HERITABILITY ESTIMATES OF MORPHOLOGICAL CHARACTERISTICS IN CENTIPEDEGRASS POPULATIONS AND EVALUATION OF FERTILITY IN POLYPLOID ZOYSIAGRASSES

by

LUELLEN SWAYZER

(Under the Direction of Brian M. Schwartz and Gerald M. Henry)

ABSTRACT

Genetic and phenotypic variation provides the opportunities for plant breeders to develop new cultivars with desirable characteristics. Variation can be found existing in wild relatives or germplasm resources. Also, variation can be induced through mutagenesis. In these studies, we assessed existing variation and induced variation in two warm season turfgrasses. Centipedegrass (*Eremochloa ophiuroides* Munro Hack) is a low input turfgrass species that is commonly grown on lawns and landscapes in the Southeastern United States. Zoysiagrass (*Zoysia spp*. Willd.) are adapted to transitional and warm climatic regions and are primarily used on golf courses, lawns, roadsides, and commercial landscapes. As the turfgrass industry shifts into an emerging era of environmental stewardship, variations that can produce new and improved cultivars are imperative to continued success in turfgrass development.

INDEX WORDS: Zoysiagrass, *Zoysia spp.*, polyploid, meiosis, centipedegrass, *Eremochloa ophiuroides*, broad-sense heritability, leaf morphology, cytology

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Purpose of the Study

The University of Georgia Tifton Campus, in cooperation with the United States Department of Agriculture- Agricultural Research Service (USDA-ARS), turfgrass breeding program has a long history of producing industry standard turf bermudagrass cultivars. In the United States, turfgrass is the largest single irrigated crop in terms of surface area with three times the acreage of maize (Milesi,et al., 2005). Growing concerns about the environmental impact of turfgrass has led plant breeders to seek genotypes that require fewer resources. Developing turfgrass cultivars that exhibit low input characteristics such as drought tolerance, reduced nitrogen requirements, and salt tolerance are imperative for the turfgrass industry (Yue et al., 2017), yet they must remain aesthetically pleasing and able to persist in environments where shade, traffic, and soil compaction often limit the performance of turf. The research included in the following studies are aimed at contributing to the turfgrass breeding program's ability to create improved cultivars with a combination of stress and quality traits by assessing the available genetic variation in the university's zoysiagrass and centipedegrass germplasm.

Literature Review

Zoysiagrasses

Zoysiagrasses are a perennial species in the family Poaceae (Gramineae), subfamily Chloridoideae, tribe Zoysieae, genus *Zoysia* (Casler and Duncan, 2003). They originated in East Asia and the Pacific Islands and there are 11 known species that differ in growth habit and

texture (Beard, 2013; Turgeon, 2011). Variability between species of the *Zoysia* genus allows a collective range of adaption that covers nearly a third of the United States (Hanna et al., 2013). Some grow especially well in transitional climates, between temperate and subtropical zones where other warm season and cool season grasses are limited. For these reasons, zoysiagrasses are increasingly being used for lawns, airports, cemeteries, coastal dunes, parks, and golf courses (Patton et al., 2017). Among the 11 known species, three have been used as turfgrass, *Zoysia japonica* Steud., known as Japanese Lawngrass, and manila grass, *Zoysia matrella* (L.) Merr, and *Zoysia pacifica* (Goudswaard) M. Hotta and Kuroki, which has been previously recorded as *Z. tenuifolia* (Chandra et al., 2017; Turgeon, 2011). Each of the three species can be distinguished from one another by their morphology (Patton et al., 2017). Average leaf widths are >2.5 mm, 1.5-2.5 mm, and <1.5 mm for *Z. japonica*, *Z .matrella*, and *Z. pacifica*, respectively (Patton et al., 2017; Yamamoto et al., 2016). The three species also vary in response to abiotic and biotic stressors. *Z. japonica* is the most cold tolerant followed by *Z. matrella* and *Z. pacifica* (Hanna et al., 2013). *Z. japonica* has moderate shade tolerance while *Z. matrella* and *Z. pacifica* have poor and very poor tolerance (Beard and Beard 2005). *Z. matrella* is the most salt tolerance of the three species (Yamamoto et al., 2016). *Z. matrella* cultivars were assessed for damage to hunting billbugs (*Sphenophorus venatus vestitus*) and appear to be more resistant and have less canopy damage when compared to *Z. japonica* cultivars (Reinert et al., 2011). They also vary in optimal mowing heights as well, *Z. japonica* range from 13 – 64 mm, *Z. matrella* range from 6.4 – 55 mm, and *Z. pacifica* range from 2.5–1.3 mm (Beard and Beard, 2005). These species have high outcrossing rates and the ability to hybridize with other *Zoysia spp*. (Forbes, 1952; Kimball et al., 2012; Tateoka, 1955). The extensive genetic diversity within the *Zoysia* genus has led to the

development of cultivars with a wide range colors, textures, and tolerances to biotic and abiotic stresses (Kimball et al. 2012).

Several morphological characteristics such as its rhizomatous and stoloniferous growth habit, unique green color, and blade rigidity due to high hemicellulose and lignin content have led to the increasing use of the species (Hanna et al., 2013; Lulli et al., 2012; McCarty, 2011; Roberts, 2014). Additionally, moderate to low fertility requirements, salt and drought tolerance of newer cultivars, weed tolerance, shade tolerance, and reduced maintenance requirements have been reasons for increased zoysiagrass production (Landry, 2010). When compared to St. Augustinegrass (*Stenotaphrum secundatum*), bermudagrass (*Cynodon dactylon*), bahiagrass (*Paspalum notatum*), and carpetgrass (*Axonopus compressus*), *Z. japonica* had the highest turfgrass quality when irrigated with highly saline water (72 dSm^{-1}) (Uddin et al., 2011). A study on tolerance of three warm season turfgrasses exposed to prolong soil water deficiency, zoysiagrass was found to have a lower water use rate and best recovery from drought potential as compared to bahiagrass and St. Augustinegrass (Cathey et al., 2011). Healthy swards of zoysiagrass are relatively weed free because of the high shoot density, however weed encroachment increases when turf is over irrigated (Fry et al., 2008; Zhang et al., 2013). *Z. matrella* was initially adopted because of its shade tolerance and it is the most shade tolerant of the three species followed by *Z. japonica* (Okeyo et al., 2011). The slow growth rate is associated with low nutritional requirements (Kussow et al., 2012). Zoysiagrass is fertilized at rates < 98 kg N ha yr throughout much of the United States as compared to 342 kg N ha yr for bermudagrass (Dunn et al., 1995; Holt 1969).

Characteristic slow growth rates contribute to the reduced maintenance requirement of the genus, which also results in slow recuperative potential (McCarty, 2011).

Despite the extensive genetic variation, improvements can still be made for the shortcomings of some zoysiagrass species including cold hardiness, insect damage susceptibility, and limited seed yield (Patton et al., 2017; Patton and Reicher, 2007). Cold tolerance varies between cultivars but even the most cold tolerant *Z. matrella* germplasm cannot survive north of the transitional climatic zone (Genovesi and Chandra, 2015). Zoysiagrass were initially thought to be pest free, however they are susceptible to various mites and insects (Christians et al., 2017). Fall armyworm (*Spodoptera frugiperfa* J.E. Smith) and tropical sod webworm (*Herpetogramma phaeopteralis* Guenee) are the most damaging to zoysiagrasses in the United States (Reinert and Engelke, 2001 2010). When compared with common bermudagrass with seed yields of 412 to 892 kg ha $^{-1}$ zoysiagrass seed production is low at 112 kg ha^{-1} annually (Ahring et al., 1974; Samudio, 1999). Low seed yield increases establishment cost for consumers.

Zoysiagrass Cytology

Cytological studies of *Zoysia spp*. have determined that the chromosome constitute is 2n = 4x = 40 with the absence of anaphase bridges, fragments, lagging chromosomes during meiosis observed in pollen mother cells (Forbes, 1952; Yaneshita et al., 1999). There have been two exceptions to this classifications, a 2n = 2x = 20 *Z. matrella* found in Sri Lanka and a natural octaploid (2n = 8x = 80) suspected to be the result of somatic chromosome doubling caused by dinitroaniline herbicides that have mitosis-inhibiting effects (Gould and Soberstrom, 1974; Harris-Shultz, 2014). A study on RFLP genetic mapping of Zoysia spp. found that there was segregation independence between linkage groups II and VI suggesting no chromosome pairing supporting disomic inheritance (Yaneshita et al., 1999). Zoysiagrasses are described as segmental allotetraploids derived from progenitors with a basic chromosome number of ten (x = 10) based on RFLP linkage mapping and analysis (Yaneshita et al., 1999). Allopolyploids

typically have two distinct subgenomes at the time of origin, are fixed heterozygotes, and do not form multivalents at meiosis (Renny-Byfield and Wendel, 2014).

Zoysiagrass Breeding

Zoysiagrass was introduced to the US from Japan in 1902 (Meyer and Funk 1989). The *Z. japonica* cultivar 'Meyer', released in 1955 by the USDA and the US Golf Association, is the most economically important cultivar to date (Patton et al., 2017). 'Meyer' was a selection in a population of *Z. japonica* for its finer texture (Grau and Radko, 1951). Soon after, the cultivar 'Emerald' was released, and it was reported to be a selection from a *Z. japonica* female parent and *Z. tenuifolia* male parent, however RFLP fingerprint analysis suggest it arose from a *Z. matrella* and *Z. pacifica* hybrid (Anderson, 2000; Forbes et al., 1955). 'Emerald' exhibited darker green color and higher density than 'Meyer', common *Z. japonica*, and common *Z. matrella* (Forbes et al., 1955). 'Meyer' and 'Emerald' became industry standards and it was not until 30 years later that many other zoysiagrass cultivars became available (Hanna et al., 2013; Patton et al., 2017). 'El Toro' was released from University of California, Riverside and was characterized by its rapid establishment rate and shorter dormancy period compared to other zoysiagrasses (Youngner, 1986). In 1993, Patten Seed Company and Seed Research of Oregon released 'Zenith' and 'Compadre', respectively. They are among the few available zoysiagrass cultivars that are propagated by seed (Patton et al., 2017). In 1996, Texas A&M University released *Z. matrella* cultivars 'Cavalier' and 'Diamond' (Patton et al., 2017). 'Diamond' was distinguished by its finer leaf texture and superior shade tolerance and 'Cavalier' was distinguished by its good to excellent salt tolerance (Engelke et al., 2002a; Engelke et al., 2002b). They also released *Z. japonica* cultivars 'Crowne' and 'Palisades' in 1996 (Patton et al., 2017). 'Crowne' has aggressive recovery growth from rhizomes and stolons and medium-course

texture and ‘Palisades’ has aggressive establishment and tolerance to low mowing (Engelke et al., 2002c; Engelke et al., 2002d). In 1996 Bladerunner Farms released *Z. matrella* cultivar ‘Zeon’, it was a selection from an open pollinated progeny (Patton et al, 2017).

