidy levels among *Poa* L. (*Poaceae*) using flow cytometry. Crop Sci. 49(4):1395-1402.

Kephart, K.D., and D.R. Buxton. Nonstructural carbohydrates in cool- and warm-season perennial grasses adapted to shaded conditions. U.S. Dairy Forage Research Center. Research summaries. 1996. pp. 85-86.

Kiellander, C.L. 1942. A subhaploid *Poa pratensis* L. with 18 chromosomes, and its progeny. Svensk Bot. Tidskr. p. 200-220.

Kindiger, B., and J. Wipff. 2009. Frequency of androgenesis in *Poa arachnifera* interspecific hybridizations. Grassland Sci. 55(4):200-205.

Kindiger, B., T. Conley, and H.W. Cai. 2013. Generation and release of molecular markers for *Poa arachnifera* Torr. Grassland Sci. 59 (3):160-165.

Kindiger, B., T. Conley, G. Keith-Stanley, and H. Cai. 2011. Isolation and characterization of microsatellite markers in the grass *Poa arachnifera* Torr. Grassland Sci. 57:173-178.

Koski, T. 2012. Hybrid (Kentucky x Texas) bluegrasses for turf use in Colorado. CMG GardenNotes #563. http://www.ext.colostate.edu/mg/gardennotes/563 (accessed 19 January 2012).

Kuang, H., C. Padmanabhan, F. Li, A. Kamei, P.B. Bhaskar, S. Ouyang, J. Jiang, C.R. Buell, and B. Baker. 2009. Identification of miniature inverted-repeat transposable elements (MITEs) and biogenesis of the siRNAs in the *Solanaceae*: New functional implications for MITEs. Genome Res. 19:42-56.

Larsen, S.U. and B.M. Bibby. 2005. Differences in thermal time requirement for germination of three turfgrass species. Crop Sci. 45:2030-2037.

Laurie, D. and M. Bennett. 1985. Nuclear DNA content in the genera *Zea* and *Sorghum*: intergeneric, interspecific, and intraspecific variation. Heredity 55:307-313.

Lickfeldt, D.W., D.S. Gardner, B.E. Branham, and T.B. Voigt. 2001. Implications of repeated trinexapac-ethyl applications on Kentucky bluegrass. Agron. J. 93:1164-1168.

Lin, C.H., R.L. McGraw, M.F. George, and H.E. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. Agroforestry Systems. 44: 109-119.

Love, A., and D. Love. 1975. Nomenclatural adjustments in some European monocotyledons. Folia Geobot. Phytotax., Praha. 10:271-276.

Mao, Q., and D.R. Huff. 2012. The evolutionary origin of *Poa annua* L. Crop Sci. 52:1910-1922.

Marcus, F., S.H. Chamberlain, C. Chu, F.R. Masiarz, S. Shin, B.C. Yee, and B.B. Buchanan. 1991. Plant thioredoxin *h*: an animal-like thioredoxins occurring in multiple cell compartments. Arch. Biochem. Biophys. 287:195-198.

Matzke, M.A., and A.J.M. Matzke. 1998. Polyploidy and transposon. Trends Ecol. Evol. 13:241.

Matzk, F., A. Meister, and I. Schubert. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. The Plant J. 21(1):97-108.

Mazzucato, A., M. Falcinelli, and F. Veronesi. 1996. Evolution and adaptedness in a facultatively apomictic grass, *Poa pratensis* L. Euphytica 92:13-19.

McCaskill, J.S. 1990. The equilibrium partition function and base pair binding probabilities for RNA secondary structures. Biopolymers. 29:1105-1119.

Meeks, M., A. Chandra, S.P. Metz, A.D. Genovesi, J.C. Read, R.H. White, G. Miller, E. Guertal, H.W. Philley, and J. Sorochan. Registration of DALBG 1201 hybrid bluegrass. J. Plant Reg. doi:10.3198/jpr2014.03.0015

Meyer, W., K. Hignight, and S. Tubbs. 2005. Boutique Kentucky bluegrass. U.S. plant variety protection cert. no. 200200112. Date Issued: March 11, 2005.

Milesi, C., C.M. Elvidge, J.B. Dieta, B.T. Tuttle, R.R. Nemani, and S.W. Running. 2005. A strategy for mapping and modeling the ecological effects of US lawns. J. Turfgrass. Manage. 1:83-97.

Mondoni, A., G. Rossi, S. Orsenigo, and R.J. Probert. 2012. Climate warming could shift the timing of seed germination in alpine plants. Annals of Bot. doi:10.1093/aob/mcs097

Morris, K.N. 2003. The National Turfgrass Research Initiative. National Turfgrass Federation, Inc., National Turfgrass Evaluation program. http://www.ntep.org/pdf/turfinitiative.pdf (accessed 1 Oct. 2014).

Morris, K.N. 2010. National Kentucky bluegrass test – 2005. Final Rpt. 2006-2010. NTEP no. 11-10.

Morris, K.N. 2013. National Kentucky bluegrass test – 2011. Prog. Rpt. 2013. NTEP no. 14-2.

Morris, K.N., M.P. Kenna, C.S. Throssell, and R.C. Shearman. 2005. The National Turfgrass Research Initiative-A national turfgrass research strategy for the USA. Int. Turfgrass Soc. Annexe-Tech. Pap. 10:63-64.