In order to create new cultivars, plant breeders must obtain and create new genetic variability (Patton et al., 2017). Over the years, variation has been achieved through sexual recombination, selection, and germplasm trips to centers of diversity for *Zoysia spp*. (Patton et al., 2017). However, while these efforts have increased variation they do not produce novel traits. Other efforts to increase variation include mutagenesis such as exposure to ^{60}Co gamma-irradiation and chromosome doubling inducing ploidy variation (Chen et al., 2011, Schwartz et al., 2013c). Harten (1998) described mutation as any heritable change in the idiotypic constitution of sporophytic or gametophytic plant tissue, not caused by normal genetic recombination. In recent years, zoysiagrass breeders have aimed their efforts at developing grasses with improved growth rates, tolerance to extreme temperatures, enhancing rooting characteristics, drought tolerance, salinity tolerance, insect and disease tolerance and low growing genotypes for putting green use (Patton et al., 2017).

Variation in ploidy levels has been impactful in the turfgrass breeding for over 50 years and have been especially important in vegetative plant propagation and creating variations in different turf types (Schwartz et al., 2013c). The annual ryegrass (*Lolium multiflor*um Lam.) cultivar ‘Jumbo’ was developed from the doubling the chromosomes of an advanced breeders population named ‘Surrey’, and the resulting tetraploid exhibited larger stems, leaves, and seed heads (Prine et al., 2002). Induced polyploids have also been used to bridge cross two species. A diploid annual ryegrass was successfully crossed with a hexaploid tall fescue (*Festuca arundinacea*) using this technique. Diploid Italian ryegrass and diploid meadow fescue

(*Festuca pratensis*) were hybridized and the resulting embryo was underwent chromosome doubling. The resulting tetraploid was then able to hybridize with hexaploid tall fescue (Acquaah, 2007).

The warm-season turfgrass industry was revolutionized by the development and release of triploid bermudagrass (2n = 3x = 27). Improved triploid hybrid bermudagrasses are made by crossing tetraploid common bermudagrass (*Cynodon dactylon* (L.) Pers.; 2n=4x=36) and diploid African bermudagrass (*Cynodon transvaalensis* Burtt-Davy; 2n=2x=18) (Hanna, 1986). Interspecific hybrids can have improved wear tolerance, cold tolerance, disease resistance, and finer turfgrass texture. Triploids have reproductive barriers in that the three sets of chromosome cannot be evenly divided during meiosis, yielding unequal segregation of the chromosomes (Ranney, 2006). Sterile bermudagrass plants propagated vegetatively have the benefit of providing a turfgrass stand with uniformity and genetic purity (Hanna and Anderson, 2008; Schwartz et al., 2013a).

In 2009, long term breeding efforts to increase vegetative growth rates were initiated by studying the effects of six colchicine treatments on the ploidy level of zoysiagrass cultivar 'Zenith'. The study resulted in four putative octaploids and one cytochimera, and with further self and cross pollination, octaploid and hexaploid genotypes were created (Schwartz et al., 2013c). Subsequent breeding and backcrossing has led to tetraploid, pentaploid, septaploid genotypes (Schwartz, unpublished). The induction of chromosome doubling has been a useful tool in plant breeding to develop sterile plants therefore there is reason to believe that pentaploid, hexaploid, septaploid, and octaploid zoysiagrasses will be valuable to a zoysiagrass breeding program (Yao et al., 2012).

Centipedegrass

Centipedegrass is classified taxonomically as Gramineae (Family), Andropogoneae (Tribe), Rottboelliinae (Subtribe), *Eremochloa* (Genus), *ophiuroides* (Species) (Duncan and Carrow, 1999). Centipedegrass is a medium-textured grass that forms a relatively dense mat with a natural light green color (McCarty 2011). Leaf blades are 15 to 30 mm long and 2 to 4 mm wide and seedheads are spike-like racemes ranging from 12 to 24 cm tall (Duble, 1989; Hanna et al., 2013). In the United States, centipedegrass usually flowers in August but will also flower in June some years and is most likely depended on day length, though very little is known about the physiology of flowering in this species (Hanna et al., 2013). Centipedegrass is a sexually reproductive diploid species with a somatic chromosome number of $2x = 2n = 18$ (Hanna and Burton, 1978; Harris-Schultz et al., 2012).

Centipedegrass is a warm season stoloniferous turfgrass (Turgeon, 2011). It is native to the warm temperate and subtropical regions of Southern China and is adapted to the humid southeastern and south central regions of the United States (Hanna, 1995). Centipedegrass was introduced into the United States in 1916 by United States Department of Agriculture (USDA) agricultural explorer Frank Meyer, and was originally used as a forage grass in Florida and Southern Georgia because of its ability to persist under low fertility (Hanna, 2000). Now centipedegrass is a cultivated turf that is known as the "the lazy man's grass" because of its ability to thrive in low fertility and reduced management in comparison with other warm season turfgrasses (Hook and Hanna, 1994; Johnson and Carrow, 1992; Yan et al., 2009). It has also been referred to as a *sleeping giant* of turfgrass highlighting the underutilization of the species (Duncan and Carrow, 1999).

Centipedegrass is considered a drought resistant turfgrass (Hanna et al., 2013). Hook et al. (1992) reported that after 9 – 13 days of drought stress centipedegrass was able to recover to its previous visual turfgrass quality when irrigation was supplied. Zhang et al. (2015) studied turf performance of bahiagrass (*Paspalum notatum* Flüggé), centipedegrass, and St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze) cultivars under a linear gradient irrigation system with treatments ranging from 0% to 120% evapotranspiration. They found that centipedegrass had less demands of supplemental irrigation to maintain acceptable turfgrass quality. Centipedegrass is also regarded as a generally pest free turf (Zheng et al., 2013). Wiseman et al. (1982) compared centipedegrass to bermudagrass and carpetgrass, (*Axonopus affinis* P.Beauv) and found that centipedegrass was highly resistant to the fall armyworm and caused high larval mortality. When compared to seven other turfgrass species in a choice test, centipedegrass exhibited the lowest of nymph and adult southern chinch bugs, *Blissus insularis* (Reinert et al., 2011). Centipedegrass generally responds well to N fertilization, however too much N can lead to centipedegrass decline characterized by failure to green-up in the spring followed by death (Johnson and Carrow, 1992). Since centipedegrass does better with no N versus excessive N, returning clippings back to the canopy following mowing usually benefits the grass by requiring no further fertilization (Hanna et al., 2013; Wiecko, 2006).

Centipedegrass' ability to produce hardy and acceptable turf in a reduced management situation is especially important with rising concerns of water use, reduced water quality, and fertilizer usage. It does well in infertile and very acidic soils (pH 4.5-6.5) (Brede, 2000). Cultural intensity is low requiring only 100 kg N ha^{-1} yr^{-1}, infrequent mowing at 2.5 to 5 cm, and watering only in drought conditions (Johnson et al., 1988; Turgeon, 2011). Centipedegrass does well in shady low traffic areas (Gannon et al. 2004). Centipedegrass can be propagated by seed, sod, or

sprigs. Sprigging establishment is slow because of the species growth rate, therefore is usually not desirable for commercial establishments (Hanna et al., 2013).

Centipedegrass Breeding

Since its introduction into the United States, very few improved centipedegrass cultivars have been released. 'Common' centipedegrass was the most widely used cultivar until the release of 'TifBlair', which was developed from recurrent ^{60}Co gamma-irradiation and has improved cold tolerance, turf performance and seed yield (Hanna et al., 1997). Before 'TifBlair', 'Oklawn' from Oklahoma State University was released in 1965 by selecting plants that showed persistence under adverse conditions. The cultivar did not become commercial successful because it did not produce many seeds (Casler and Duncan, 2003). In 1983, 'Centennial' was developed from ^{60}Co gamma-irradiation of 'Common' at Auburn University and has darker green denser foliage, dwarf characteristics, and is vegetatively propagated (Pedersen and Dickens, 1985). In 1997, 'TennTurf' from the University of Tennessee was released and has improved cold hardiness that traces back to a single sprig, presumably from 'Common' that survived winter kill in 1955 (Callahan, 1999; Casler and Duncan, 2003). In 2006 'Hammock' was released from the University of Florida and was developed from an open pollinated progeny of unimproved 'Common'. It has more compact leaf structure, faster establishment rate, and darker color compared to common centipede (Scully et al., 2012).

The most frequently used cultivars to date all have similar backgrounds, increasing the chances of genetic vulnerability within the species. Currently (February 2018), there are only five *Eremochloa* accessions listed in the Germplasm Resources Information Network (GRIN) database as active (USDA-ARS, 2016). There are seven other species of *Eremochloa*, but their turfgrass potential and cross compatibility with centipedegrass has not been explored (Hanna et

al., 2013). *Eremochloa ciliaris* is a diploid with a bunch growth habit that appears to have a limited potential as a turfgrass.