Murovec, J., D. Kastelec, B. Vilhar, J. Cop, and B. Bohanec. 2009. High variability of nuclear DNA content in cultivars and natural populations of *Poa pratensis* L. in relation to morphological characters. Acta Biologica Cracoviensia series Botanica 51:45-52.

Myers, W.M. 1947. Cytology and genetics of forage grasses. Bot. Rev. 13:319-367.

National Climatic Data Center. 2011. State of the Climate. National Oceanic and Atmospheric Administration. http://www.ncdc.noaa.gov/sotc/drought/2011/8 (accessed 8 Jan. 2015).

National Climatic Data Center. 2012. National Oceanic and Atmospheric Administration. http://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00417588/detail (accessed 8 Jan. 2015).

Patterson, J.T., S.R. Larson, and P.G. Johnson. 2005. Genome relationships in polyploidy *Poa pratensis* and other *Poa* species inferred from phylogenetic analysis of nuclear and chloroplast DNA sequences. Genome 48:76-87.

Pepin, G.W., and C.R. Funk. 1974. Evaluation of turf, reproductive, and disease-response characteristics in crossed and selfed progenies of Kentucky bluegrass. Crop Sci. 14:356-359.

Piriyapongsa, J., and I.K. Jordan. 2007. A family of human microRNA genes from miniature inverted repeat transposable elements. PLoS One 2:e203.

Piriyapongsa, J., and I.K. Jordan. 2008. Dual coding of siRNAs and miRNAs by plant transposable elements. RNA 14:814-821.

Qian, Y.L., and M.C. Engelke. 1999. Influence of trinexapac-etyl on Diamond zoysiagrss in a shade environment. Crop Sci. 39:202-208.

Qian, Y.L., M.C. Engelke, M.J.V. Foster, and S.M Reynolds. 1998. Trinexapac-ethyl restricts shoot growth and improves quality of Diamond zoysiagrass under shade. HortScience 33:1019-1022.

Read, J.C. 1988. Sex expression in Texas bluegrass. Texas A&M AgriLife. http://agrilife.org/foragesoftexas/files/2011/01/SupplementalFishmeal1.pdf (accessed 19 Jan. 2012).

Read, J.C. 1994. Potential of Texas bluegrass x Kentucky bluegrass hybrids for turf in north central Texas. In: Texas Turfgrass Research - 1993, Consolidated Progress Report. Texas Agricultural Experiment Station, Dallas, TX. p. 11–12.

Read, J.C. 2001. Utilization of apomictic and dioecious method of reproduction in breeding of *Poa* spp. Int. Turfgrass Soc. Res. J. 9:202-205.

Read, J.C., J.A. Reinert, P.F. Colbaugh, and W.E. Knoop. 1999. Registration of 'Reveille' hybrid bluegrass. Crop Sci. 39:590.

Reid, J. B. and J. J. Ross. 1991. Gibberellin mutants in Pisum and Lathyrus, p. 40-50. In Takahashi et al. (eds.). Gibberellins. Springer Verlag, New York.

Renganayaki, K., J.C. Read, A.K. Fritz. 2001. Genetic diversity among Texas bluegrass genotypes (*Poa arachnifera* Torr.) revealed by AFLP and RAPD markers. Theor. Appl. Genet. 102:1037-1045.

Rhoads, J.L., J.H. Dunn, D.D. Minner, and K.L. Hunt. 1992. Reproductive morphology of five Kentucky bluegrass cultivars. Agron. J. 84(2):144-147.

Richardson, M., E. Slack, T. Fermanian, S. Reid, E. Watkins, W. Meyer, B. Meyer, S. Langlois, M. Fraser, K. Hignight, and M. Goatley. 2010. Cooperative Turfgrass Breeders Test Report - 2010. 21 Jan 2015. <http://www.ctbt-us.info/>.

Rohlf, F.J. 2005. NTSYSpc: Numerical taxonomy system, ver. 2.20. Exeter Publishing, Ltd., Setauket, NY.

Rose-Fricker, C.A., D.A. Smith, S.A. Bonos and W.A. Meyer. 2007. Longhorn Kentucky bluegrass. U.S. Plant variety protection application no. 200700091. Applied for.

Savidan, Y., J.G. Carman, and T. Dresselhaus. 2001. The flowering of apomixes: from mechanisms to genetic engineering. CIMMYT, Mexico.

Serrato, A.J., and F. J. Cejudo. 2003. Type-*h* thioredoxins accumulate in the nucleus of developing wheat seed tissues suffering oxidative stress. Planta. 217:392-399.

Shortell, R.R. 2009. Genetic diversity of Kentucky bluegrass genotypes in morphological, agronomic, and abiotic stress tolerance characteristics. Ph.D. diss., Rutgers Univ., New Brunswick, NJ.

Simpson, G.M. 1990. Seed dormancy in grasses. Cambridge University Press, Cambridge, New York.

Small, R.L., R.C. Ryburn, R.C. Cronn, T. Seelanan, and J.F. Wendel. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear *Adh* sequences for phylogeny reconstruction in a recently diverged plant group. Am. J. Bot. 85:1301-1315.

Smith, D. A., and W. A. Meyer. 2009. Spitfire Kentucky bluegrass. U.S. Plant variety protection application no.200900426. Applied for.

Smith, D. A., L. Brilman, E. Szerszen, S. A. Bonos, and W. A. Meyer. 2008. United States Plant Variety Protection Application no. 200800129. Bandera Kentucky bluegrass.

Soltis D.E., and P.S. Soltis. 1999. Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. 14:348-352.

Steinke, K., and J.C. Stier. 2003. Nitrogen selection and growth regulator applications for improving shaded turf performance. Crop Sci. 43:1399-1406.

Stier, J.C., J.N. Rogers, III, J.R. Crum, and P.E. Rieke. 1999. Flurprimidol effects on Kentucky bluegrass under reduced irradiance. Crop Sci. 39: 1423-1430.

Stier, J.C., and J.N. Rogers, III. 2001. Trinexapac-ethyl and iron effects on supina and Kentucky bluegrasses under low irradiance. Crop Sci. 41: 457-465.

Stowell, L., and W. Gelernter. 1997. *Rhizoctonia* biology and management. Pace consulting, San Diego, CA.

Su, K., D.J. Bremer, S.J. Keeley, and J. Fry. 2007. Effects of high temperature and drought on a hybrid bluegrass compared with Kentucky bluegrass and tall fescue. Crop Sci. 47:2152-2161.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512-526.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725-2729.

Tan, Z.G., and Y.L. Qian. 2003. Light intensity affects gibberellic acid content in Kentucky bluegrass. HortScience 38(1):113-116.

Tegg, R.S., and P.A. Lane. 2004. A comparison of the performance and growth of a range of turfgrass species under shade. Aust. J. of Exp. Agric. 44:353-358.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

USDA-ARS National Genetic Resources Program. 1997. Germplasm Resources Information Network (GRIN) database. Western Regional Plant Introduction Station, Pullman, WA. http://www.ars-grin.gov/npgs/acc/acc_queries.html (accessed 4 Jan. 2012)

Vance, N.C. 2010. Evaluation of native plant seeds and seeding in the east-side central cascades ponderosa pine zone. Gen. Tech. Rep. PNW-GTR-823. Portland, OR: USDA, Forest Service, Pacific North-west Research Station. p. 85.

Vieira Dos Santos, C. and P. Rey. 2006. Plant thioredoxins are key actors in the oxidative stress response. Trends in Plant Sci. 11(7):329-334.

Vinall, H.N., and M.A. Hein. 1937. Breeding miscellaneous grasses. In: Yearbook of Agriculture, 1937. USDA, U.S. Gov. Print. Office, Washington, DC.

Wallace, V., T. Fermanian, K. Blanchet, S. Ebdon, E. Watkins, W. Meyer, S. Langlois, M. Fraser, K. Hignight, C. Fricker. 2013. Cooperative Turfgrass Breeders Test Report – 2013. 13 June 2014. <http://www.ctbt-us.info/>.

Wang, X.Y., T.M. Hu, Q.Z. Wang, L.M. Tian, X.L. Zhang, and K. Tian. 2009. Growth of Kentucky bluegrass as influenced by nitrogen and trinexapac-ethyl. Agr. Sci. China. 8(12):1498-1502.

Watkins, E., and W.A. Meyer. 2004. Morphological characterization of turf-type tall fescue genotypes. HortScience 39(3):615-619.

Watson, V.H., G. Hagedorn, W.E. Knight, and H.A. Pearson. 1984. Shade tolerance of grass and legume germplasm for use in the southern forest range. J. Range Mgt. 37:229-232.

Wherley, B.G., D.S. Gardner, and J.D. Metzger. 2005. Tall fescue photomorphogenesis as influenced by changes in the spectral composition and light intensity. Crop Sci. 45:562-568.

Wherley, B.G., A. Chandra, A. Genovesi, M. Kearns, T. Pepper, and J. Thomas. 2013. Developmental response of St. Augustinegrass cultivars and experimental lines in moderate and heavy shade. HortScience. 48:1047-1051.

Wieners, R., S.Z. Fei, and R.C. Johnson. 2006. Characterization of a USDA Kentucky bluegrass (*Poa pratensis* L.) core collection for reproductive mode and DNA content by flow cytometry. Genet. Res. Crop Evol. 53:1531-1541.

Williams, C.B. 1916. How to secure better lawns in North Carolina. N.C. College of Agric. and Mech. Arts. Ext. Cir. No. 28.

Wong, J. H., Y.B. Kim, P.H. Ren, N. Cai, M.J. Cho, P. Hedden, P.G. Lemaux, and B.B. Buchanan. 2002. Transgenic barley grain overexpressing thioredoxin shows evidence that the starchy endosperm communicates with the embryo and the aleurone. Proceedings of the National Academy of Science, U.S.A. 99:16325-16330.

Wood, G.M. 1968. Evaluating turfgrasses for shade tolerance. Agron. J. 61:347-352.

Wu, L., D. Huff, and W.B. Davis. 1985. Tall fescue turf performance under a tree shade. HortScience 20:281-282.

Yang. R. 2014. Analysis of linear and non-linear genotype×environment interaction. Front Genetic 2014. 5:227.

Zeid, M., J.K. Yu, I. Goldowitz, M.E. Denton, D.E. Costich, C.T. Jayasuriya, M. Saha, R. Elshire, D. Benscher, F. Breseghello, J. Munkvold, R.K. Varshney, G. Belay, and M.E. Sorrells. 2010. Cross-amplification of EST-derived markers among 16 grass species. Field Crops Res. doi:10.1016/j.fcr.2010.03.014

Zhang, W.D., G.S. Liu, S.Y. Chen, X.F. Li, and H.J. Li. 2004. Molecular cloning, expression and activity assay of thioredoxin *h*: a gene related to self-incompatibility. Acta Agronomica Sinica. 30(12):1192-1198.