In order to broaden the variation of the United States germplasm, a collection trip was conducted in 1999 in central and southern China collecting germplasm from seven geographical regions. Morphological variation was assessed in 31 accessions and seed set variation was measured in 58 accessions from the six regions of China. The Chinese collections were then evaluated for morphological and seed set characteristics as they compared to 'Common' and 'TifBlair' (Liu et al., 2003). The resulting data showed that 'TifBlair' and 'Common' had similar morphological characteristics further illustrating the limited variation of cultivars derived from 'Common'. The resulting data also showed that there was variation in stolon number, internode length, leaf length, and width indicating that variation of the Chinese accessions will provide new sources of variation for centipedegrass breeding (Liu et al., 2003).

These Chinese accessions were further investigated for their potential to bring new molecular variability to the United States germplasm using sequence-related amplified polymorphism (SRAP) markers. The results showed that there are alleles in the Chinese group that are unique and not represented in the United States germplasm and these materials can be used in cultivar development (Milla-Lewis et al., 2012). In another study assessing the genetic diversity of 55 centipedegrass accessions and one *Eremochloa zeylanica* Hack. accession, principle coordinate analysis found that the grasses were divided into three distinct groups (Harris-Shultz et al., 2012). Additionally, ploidy analysis revealed that all centipedegrass accessions were diploid and *E. zeylanica was* a putative tetraploid, implying this germplasm could be useful in a breeding program (Harris-Schultz et al., 2012).

In the spring of 2009, efforts to increase the variation in centipedegrass by inducing polyploidy in 'TifBlair' using six colchicine treatments were initiated (Schwartz et al., 2013b). One putative tetraploid with 2C nuclear DNA content twice that of 'TifBlair' and one cytochimera with DNA content that had both diploid and tetraploid cells were confirmed by cytological and flow cytometry analyses. Stomata length and pollen diameter where used to determine which histogenic layers were affected by the treatments. The tetraploid exhibited an increase in stomata length by 12% and the cytochimera's pollen had a diameter that was significantly larger than that of the 'TifBlair' and the tetraploid. The tetraploid was shown to be unstable, which provided evidence that colchicine treatment may only work if mitosis is arrested in cells at the exact right stage of the process. Further work to induce variation by colchicine treatments could result in heterosis stemming from the accumulation of favorable dominant alleles, which would increase variation in centipedegrass (Schwartz et al., 2013b).

In 2011, improved populations from the University of Georgia Turfgrass Breeding program in Tifton, GA were investigated for survival rate and response to subfreezing temperatures. Ten seeds from each accession were planted in pots in Lubbock, TX and each germinated seedling was transplanted into individual pots and became a clonal entry. Clonal entries were grown in the greenhouse for four months and then transplanted outside into bare soil where they were subjected to subfreezing temperatures. Digital photographs were taken after spring green up to analyze green leaf tissue and turfgrass cover. 'Common' centipedegrass did not survive and 'TifBlair' exhibited 7% turfgrass cover and 27% green leaf tissue. The highest percent of turfgrass cover of the clonal entries was found in TC 437-33 (49%) and TC 437-25 (35%). The highest percent of green leaf tissue of the clonal entries was exhibited by TC 437-26 (72%), TC 437-25 (46%), TC 437-1 (40%), and TC 437-36 (40%) (Copper et al., 2012). The

same populations were also assessed for tolerance to high soil pH and 'TifBlair' and 'Common' exhibited 47% cover and 50 to 57% green tissue 2 months after transplant (MAT). TC 437-2, TC 427-7, and TC-437-3 exhibited 80 to 87% cover and 90 to 92% green tissue 2 MAT, while TC 434-3, TC 427-1 and TC 434-4 exhibited 71 to 74% cover and 77 to 82% green tissue. TC 427-99 and TC 437-8 exhibited slow growth (56 to 59% cover), but good color (75 to 86% green tissue) (Henry and Schwartz, 2010) The TC-437 population has exhibited better turfgrass performance in stressful environments to date. Broadening the genetic diversity available to turfgrass growers and managers is beneficial because it allows the species to cope with current environment variability affecting widespread application of this species.

Morphological and agronomic characteristics are used by breeders in development of improved cultivars and by managers for specific cultivar selection (Shortell et al., 2009). Success in the breeding process largely depends on heritability because it determines how quickly the mean phenotype evolves in response to artificial selection (Conner and Hartl, 2003; Jockovic et al., 2013). Broad-sense heritability estimates the total genetic effects influencing a trait and includes additive, dominance, and epistatic effects (Shortell et al., 2009). Heritability partitions the phenotypic variation into genetic and environmental components so that the effect of the environment on a specific trait can be determined (Shortell et al., 2009). In other words, broad-sense heritability estimates are the fraction of the variance which is genetic. Broad-sense heritability has been calculated for rose bush architecture characteristics (Crespel et al., 2014), fruit and flower characteristics in strawberry (*Fragaria x ananassa* Duch.) (Mishra et al., 2015), and plant height and head diameter in sunflower (*Helianthus annuus* L.) (Jockovic et al., 2013). Broad-sense heritability has been calculated for turfgrasses on single plant basis, in addition to clonal mean performance. Prairie junegrass (*Koeleria macrantha*) broad sense heritability

estimates were calculated on a clonal mean (H_c) and single-plant (H_{sp}) basis for turfgrass quality, crown density, and mowing quality and ranged from 0.06 – 0.62. The high heritability estimates suggested that selection for these traits should results in significant gains in germplasm improvement (Clark and Watkins, 2012). In zoysiagrass, broad-sense heritability values indicated that conventional breeding could be used to enhance salt tolerance (Qian et al., 2000). High genotypic variance was expressed in zoysiagrass turf density, turf quality, genetic color, and seedhead and the higher heritability values indicates the potential for improving traits for superior hybrids (Schwartz et al., 2009).

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CHAPTER 2

HERITABILITY ESTIMATES OF MORPHOLOGICAL CHARACTERISTICS IN CENTIPEDEGRASS POPULATIONS[1]

[1] Swayzer, L., B.M. Schwartz, and G.M. Henry. To be submitted to *Crop Science*

Abstract

Centipedegrass (*Eremochloa ophiuroides* Munro Hack) is a low input, warm season turfgrass species that is commonly grown in the Southeastern United States. However, since its introduction from China, few improved cultivars have been released. The purpose of this study was to assess the broad sense heritability of morphological traits and growth characteristics including leaf width, leaf length, internode length, turfgrass plot coverage, leaf color, and inflorescence density at the population level, and also to determine whether there is variation for these traits in individual plants. Field trials were established during June 2016 in Athens, GA and May 2017 in Tifton, GA. Broad sense heritability estimates for all traits was ($H^2 = 0$). The results indicate that selection for these traits would not result in significant changes in these populations of centipedegrasses if only mass selection is utilized. Additionally, this study aimed to identify the percentage of individual plants within each population that were classified to be in the most desirable 90th percentile of each trait. Population TC 434 and TC 437 had the highest percentage of plants with a leaf width ≤ 3.06 mm, TC 434 had the highest amount of plants with internode length ≤ 14.76 mm and darkest green color mid-season in both Athens and Tifton, but TC 437 had a slightly higher percentage of plants with darker green color late season in both years. TifBlair had the highest percentage of plants with mid-season turfgrass plot coverage ≥85.67%, where TC 434 had more plants with ≥87.94% green plot coverage later in the season. Mean inflorescence density was rated on a scale of 1 to 5. TC 196 had the highest amount of plants (16.7%) with a rating of 5, where TC 428 had the highest amount of plants (11.9%) with a rating of 1. Results from this study showed that within each population there is individual plant variation, however, broad sense heritability estimates calculated using population means do not reflect this. In order to utilize this variation in future cultivar development, individual plants

should be selected an allowed to inter-mate in isolation using in a recurrent selection breeding scheme.

Introduction

Centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] is a warm season perennial turfgrass grown throughout southeastern United States (Hook and Hanna, 1994). The center of origination based on phenotypic and genetic diversity is the temperate and subtropical regions of Southern China and Taiwan (Gould and Soberstrom, 1975). The successful adaption to the southeastern United States may be related to the climatic similarities found in southern China (Scully et al., 2012). Centipedegrass is medium-textured and stoloniferous and forms dense mat with a natural light green color (McCarty 2011). Leaf blades are 15 to 30 mm long and 2 to 4 mm wide and seedheads are spike-like racemes ranging from 12 to 24 cm tall (Duble, 1989; Hanna et al., 2013). In the northern hemisphere, centipedegrass usually flowers in August and is most likely dependent on day length though little is known about the physiology of flowering in this species (Hanna et al., 2013). It can be propagated by seed or sod and is a sexually reproducing diploid ($2n = 2x = 18$) with some self-incompatibility (Beard, 2013; Hanna and Burton, 1978; Liu et al., 2003).

Since its introduction to the United States, centipedegrass has grown in popularity and is an excellent choice for a low-maintenance lawn, landscape, or roadside turfgrass (Hanna, 2000; Hanna et al., 2013; Scully et al., 2012). Amidst growing concerns of excessive water, pesticide, and fertilizers use, centipedegrasses' characteristically low maintenance requirements are especially advantageous. Centipedegrass is considered to be a drought resistant turfgrass (Hanna et al., 2013). Hook et al. (1992) reported that after 9 – 13 days of drought stress, centipedegrass was able to recover to its previous visual turfgrass quality when irrigation was supplied.

Centipedegrass is regarded as a generally pest free turf (Zheng et al., 2013). Centipedegrass grows best in sandy, acid soils with low fertility and can live in slightly neutral soils and coastal areas with soil pH greater than 7.

Despite the benefits of centipedegrass, relatively little genetic improvement or selective breeding efforts have been made due to the low germplasm variation available (Liu and Hanna, 2003). Most centipedegrass cultivars are believed to be related to the initial plant introduction by USDA explorer Frank Meyer in 1916 known as 'Common' (Hanna, 1995). 'Common' centipedegrass was the most widely used cultivar until the release of 'TifBlair', which was developed from recurrent ^{60}Co gamma-irradiation and has improved cold tolerance, turf performance and seed yield (Hanna, 1997). Before 'TifBlair', Oklawn' from Oklahoma State University was released in 1965 by selecting plants that showed persistence under adverse conditions. 'Centennial' was also developed from ^{60}Co gamma-irradiation of 'Common' and has darker green denser foliage, dwarf characteristics, and is vegetatively propagated (Pedersen and Dickens, 1985). 'TennTurf' has improved cold hardiness that traces back to a single sprig presumably from 'Common' that survived winter kill in 1955 (Callahan, 1999; Hanna and Liu, 2003). 'Hammock' was developed from an open pollinated progeny of unimproved 'Common' centipedegrass and was identified as phenotypically different (Scully et al., 2012). Although these cultivars have improved pest resistance and require reduced fertility, centipedegrass is still plagued by its inability to grow in alkaline soils and lacks cold tolerance.

In efforts to add variability to the centipedegrass germplasm, collection trips were conducted around the United States and the center of origin China. Morphological variation in stolon number, stolon length, internode length, and leaf width was assessed in 31 accessions and seed set variation was measured in 58 accessions from the six regions of China. The results

showed that ‘Common’ and ‘TifBlair’ had similar morphological characteristics and the Chinese accession could provide a valuable source of germplasm for the species (Hanna, 1995; Liu et al., 2003). In 2011, these accessions and improved populations from the University of Georgia Turfgrass Breeding program in Tifton, GA were investigated for survival rate and response to subfreezing temperatures. ‘Common’ centipedegrass did not survive and ‘TifBlair’ exhibited 7% turfgrass cover and 27% green leaf tissue. The highest percent of turfgrass cover of the clonal entries was found in individual plants TC 437-33 (49%) and TC 437-25 (35%). The highest percent of green leaf tissue of the clonal entries was exhibited by TC 437-26 (72%), TC 437-25 (46%), TC 437-1 (40%), and TC 437-36 (40%) (Copper et al., 2012). The same populations were also assessed for tolerance to high soil pH and ‘TifBlair’ and ‘Common’ exhibited 47% cover and 50 to 57% green tissue 2 months after transplant (MAT). Individual plants TC 437-2, TC 427-7, and TC-437-3 exhibited 80 to 87% cover and 90 to 92% green tissue 2 MAT, while TC 434-3, TC 427-1 and TC 434-4 exhibited 71 to 74% cover and 77 to 82% green tissue. TC 427-99 and TC 437-8 exhibited slow growth (56 to 59% cover), but good color (75 to 86% green tissue) (Henry and Schwartz, 2010)

In assessing variation, knowledge of how the genetic and environmental effects will be expressed in the phenotype is important (Schwartz et al., 2009). Broad-sense heritability (H^2) estimates the total genetic effects influencing a trait and includes additive, dominance, and epistatic effects (Bernardo, 2014; Shortell et al., 2009). The success of selection in plant breeding is largely dependent on the calculated heritability of a specific trait. In other words, broad-sense heritability estimates are the fraction of the variance that is genetic in a population. Characteristics with higher broad-sense heritability are expected to be consistent in multiple environments. Broad-sense heritability estimates have been used in carpetgrass for leaf length,

leaf width, inflorescence density, genetic color, and turf density (Greene et al., 2008) and zoysiagrass for turf quality, genetic color, and seedhead density (Schwartz et al., 2009). Broad-sense heritability has also been estimated in cool season turfgrasses such as perennial ryegrass for gray leaf spot (*Pyricularia grisea*) resistance (Bonos et al., 2004), and Kentucky bluegrass (*Poa pratensis*) for leaf length, leaf width, rhizome spread, and panicle length (Shortell et al. 2009)

The objectives of this study were to 1) determine the ability to detect genetic variation for morphological characteristics in several University of Georgia centipedegrass populations when evaluated by population mean responses, and 2) determine the range of these characteristics when reported at the individual plant level.

Materials and Methods

Plant Material

Five experimental populations (TC-196, TC-427, TC-428, TC-434, and TC-437) (Fig. 2.1) along with the cultivar 'TifBlair' were evaluated in Athens, GA and Tifton, GA. Seeds were obtained from the University of Georgia germplasm collection in Tifton, GA. The population TC-196 was derived in 1981 from a random seed of the cultivar 'Oklawn' that was released from Oklahoma State University. In 2001, the population TC-434 was derived from seed that was harvested from a random mating population of Oklawn x 5 persistent Oklawn progeny. The populations TC-427 and TC-428 were derived from seed harvested from a random mating population from Southern Tennessee and Northern Tennessee, respectfully. The population TC-437 was derived from a seed bulk of populations TC-434, TC-427, TC-428, and TifBlair in 2002. Experiments were conducted at the Plant Sciences greenhouse complex at the University of Georgia in Athens, GA in 2015 and 2016. On December 14, 2015, each population was

seeded at 12 kg ha^{-1} into pots (25.4 cm diameter) containing a steamed 2:1 mixture of Cecil sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludults) and Wakulla sand (siliceous, thermic Psammentic Hapludults) with a pH of 5.7 and organic matter content of 21.1 g kg^{-1}. Fertilizer (14N – $14P_2O_5$ – $14K_2O$) (Osmocote Classic; Scotts Miracle-Gro Company, Marysville, OH) was applied at the time of seeding at a rate of 24.4 kg N ha^{-1}. Greenhouse temperatures were maintained at 32/24 °C (day/night). Natural light was supplemented with artificial light at 500 μmol m^{-2} s^{-1} photosynthetic photon flux in a 12-h day to approximate summer light intensity and photoperiod.

On January 27, 2016, germinated seedlings were randomly selected from each population and transplanted into individual 10.2 cm pots containing the same soil media previously described. Plants were fertilized (20N – $20P_2O_5$ – $20K_2O$) (Masterblend Water Soluble Fertilizer; Masterblend International, Morris, IL) at transplant and weekly thereafter at a rate 3.6 kg N ha^{-1}. Once leaf length reached > 12 cm, plants were trimmed weekly with grass shears to a height of 7.6 cm. Clippings were not returned to the turfgrass canopy. Plants were surface irrigated with tap water as needed in order to encourage uniform recruitment. Conditions in the greenhouse were similar to those previously described. Pots were grown in the greenhouse for approximately 4 months until reaching 100% cover.

Field Experiment

Centipedegrass plants (10.2 cm plug) were transplanted into the field on June 9, 2016 at the Athens Turfgrass Research and Education Center, in Athens, GA. The soil was a Cecil sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludults) with a pH of 5.8 and organic matter content of 23.1 g kg^{-1}. The research area was cultivated in two directions with a tractor-mounted rototiller to a depth of 30.5 cm and graded at a 1% slope in 2 directions to allow for surface

drainage. Plugs (10.2 cm) of each genotype were transplanted into the center of 0.9 m x 0.9 m plots arranged in a randomized complete block design with seven replications and nine subsamples within each replication. Each subsample represents an individual plants and a unique genotype in the population. Fertilizer (16N–10.5P–9.9K) (The Andersons, Inc., Maumee, OH) was applied at the time of transplant at a rate of 12.2 $kg \cdot ha^{-1}$ N. and monthly thereafter throughout the duration of the study. Plants were mowed weekly (clippings returned) to a height of 7.6 cm with a ride-on rotary mower (Z535R ZTrak Residential Mower; John Deere, Moline, IL); however, mowing was terminated at the initiation of flowering. Irrigation was applied as a supplement to rainfall at a rate of 2.5 to 3.0 cm wk^{-1}. Plots were maintained weed free by hand-weeding and through applications of glyphosate (Roundup PRO; Monsanto Company, St. Louis, MO) at 10.1 kg ai ha^{-1}, mesotrione (Tenacity; Syngenta Crop Protection, Greensboro, NC) at 0.28 kg ai ha^{-1}, thiencarbazone-methyl + iodosulfuron-methyl-sodium + dicamba (Celsius; Bayer Environmental Science, Research Triangle Park, NC) at 0.18 kg ai ha^{-1}, clethodim (Envoy Plus; Nufarm Americas Inc., Alsip, IL) at 0.2 kg ai ha^{-1}, and oxadiazon (Ronstar FLO; Bayer Environmental Science, Research Triangle Park, NC) at 2.24 kg ai ha^{-1} with a handheld pump sprayer (Solo Pro Backpack Sprayer; Solo, Newport News, VA) or CO_2 powered backpack sprayer (R&D Sprayers, Opelousas, LA) to reduce the effects of weed competition. Acephate (Ortho Orthene Fire Ant Killer; The Scotts Company, Marysville, OH) was applied to mounds to control fire ants in plots.

On May 22, 2017, centipedegrass plants (10.2 cm plug) from Athens, GA greenhouses were transplanted into a field at the Coastal Plain Experimental Station in Tifton, GA comprised of a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with a pH of 6.2. Plots were arranged in a randomized complete block design with seven replications and nine

subsamples within each replication. Fertilizer (16N–1.7P–6.6K) (Super Rainbow Plant Food; Agrium U.S. Inc., Denver, CO) was applied at a rate of 24 $kg \cdot ha^{-1}$ N at the time of transplant and again on 6 July 2017. Plots were not mowed. Irrigation was applied as a supplement to rainfall using a water cannon at a rate of 1 to 2 cm wk^{-1}. All weeds were mechanically removed with a hoe or pulled by hand. No pesticides were applied to the plots during the length of the trial.