Zuker, M., and P. Stiegler. 1981. Optimal computer folding of large RNA sequences using thermodynamic and auxiliary information. Nucleic Acids Res. 9:133-148.

APPENDIX A

Table A-1. Air temperature data recorded monthly for the years 2010 to 2012 for each test location.

		Air Temperature (°C - Max, Min, Avg)																	
		Jan			Feb			Mar			Apr			May			Jun		
Location	Year	Max†	Min‡	Avg§	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg
Auburn, AL¶	2010	20.0	-8.9	5.5	21.1	-4.4	5.7	24.4	-1.7	11.2	31.1	7.8	19.1	33.3	10.6	23.6	35.6	20.0	27.3
	2011	22.2	-7.2	6.3	25.6	-3.9	10.9	30.0	2.2	15.3	30.6	3.3	19.5	34.4	6.7	22.2	37.8	18.3	27.6
	2012	24.4	-5.0	11.0	24.4	-5.6	12.2	29.4	4.4	18.7	31.1	5.0	18.9	34.4	13.3	23.6	38.3	13.3	25.1
Starkville, MS¶	2010	23.3	-11.1	3.5	21.1	-6.7	4.0	25.0	-3.3	10.7	31.1	5.0	18.4	23.8	23.8	23.8	37.2	18.9	28.4
	2011	24.4	-9.4	5.4	26.1	-8.3	9.2	30.0	1.1	13.8	32.2	3.9	19.3	33.9	6.7	21.7	37.8	17.8	28.0
	2012	22.8	-6.7	9.5	28.3	-6.7	9.6	30.0	0.6	18.3	30.0	3.9	18.9	35.0	12.8	23.8	39.4	15.0	25.6
Raleigh, NC¶	2010	20.7	-8.9	3.2	18.2	-6.6	3.1	25.2	-2.9	11.2	31.8	3.2	17.3	33.4	8.6	21.7	36.1	16.4	26.3
	2011	19.6	-9.3	3.1	27.0	-5.8	8.7	28.3	-1.9	10.8	30.1	0.4	17.1	34.6	7.0	20.4	34.9	13.4	25.6
	2012	20.4	-7.5	7.6	23.9	-5.8	8.4	28.3	-2.0	15.5	29.6	2.3	15.6	33.5	8.6	21.4	40.0	11.8	23.2
Knoxville, TN¶	2010	16.1	-13.9	0.8	18.3	-7.8	1.4	22.8	-6.7	7.8	29.4	1.7	15.9	31.1	5.6	20.8	35.6	13.9	25.8
	2011	20.0	-11.7	2.4	22.2	-8.3	6.6	26.7	-1.1	10.3	30.0	-0.6	15.3	33.3	6.1	19.8	35.6	16.1	25.6
	2012	18.3	-8.3	5.5	25.0	-9.4	6.9	30.0	-3.9	14.8	28.9	-0.6	15.6	32.8	7.2	21.5	38.3	9.4	23.5
Dallas, TX#	2010	23.3	-11.7	6.2	17.8	-4.4	5.0	28.3	-1.7	12.7	28.3	5.0	18.7	36.1	10.0	24.1	36.7	19.4	29.2
	2011	23.3	-7.2	5.8	27.2	-12.8	9.1	28.9	-1.7	15.9	31.7	2.8	21.2	35.0	5.0	22.1	38.3	18.9	29.6
	2012	26.7	-3.3	10.2	26.7	-3.3	10.7	28.3	3.9	17.8	31.1	7.8	20.8	33.9	12.2	24.5	38.9	16.1	28.0

† The maximum (Max) air temperature was the highest value recorded for the entire month.
‡ The minimum (Min) air temperature was the lowest value recorded for the entire month.
§ The average (Avg) air temperature was the mean of each daily recorded average air temperature for the entire month.
¶ Data were provided from weather stations at each test location.
# Data for Dallas, TX was obtained from a National Climatic Data Center (NCDC) weather station in Richardson, TX (GHCND:USC00417588) located approximately 2.4 kilometers northeast from the test location.