Morphological Data Collection

Several commonly used morphological parameters were utilized to evaluate the phenotypic diversity within the centipedegrass germplasm. Leaf width (mm), leaf length (mm), and internode length (mm) measurements were obtained with a digital caliper (15.2 cm Pittsburgh Digital Caliper; Pittsburgh Automotive, Camarillo, CA) 64 and 56 days after transplant in Athens and Tifton, GA, respectively. Leaf width was measured at the widest point of fully expanded leaf blades. Those same leaves were used to measure leaf length. Internode length was recorded 126 and 56 days after transplant in Athens and Tifton, respectively. Three measurements were taken in each subsample plot and averaged to produce one value. Inflorescence density was rated on two separate occasions. Early season, a rating of 0 or 1 was assigned to indicate the absence or presence of an inflorescence. The late season rating was conducted on a scale of 1 to 5 (1 = no inflorescence, 5 = maximum inflorescence) (Table 2.1). Percent green plot coverage and green color were assessed in July and September of both years using digital image analysis (Karcher and Richardson, 2003; Richardson et al., 2001). Images were acquired using a digital camera (Nikon 10.0 megapixel Coolpix; Nikon Inc., Melville, NY) mounted on a light box equipped with four compact fluorescent light bulbs (550 lumens, EcoSmart) that provided consistent lighting throughout image acquisition. The camera setting remained constant during the course of the study: aperture of F3.2 and white balance set to

fluorescent. Images were captured in JPEG (joint photographic experts group, or "jpg.") format with an image resolution of 640 x 480. Image analysis was conducted with SigmaScan software (SigmaScan Pro version 5.0; Systat Software Inc., Chicago, IL).

Statistical Analysis

Leaf width, leaf length, internode length, green plot coverage, and green color data were analyzed as a randomized complete block design across locations. Analysis of variance was calculated to compare genotypes using PROC MIXED in SAS (SAS Institute, Cary, NC) with genotype, location, and interactions as fixed effects. Inflorescence densities (Table 2.2) were compared among cultivars using PROC GLIMMIX with genotype, location, and interactions as fixed effects. PROC GLIMMIX was conducted with a binary distribution for inflorescence rating mid-season and a binomial distribution for late season. Estimates of variance components were determined using PROC VARCOMP (Greene et al, 2008). Variance allowed for calculation of broad-sense heritability (H^2) using the following formula:

$$H^2 = \frac{\sigma^2_g}{\sigma^2_G + \sigma^2_{GL} + \sigma^2_{(R)L} + \sigma^2_{GL(R)} + \sigma^2_E}$$

where σ^2_G equals the variance of genotypes, σ^2_{GL} equals thevariance of genotypes × location, $\sigma^2_{(R)L}$ equals the variance of replication (location), $\sigma^2_{GL(R)}$ equals the variance of genotype x location (replication) σ^2_E equals the error variance (Bonos et al., 2004). A Kruskal-Wallis test was used to determine if there was a significant difference between population medians.

Results and Discussion

Analysis of variance

Genotypic differences at the population level were not significant according to an analysis of variance of leaf width, leaf length, internode length, green plot coverage early and late season, green color early and late season (Table 2.2), and inflorescence density mid-season (Table 2.3). Inflorescence density late season differences were significant among populations ($p < 0.05$) (Table 2.3). Location was shown significant ($p<0.05$) for all traits measured and population × location interactions were significant for internode length and plot coverage mid-season (Table 2.2). The significant differences in location can be due to the differences in data collection dates of evaluation. For example, leaf width was taken 64d after planting Athens and 36d after planting in Tifton. The population × replication (location) interactions for all evaluated characteristics, which represent the variability of individual plants within each centipedegrass population, were also significant ($p<0.05$). The lack of genotypic variances within populations in this study supports the general statement that centipedegrass does not appear to have a large range of morphological variation at the population level (Hanna and Liu, 2003).

Heritability

The characteristics studied in this investigation expressed very low or null broad sense heritability as estimated from REML variance components. Heritability for all traits was 0.00 with the exception of leaf width and inflorescence density late season ($H^2 = 0.03$) (Table 2.3). The null or very low heritability estimates correlate to the low population variance estimates ranging from 0.00 to 0.04. Low variance estimates represent independence between two variables (Serfling, 2006). This suggests that for nearly all morphological characteristics, there is no correlation with a particular population. The higher variance estimates for location, location x

population, and population x replication (location) indicate a higher correlation between population and morphological trait expression. Ma et al. (2014) also found low broad sense heritability for number of spikelets per branch in carpetgrass. A study in weedy rice showed that in 370 accessions seed width had a low heritability ($H^2 = 0.07$), and was shown to have a high influence from the environment (Perera et al., 2012). A study on zoysiagrass stress response showed medium to high heritability estimates ($H^2 = 0.32 – 0.83$) for turfgrass color characteristics. The study suggested that the lower heritability estimates had larger environmental influence. In this study, the low heritability could be due to the populations all being derived from a common ancestor meaning there was little variation at the population level for these traits. These studies usually describe the environmental influence as location and the genotype x location interaction. This means the influence of the location and the location x genotype interaction had a greater effect on the trait than the genetic (Schwartz et al., 2009).

The reason for null broad-sense heritability estimates in our research was not solely due to large environmental effects, but 0.00 population variance estimates. Although mean values for all traits across populations were similar, minimum and maximum ranges indicate there was significant variation at the individual plant level (Table 2.2). Heritability estimates values are dependent on genetic variance. Genetic variance can change if allele frequency changes due to selection, or if new variance comes in to the population (Wray and Visscher, 2008). This means that even though heritability estimates are low or null for centipedegrass morphological traits this can change as genetic variance is changed. One way to increase genetic variance in this population is recurrent selection, which is the cyclical improvement of a population. Individual plants that have the desired traits are recombined or intermated to form the next generation (Bernardo, 2014). In the past, centipedegrass breeding populations have been developed by mass

selection methods that allow natural selection for resistance to abiotic and biotic stresses (Hanna et al. 1997; Callahan, 1999). These populations were developed by the same methods. This is beneficial when selecting for plants that can survive freezing temperatures or have drought tolerances in centipedegrass populations because weaker plants do not survive, however this is not beneficial for the morphological traits assessed in this study. Failing to control pollen causes plants to be pollinated with both desirable and undesirable individuals (Fehr et al, 1987). Desirable turfgrass qualities such as smaller leaves and shorter internodes may be at a fitness disadvantage in regards to light capture, gas exchange, and thermoregulation compared to longer leaves and larger internodes and have a moderate probability of being underrepresented in the next generation (Bernardo, 2014; Chitwood and Sinha, 2016) Recurrent selection based on the phenotype of individual would increase the frequency of favorable morphological traits in the population. This population was naturally selected based on the soil conditions and climates of South and North Tennessee, therefore there might be higher broad-sense heritability estimates if the traits we were assessing were persistence in various soil pHs and low temperatures. A study on F1 segregating populations from two centipedegrass accessions measured similar morphological characteristics (leaf length, leaf width and internode length and diameter) and found that internode length heritability was $H^2 = 0.47$, leaf length was $H^2 = 68.98$ indicating that the population structure is likely contributing to the low heritability and in turn the low variation between genotypes (Zheng et al., 2009).

Breeding progress based on selection at the population level cannot be expected in the material evaluated in this study because significant genetic variability for desired traits was not observed. But, there was variance within each population as shown in the differences between subsamples. An analysis of the distribution of individual plants from each population that

performed in the top 10% of each desired characteristic is reported in Table 2.4. This information can be further dissected to identify specific genotypes that possess several desirable characteristics. Building new populations from plants that show narrow leaf width and short internode length, or any other combination of superior traits, could lead to the development of a new populations that will be significantly different from those researched in this study.

Morphological data

Morphological data were collected on five centipedegrass populations and the cultivar TifBlair. Plant leaf length for individual genotypes across both locations ranged from 18.5 mm (TC 428) to 145 mm (TC 196), leaf width ranged from 1.9 mm (TC 434) to 5.5 mm (TC 428), and internode length ranged from 19.1 mm (TC 428) to 54.0 mm (TC 427). Green color mid-season ranged from 0.26 (TC 343) to 0.74 (TC 427) and green color late season ranged from .40 (TC 434) to .77 (TC 437). Green plot cover ranged mid-season from <1% (TC 434) to 96.2% (TC 196) and green plot cover ranged late season <1% (TC 434) to 96.8 % (TifBlair) (Fig 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8).

Desired characteristics included thinner leaves, shorter internodes, darker green color, high plot coverage, and high or low inflorescence density depending on the future use. Populations TC 437 and TC 434 had the greatest number of plants with the smallest 10% of leaf widths (<3.06 mm). TC 196 and TC 434 had the greatest number of plants in the shortest 10% of internode lengths (<14.76 mm). TC 434 and TC 437 had the greatest number of plants with green color mid-season (>0.61) and TC 437 for green color late season (>0.70). 'TifBlair' and TC 428 had the most plants with highest percent green plot coverage mid-season (>85.67) and TC 434 plants had greater percent green plot coverage late season (>87.67%). TC 196 consisted of the highest number of plants rated at inflorescence density of 5. Populations TC 428 and TC

434 have the lowest percentages of plants at an inflorescence density of 5 (Table 2.4). According to the Kruskal-Wallis test, the medians between populations are significantly different ($p < 0.05$) for leaf width, internode length, and late season inflorescence density.

Conclusion

Selection on a single plant basis would allow for choosing the best plants and crossing of "good by good", or in this case, desired characteristic by desired characteristic (Bernardo, 2014). In the future, it will be important to choose single plants or genotypes that exhibit superior performance for several desired characteristics to use as parents for future cultivar development. For many years, centipedegrass breeding methods have been primarily mass selection and natural selection. These methods have been efficient, however phenotypic outliers are underrepresented in subsequent generations and the variation for traits is lost. This can be seen in this study as the mean genotypic values have distorted the variation in each genotype. This is also evident in Green and Beard (1991), were 'Oklawn', 'TennTurf', 'Centennial' and two experimental lines of centipedegrass were assessed for turf quality and there were no differences found. Liu et al. (2003) found there were morphological and seed set characteristic variations in accession from China and this signified that variation is available in the species. Hanna and Liu (2003) stated that if individual plants are space planted, one could observe some variation for internode length as well as leaf characteristics. Turfgrass breeders must utilize plant breeding methods to exploit this variation to benefit from for future centipedegrass development.

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Table 2.1: Date of Morphological trait evaluation in centipedegrass field experiment in Athens, GA and Tifton, GA

Characteristic	Date of Evaluation	
	Athens, GA	Tifton, GA
% Green plot coverage mid-season	25 July 2016	13 July 2017
% Green plot coverage late season	4 Sept. 2016	8 Sept. 2017
Green color mid-season	25 July 2016	13 July 2017
Green color late season	4 Sept. 2016	8 Sept. 2017
Leaf width (mm)	12 Aug. 2016	27 June 2017
Leaf length (mm)	12 Aug. 2016	27 June 2017
Internode length (mm)	13 Oct. 2016	27 June 2017
Inflorescence density mid-season (0 or 1)	12 Aug. 2016	30 June 2017
Inflorescence density late season (1-5)	13 Oct. 2016	28 Aug. 2017

Table 2.2: Variance component estimates and descriptive statistics for morphological traits evaluated in Athens, GA and Tifton, GA

Variance Estimates

Source	Length	Width	Internode Length	Green Plot Coverage 1	Green Color Mid-Season	Green Plot Coverage 2	Green Color Late-Season
Genotype (G)	0.00±5.51	0.004±0.006	0.00±1.00	0.00±8.44*	0.00±0.00*	0.00±8.62	0.00±0.00
Location (L)	1640.29±2323.27*	.20±0.29*	85.43±121.34*	1824.41±2585.64*	0.02±0.03*	726.31±1035.27*	0.02±0.02*
G x L	4.72±8.80	0±0.006	1.69±1.74*	18.20±13.41*	0.00±0.00	2.51±16.67	0.00±0.00
Rep (L)	4.13±6.24	0±0.003	0.00±0.318	2.69±2.60	0.00±0.00	10.37±15.97	0.00±0.00
G x R (L)	41.14 ±11.50*	0.05±0.01*	4.18±1.34*	11.10±3.85*	0.00±0.00*	138.49±29.43*	0.00±0.00*
Error	193.47±10.56	0.17±0.009	27.94±1.52	88.39±4.82	0.00±0.00	203.17±11.08	0.00±0.00
Mean	65.35	3.80	23.84	42.92	0.47	57.80	0.58
Min.	18.50	1.91	11.11	0.79	0.27	0.02	0.41
Max.	145.03	5.47	54.03	96.29	0.75	96.89	0.78
Std. Dev.	32.60	0.56	8.67	32.09	0.1	26.62	0.1

§Variance components ± standard errors. * = (P<0.05). Internode length, width, length (mm), plot coverage (%). Green plot coverage 1 is mid-season and 2 is late season.

Table 2.3: Variance component estimates and descriptive statistic for centipedegrass inflorescence density rating at mid-season and late season evaluated in Athens, GA and Tifton, GA

Variance Estimates

	Inflorescence Density	
	Mid-Season	Late Season
Source		
Genotype (G)	0.000±0.0014	0.0402±0.0377*
Location (L)	0.0934±0.1331*	0.0933±0.1414*
G x L	0.00±0.002	0.0100±0.0218*
Rep (Location)	0.001±0.002	0.006±0.150
G x R (L)	0.00±0.01023	0.0457±0.0311
Error	0.1875±0.01023	1.089±0.0594
Mean	1	3

§Variance components ± standard errors * = (P<0.05). Date 1 (0 or 1) Date (1-5)

Table 2.4: Individual plants within each centipedegrass populations with most desirable 90th percentile of turfgrass quality traits

	Most desirable 90th percentile of each trait					
Genotype	Leaf Width <3.06 mm	Internode Length <14.76 mm	Green Color Mid-Season >0.611	Green Plot Coverage Mid-Season >85.67%	Green Plot Coverage Late Season >87.94%	Green Color Late Season >0.709
TC 196	8%	14%	8%	10%	10%	9%
TC 427	0	13%	5%	16%	17%	13%
TC 428	9.5	7%	8%	19%	18%	13%
TC 434	12%	15%	23%	17%	22%	13%
TC 437	12%	12%	16%	10%	12%	15%
TifBlair	8%	9%	6%	28%	18%	13%

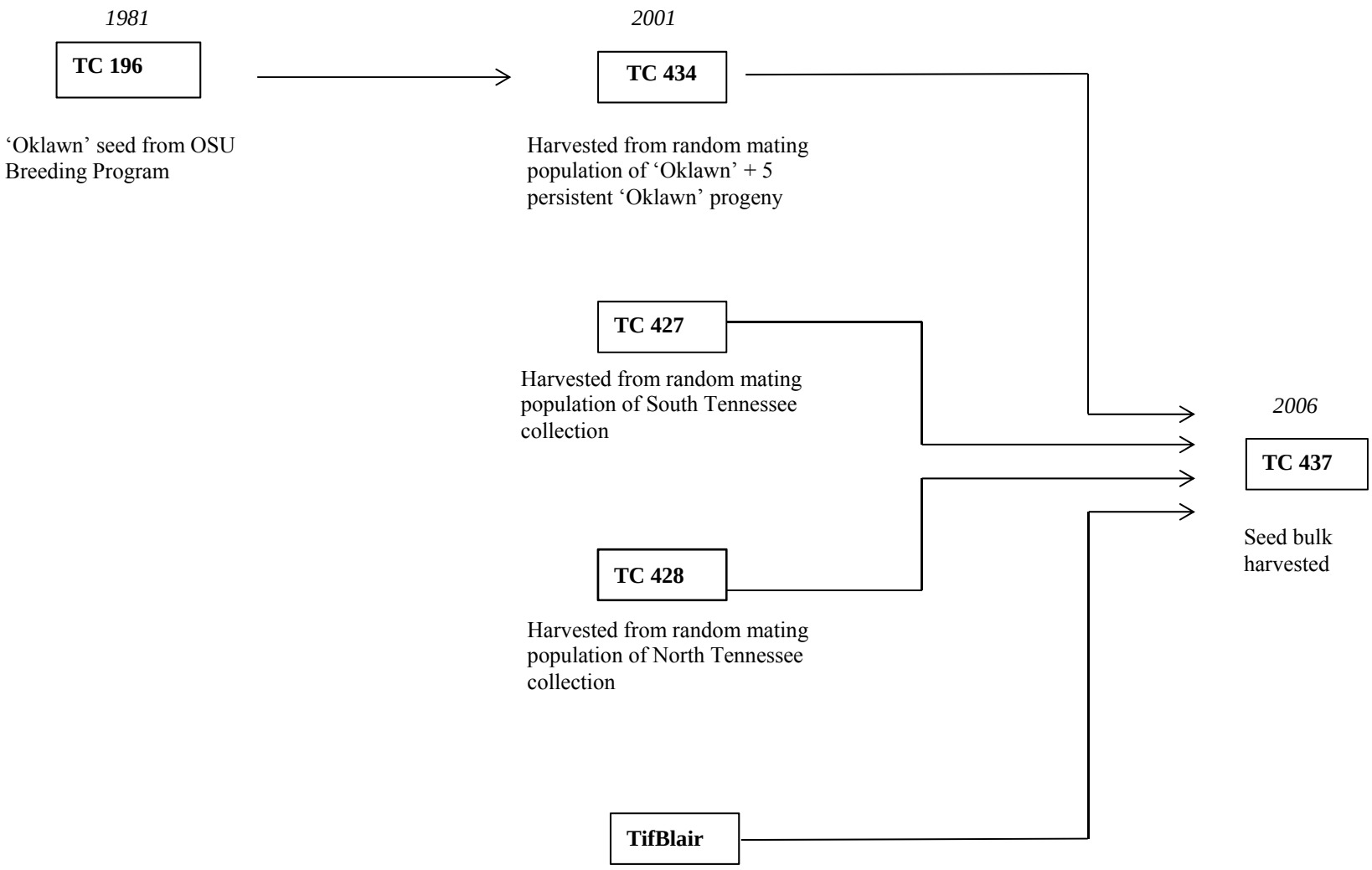

Figure 2.1: Population origin for centipedegrass genotypes assessed in Athens, GA and Tifton, GA field trials

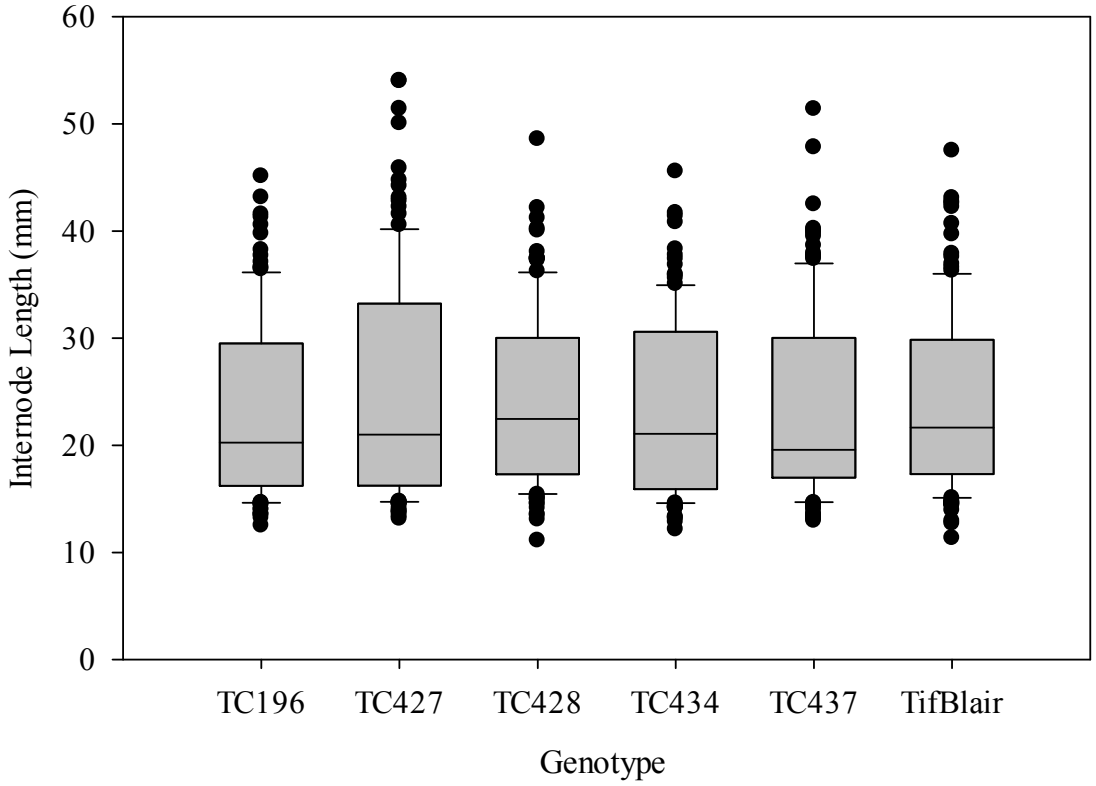

Figure 2.2: Internode length (mm) distribution of all centipedegrass genotypes. mean = 23. 84