Table A-1. continued…

Location	Year	Air Temperature (°C - Max, Min, Avg)																	
		Jul			Aug			Sep			Oct			Nov			Dec		
		Max†	Min‡	Avg§	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg
Auburn, AL¶	2010	38.3	20.0	28.6	37.2	21.1	28.2	36.1	13.9	26.2	31.7	7.2	19.6	26.7	0.0	13.9	23.3	-8.3	5.6
	2011	36.7	21.1	27.1	37.8	19.4	28.4	35.6	13.9	23.1	30.6	3.9	33.9	27.2	1.1	14.2	22.8	-2.2	11.4
	2012	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starkville, MS¶	2010	29.2	29.2	29.2	40.0	19.4	29.7	38.3	9.4	25.4	33.3	0.6	18.3	26.1	-2.2	12.9	5.0	5.0	5.0
	2011	38.3	18.3	28.3	38.3	16.7	28.1	37.8	9.4	21.8	31.1	-2.2	15.2	27.2	-5.0	12.4	23.3	-5.6	7.7
	2012	38.9	20.0	28.2	37.8	15.6	26.1	34.4	8.9	23.2	30.0	1.7	16.3	28.9	-3.9	10.7	24.4	-5.6	10.2
Raleigh, NC¶	2010	38.4	13.3	27.1	34.7	18.1	26.1	35.1	13.4	23.6	29.7	2.4	16.7	23.5	-1.4	10.5	20.9	-8.6	1.4
	2011	39.2	16.8	27.4	38.0	16.8	25.9	31.6	12.6	22.2	29.8	-0.1	14.9	25.1	-2.2	12.1	21.5	-2.6	9.3
	2012	39.1	19.7	26.9	33.5	16.7	24.6	33.6	8.7	21.2	29.7	3.0	15.5	23.2	-4.6	8.6	23.8	-4.2	9.5
Knoxville, TN¶	2010	36.7	15.6	26.9	37.2	16.1	26.8	34.4	8.9	22.3	28.9	0.6	14.8	23.3	-3.9	9.7	16.1	-12.8	-0.1
	2011	36.1	17.2	27.7	35.0	13.9	25.2	36.1	11.1	20.8	27.8	0.0	14.4	23.9	-3.9	10.7	22.2	-5.0	6.5
	2012	40.6	18.3	26.8	33.3	13.3	24.1	32.8	5.6	21.3	26.1	3.9	14.2	23.3	-5.6	7.6	22.2	-6.1	7.4
Dallas, TX#	2010	38.3	22.2	29.5	41.1	18.3	31.7	34.4	11.1	25.8	29.4	3.9	19.6	27.8	-1.1	14.1	28.3	-4.4	8.9
	2011	40.6	24.4	32.5	42.2	22.8	33.5	41.1	13.3	26.1	30.6	3.9	19.1	27.2	0.0	14.0	23.3	-5.6	8.3
	2012	40.6	21.1	30.2	41.1	17.2	29.2	38.3	13.9	25.4	30.6	1.7	18.2	30.0	-0.6	14.6	27.2	-6.7	9.5

Table A-2. Precipitation recorded monthly for the years 2010 to 2012 from each location.

		Precipitation (mm)												
Location	**Year**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total**
Auburn, AL†	2010	134	84	118	28	143	107	32	67	53	33	45	54	899
	2011	52	107	131	52	28	75	164	26	162	36	83	99	1014
	2012	119	86	93	55	68	55	-	-	-	-	-	-	476
Starkville, MS†	2010	154	69	93	108	163	64	77	43	60	32	171	31	1064
	2011	138	67	165	306	53	131	109	46	182	26	89	165	1477
	2012	77	103	188	95	84	72	237	197	136	125	57	176	1546
Raleigh, NC†	2010	97	68	79	41	147	77	53	168	154	22	20	36	962
	2011	29	52	71	81	78	94	45	131	98	51	80	36	846
	2012	52	50	107	38	106	65	134	69	164	64	15	76	939
Knoxville, TN†	2010	158	71	73	64	107	32	150	71	110	101	171	56	1163
	2011	87	104	162	231	42	76	63	27	222	121	180	125	1439
	2012	144	103	154	92	92	64	159	105	194	71	27	157	1362
Dallas, TX‡	2010	90	75	89	43	39	56	98	28	222	22	77	49	886
	2011	40	33	2	134	180	62	4	10	36	119	38	121	777
	2012	149	50	168	45	76	100	5	69	72	33	0	72	838

† Data were provided from weather stations at each test location.
‡ Data for Dallas, TX was obtained from a National Climatic Data Center (NCDC) weather station in Richardson, TX (GHCND:USC00417588) located approximately 2.4 kilometers northeast from the test location.

APPENDIX B

Table B-1. Experimental photosynthetic photon flux (*PPF*) and daily light integrals (DLI) calculated for each light environment.

	PPF (%)[†]		
Experiment	Full Sunlight	Moderate Shade	Heavy Shade
May - July	89.32 ± 10.17	46.45 ± 5.70	19.52 ± 3.05
August - October	76.48 ± 4.53	28.83 ± 12.42	8.57 ± 3.21

	DLI (mol m^{-2} d^{-1})[‡]		
	Full Sunlight	Moderate Shade	Heavy Shade
May - July	29.94 ± 10.12	14.67 ±5.02	6.33 ± 2.05
August - October	23.54 ± 6.81	9.93 ± 3.07	4.08 ± 1.63

[†]Average *PPF*, or the amount of light transmitted, for each experiment x light environment were calculated from fourteen daily measurements at 1:00PM on sunny days. Averages are presented with the standard deviations.

[‡]The average daily light integral were calculated from each individual DLI across the experiments. Standard deviations are presented with the averages.

Table B-2. Daily leaf elongation rates calculated every two weeks during experiment 1.

	Daily leaf elongation rate ($cm \cdot d^{-1}$)					
	Collection Week					
Genotype[†]	2	4	6	8	10	AVG[y]
Rebel Exeda	0.6 d	0.8 e	1.0 c	1.0 bc	0.8 c	0.8 d
Kenblue	1.8 a	1.8 a	2.0 a	2.0 a	1.5 a	1.8 a
CS#4	0.7 d	1.2 c	1.4 b	1.2 b	0.9 bc	1.1 c
Thermal Blue	1.3 b	1.5 b	1.8 a	1.6 a	1.5 ab	1.5 b
Reveille	1.1 bc	1.1 cd	1.1 c	1.0 bc	0.7 c	1.0 c
DALBG 1201	0.7 d	0.9 de	0.9 c	0.8 bc	0.7 c	0.8 d
TAES 5654	0.6 d	0.7 e	0.9 c	0.7 c	0.5 c	0.7 d
TAES 5655	0.9 cd	1.1 cd	1.1 c	0.7 c	0.7 c	0.9 c
Model R^2	0.94	0.96	0.94	0.85	0.22	-
r[x]	0.96	0.99	0.96	0.94	0.91	-

[†]Genotype means were separated using Tukey's honestly significant difference test at a significance level of $P \leq 0.05$.
R^2 = Coefficient of determination; CV = Coefficient of variation.