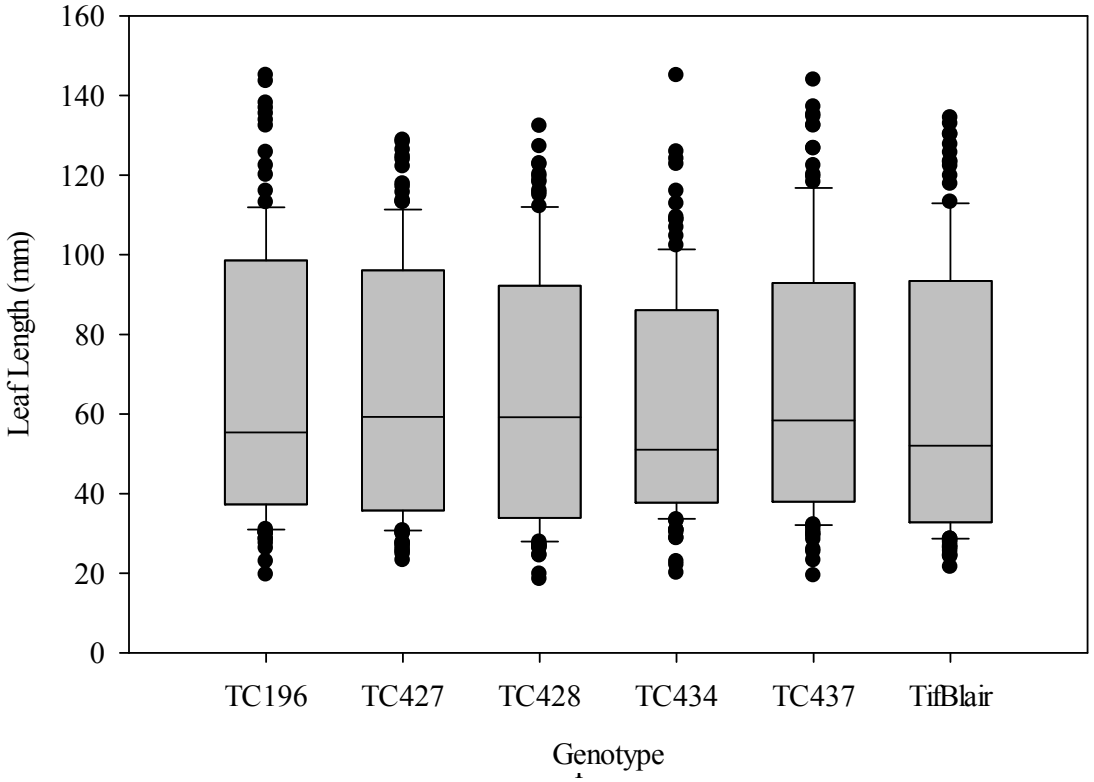

Figure 2.3: Leaf length (mm) distribution of all centipedegrass genotypes. mean = 65.35 mm.

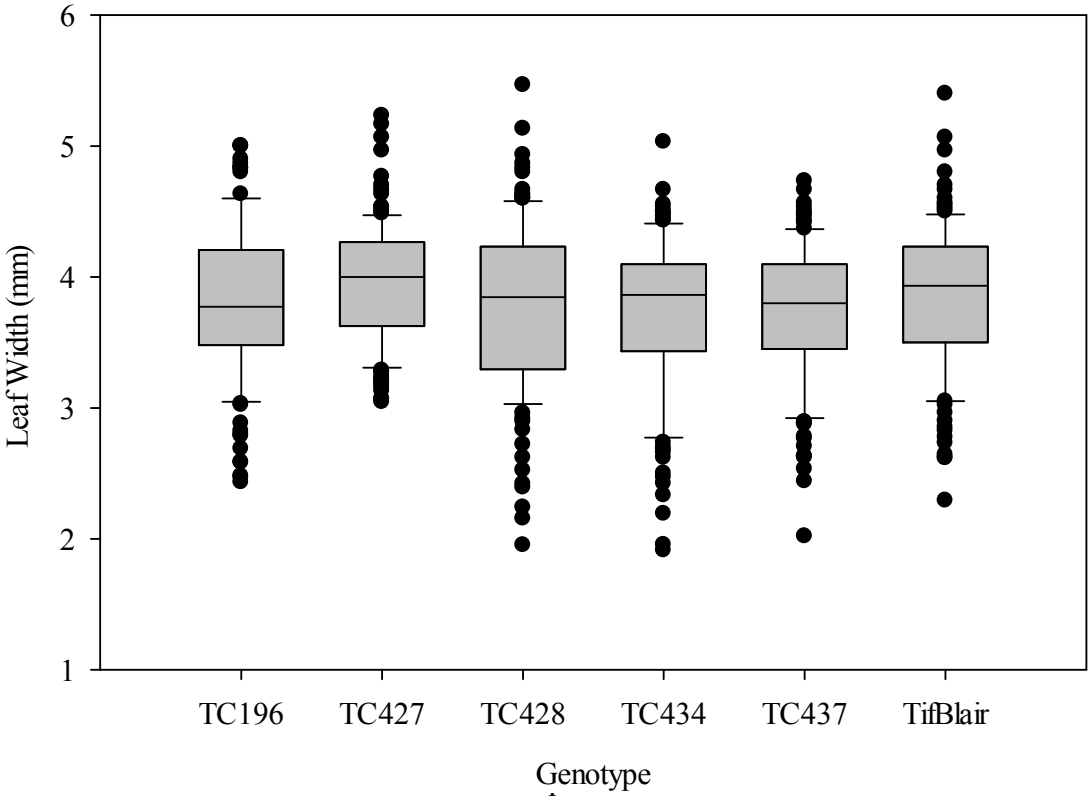

Figure 2.4: Leaf width (mm) distribution of all centipedegrass genotypes. mean = 3.80 mm

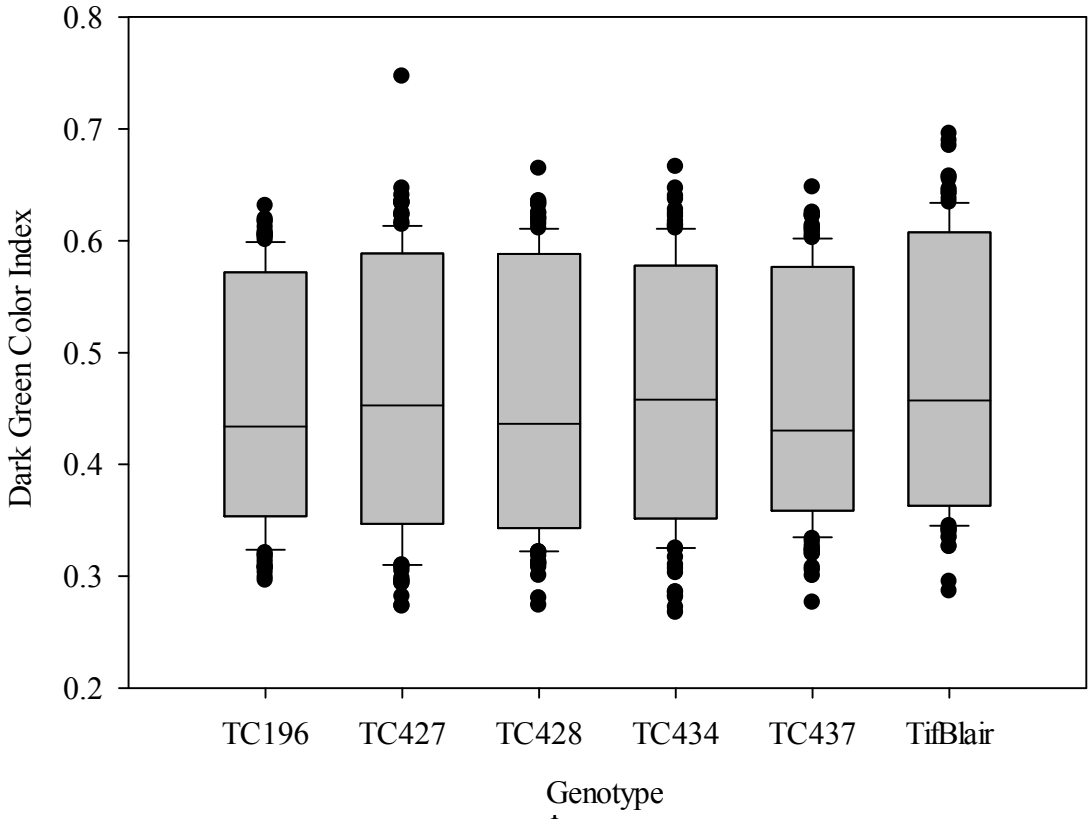

Figure 2.5: Green color mid-season distribution of all centipedegrass genotypes. mean = 0.47