Table B-3. The effect of TE on turfgrass quality of individual genotypes under full sunlight, moderate shade, and heavy shade.

	Final turfgrass quality (1-9 scale)[†]											
	Full sunlight				Moderate shade				Heavy shade			
Genotype	Control	TE	Mean difference[‡]	%Δ[§]	Control	TE	Mean difference	%Δ	Control	TE	Mean difference	%Δ
Rebel Exeda	7.3	7.5	0.2 NS	3	5.8	6.5	0.7 NS	12	4.0	3.8	-0.2 NS	-5
Kenblue	5.2	6.0	0.8 NS	15	3.7	4.7	1.0 NS	27	2.5	2.3	-0.2 NS	-8
CS#4	7.3	7.2	-0.1 NS	-1	5.0	5.7	0.7 NS	14	3.3	4.0	0.7 NS	21
Thermal Blue	6.3	6.5	0.2 NS	3	4.0	5.2	1.2 *	30	3.2	2.7	-0.5 NS	-16
Reveille	6.8	6.5	-0.3 NS	-4	4.5	4.7	0.2 NS	4	4.0	1.8	-2.2 *	-55
DALBG 1201	8.8	8.2	-0.6 NS	-7	7.2	6.7	-0.5 *	-7	5.8	5.5	-0.3 NS	-5
TAES 5654	9.0	7.3	-1.7 *	-19	6.7	6.5	-0.2 NS	-3	5.5	4.0	-1.5 NS	-27
TAES 5655	6.7	7.0	0.3 NS	4	5.5	4.3	-1.2 *	-22	3.5	2.7	-0.8 NS	-23
ANOVA												
Experiment[¶]			NS				NS				**	
Treatment[#]			NS				NS				NS	
Experiment x Treatment			NS				NS				NS	

NS, * Nonsignificant or significant at $P \leq 0.05$, respectively.

[†] Turfgrass quality ratings were assessed on a 1-9 scale (1 = brown/tan or dead; 5 = minimum acceptable quality).

[‡] Mean differences for turfgrass quality were calculated by subtracting mean control ratings from mean TE ratings.

[§] The percentage in change was calculated by dividing the mean difference over the control mean and multiplying by one-hundred.

[¶] Turfgrass quality between experiments were determined using Student's t-test at a significance level of $P \leq 0.05$. Data was pooled for all environments.

[#] Differences in turfgrass quality between the control and TE treatment means for each individual genotype were determined using Student's t-test at a significance level of $P \leq 0.05$.

Table B-4. The effect of TE on leaf elongation rate of individual genotypes under full sunlight, moderate shade, and heavy shade.

		Daily leaf elongation rate ($cm \cdot d^{-1}$)[†]											
		Full Sunlight				Moderate Shade				Heavy Shade			
	Genotype	Control	TE	Mean difference[‡]	%Δ[§]	Control	TE	Mean difference	%Δ	Control	TE	Mean difference	%Δ
Experiment 1	Rebel Exeda	0.7	0.5	-0.2 *	-29	0.8	0.4	-0.4 *	-50	0.8	0.3	-0.5 *	-63
	Kenblue	1.8	1.1	-0.7 *	-39	1.8	0.7	-1.1 *	-61	1.5	0.6	-0.9 *	-60
	CS#4	1.0	0.6	-0.4 *	-40	1.1	0.4	-0.7 *	-64	1.1	0.3	-0.8 *	-73
	Thermal Blue	1.3	0.7	-0.6 *	-46	1.5	0.6	-0.9 *	-60	1.3	0.4	-0.9 *	-69
	Reveille	1.0	0.6	-0.4 *	-40	1.0	0.4	-0.6 *	-60	0.7	0.2	-0.5 *	-71
	DALBG 1201	0.7	0.4	-0.3 *	-43	0.8	0.3	-0.5 *	-63	0.7	0.2	-0.5 *	-71
	TAES 5654	0.6	0.4	-0.2 *	-33	0.7	0.3	-0.4 *	-57	0.6	0.2	-0.4 *	-67
	TAES 5655	0.9	0.6	-0.3 *	-33	0.9	0.4	-0.5 *	-56	0.7	0.2	-0.5 *	-71
Experiment 2	Rebel Exeda	0.7	0.5	-0.2 *	-29	0.8	0.4	-0.4 *	-50	0.5	0.1	-0.4 *	-80
	Kenblue	1.6	0.9	-0.7 *	-44	1.6	0.6	-1.0 *	-63	1.0	0.1	-0.9 *	-90
	CS#4	1.1	0.6	-0.5 *	-45	1.0	0.3	-0.7 *	-70	0.8	0.2	-0.6 *	-75
	Thermal Blue	1.4	0.7	-0.7 *	-50	1.3	0.4	-0.9 *	-69	1.3	0.3	-1.0 *	-77
	Reveille	0.7	0.5	-0.2 *	-29	0.8	0.2	-0.6 *	-75	0.6	0.2	-0.4 *	-67
	DALBG 1201	0.7	0.3	-0.4 *	-57	0.7	0.2	-0.5 *	-71	0.6	0.2	-0.4 *	-67
	TAES 5654	0.6	0.3	-0.3 *	-50	0.7	0.2	-0.5 *	-71	0.7	0.1	-0.6 *	-86
	TAES 5655	1.0	0.4	-0.6 *	-60	1.0	0.3	-0.7 *	-70	0.8	0.2	-0.6 *	-75
ANOVA													
Experiment[¶]				**				NS				NS	
Treatment[#]				****				****				****	
Experiment x Treatment				NS				NS				NS	

* Significant at $P \leq 0.05$.

† Average daily leaf elongation rate was obtained by diving total elongation over the growth period by the number of days since trimming.

‡ Mean differences for average daily leaf elongation rates were calculated by subtracting mean control ratings from mean TE ratings.

§ The percentage in change was calculated by dividing the mean difference over the control mean and multiplying by one-hundred.

¶ Leaf elongation rate differences between experiments were determined using Student's t-test at a significance level of $P \leq 0.05$. Experiments were significantly different in all environments, so data was not pooled.

Differences in leaf elongation rate between the control and TE treatment means for each individual genotype were determined using Student's t-test at a significance level of $P \leq 0.05$.

APPENDIX C

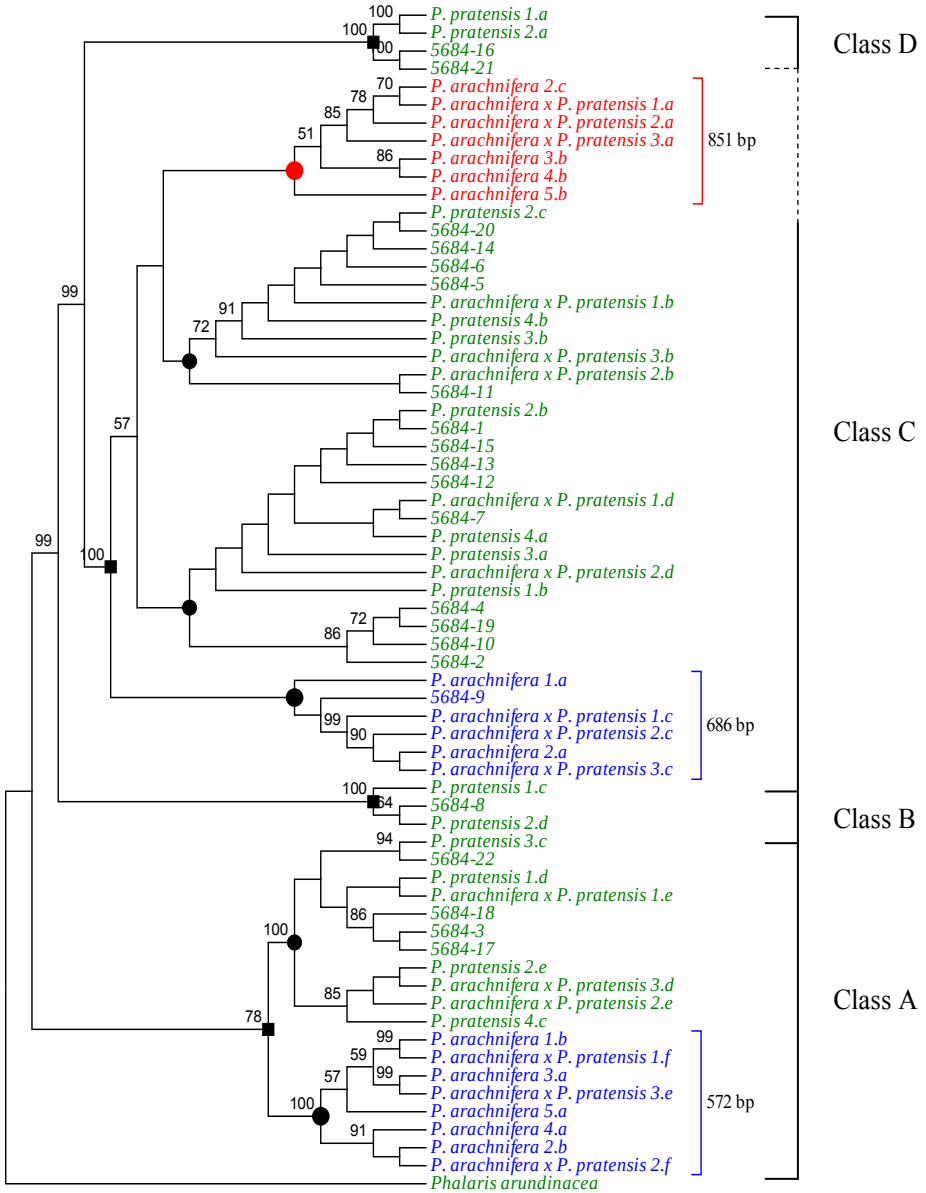


Fig. C-1. The most parsimonious tree produced for the comparison of 5684 trx sequences with *P. arachnifera, P. pratensis* sequences from Table 4-1 using Mega 6.0. The tree is rooted to *Phalaris arundinacea*. Clusters (●) in blue indicate new sequences from *P. arachnifera*, and clusters in green indicate sequences from *P. pratensis*. The red cluster (●) shows the 851 bp *P. arachnifera* sequence group containing the 163 bp insertion. Nodes shown as ■ represent one of four *P. pratensis* classes as reported in Patterson et al. (2005). The numerical values followed by the lower case alphabetical letter correspond to GenBank accession numbers in Table 4-1. Numbers following dashes for 5684 are clone sequence identifying numbers. Numbers above the tree branch indicates the bootstrap values from 1,000 bootstrap replications.

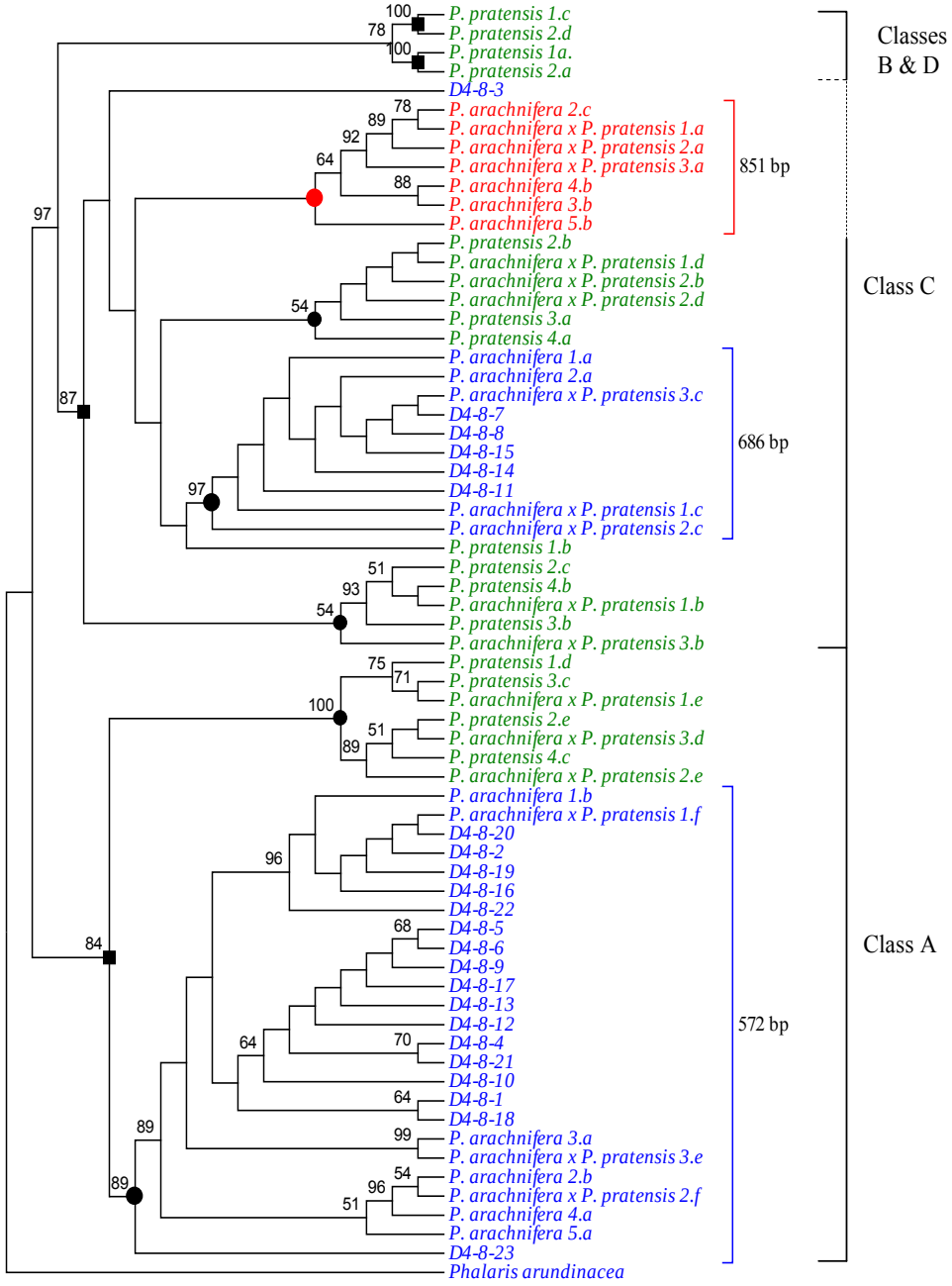


Fig. C-2. The most parsimonious tree produced for the comparison of D4 Isolate#8 (D4-8) trx sequences with P. arachnifera, P. pratensis sequences from Table 4-1 using Mega 6.0. The tree is rooted to Phalaris arundinacea. Clusters (●) in blue indicate new sequences from P. arachnifera, and clusters in green indicate sequences from P. pratensis. The red cluster (●) shows the 851 bp P. arachnifera sequence group containing the 163 bp insertion. Nodes shown as ■ represent one of four P. pratensis classes as reported in Patterson et al. (2005). The numerical values followed by the lower case alphabetical letter correspond to GenBank accession numbers in Table 4-1. Numbers following dashes for D4-8 are clone sequence identifying numbers. Numbers above the tree branch indicates the bootstrap values from 1,000 bootstrap replications.

APPENDIX D

Availability of DALBG 1201

Texas A&M AgriLife Research-Dallas, TX, will maintain Breeder stock of DALBG 1201. Only Foundation, Registered, and Certified classes of sod are recognized for DALBG 1201. All Certified production must be directly from Registered, Foundation, or Breeder stock. Vegetative propagules of DALBG 1201 are available from the corresponding author for research purposes.