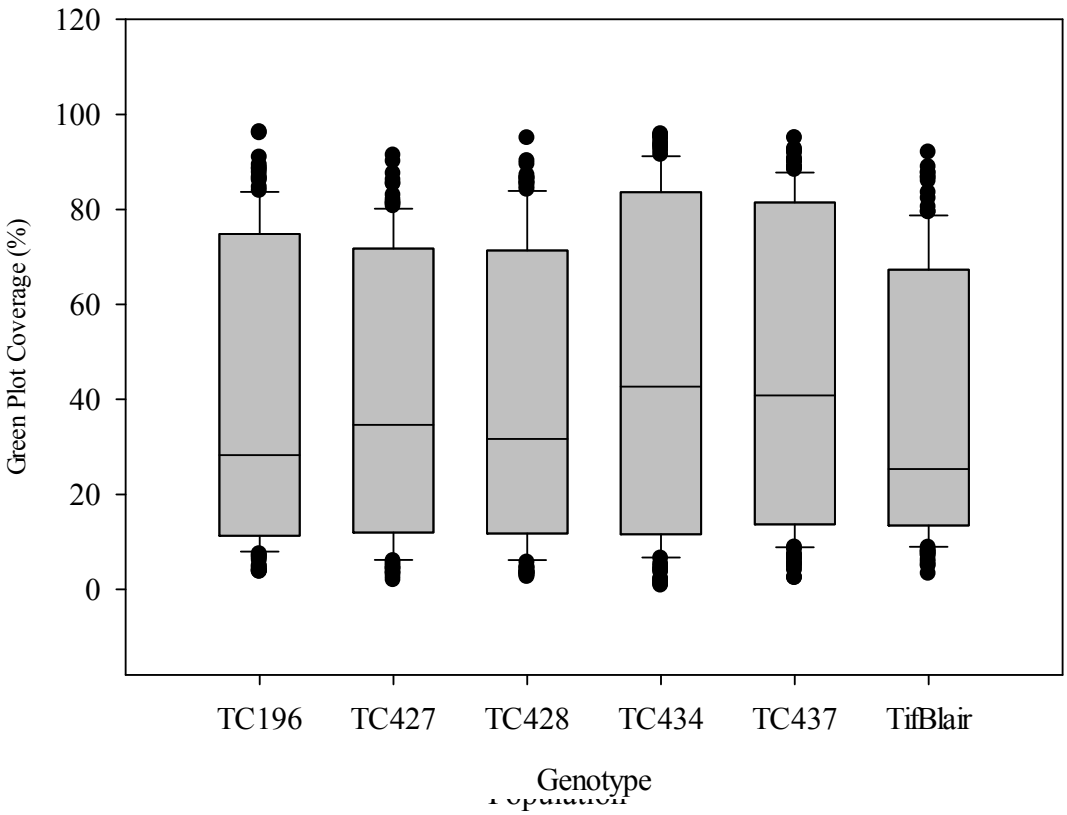

Figure 2.6: Green plot coverage mid-season(%) distribution of all centipedegrass genotypes. mean = 42.92%.

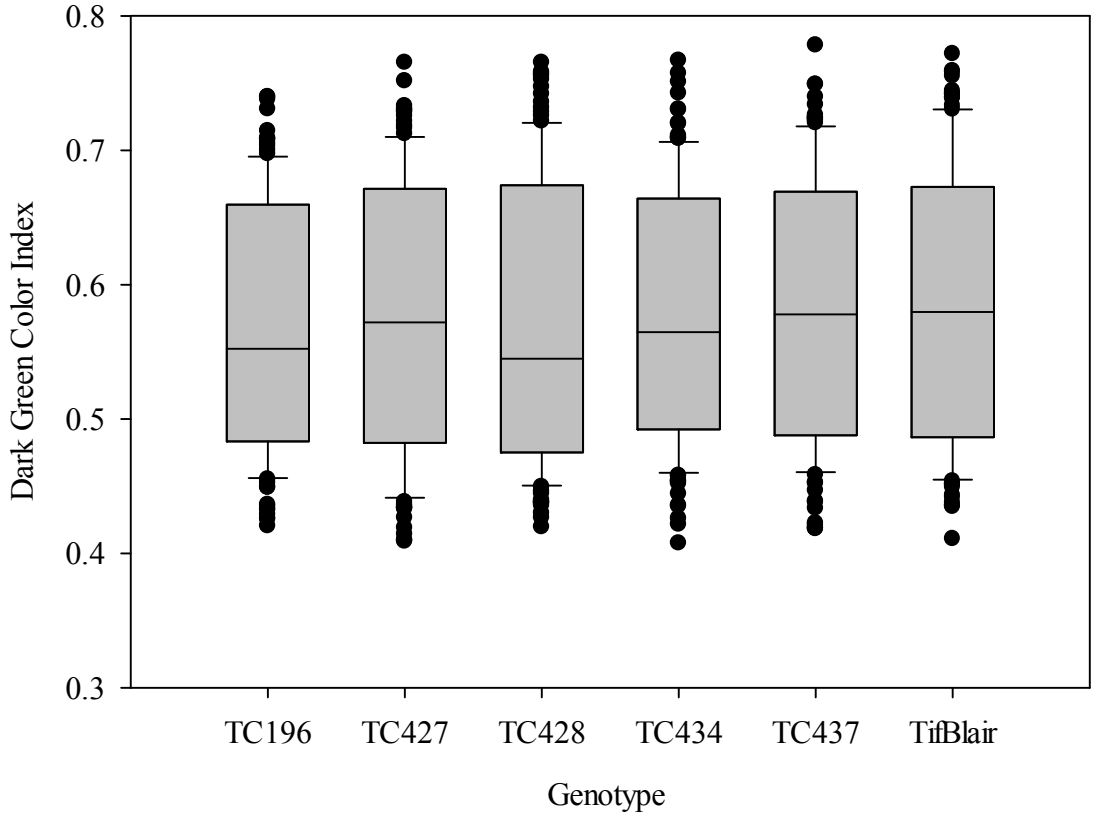

Figure 2.7: Green color late season distribution of all centipedegrass genotypes. Mean =0.58

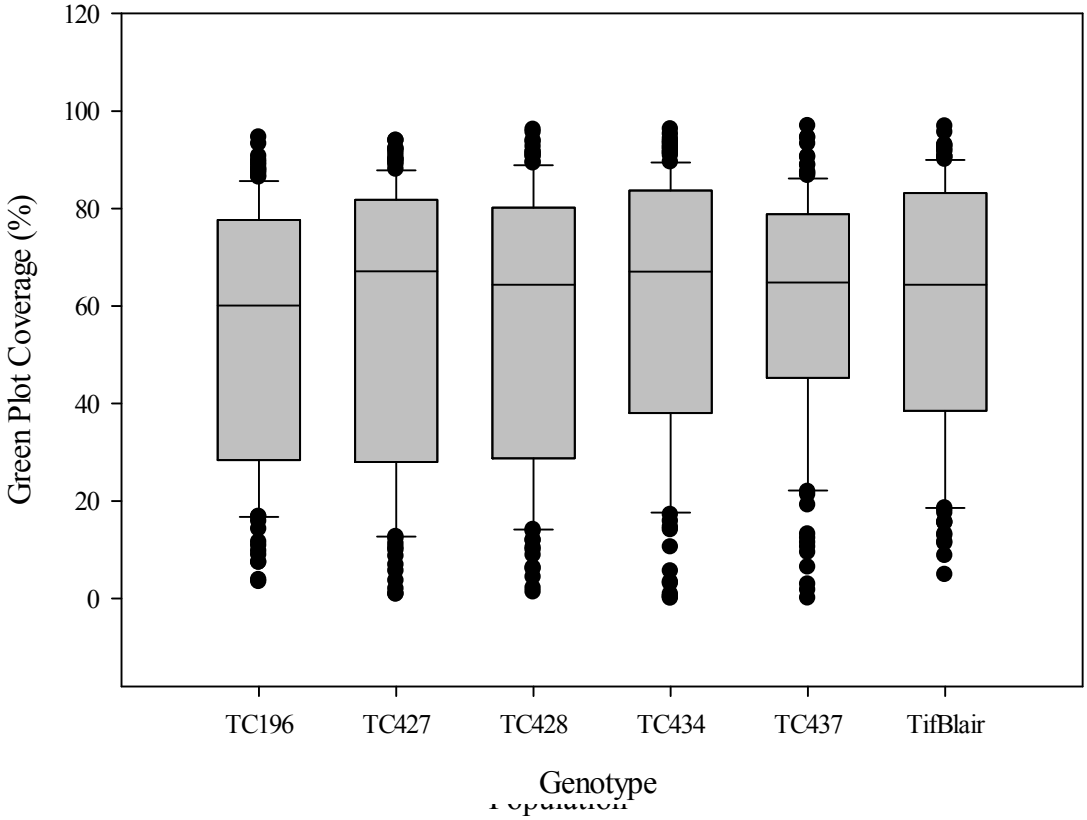

Figure 2.8: Green plot coverage late season (%) distribution of all centipedegrass genotypes. mean = 57.8%

CHAPTER 3

EVALUATION OF FERTILITY IN POLYPLOID ZOYSIAGRASSES[1]

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Abstract

Zoysiagrasses (*Zoysia* spp.) are typically characterized as allotetraploids ($2x = 4x = 40$) with high outcrossing rates and the ability to hybridize with other members of the genus. Despite all the morphological and genetic variation available from interspecific crosses, the ability to recuperate quickly from damage and wear is still lacking. Additionally, vegetative propagation is used to increase planting stocks of most *Zoysia* cultivars even though they can produce seed. Chemical mutagenesis has long been used in plant breeding to produce crops that have novel traits such as larger flowers or compact growth habit, however it can also be used to produce sterile plants. Sterility is useful in vegetatively propagated turfgrasses to maintain uniformity and stability in farm fields, golf courses, and landscapes by reducing or eliminating the production of segregating seedlings. In a long-term breeding effort initiated in 2009 at the University of Georgia to increase vegetative growth rates, tetraploid ($4x$) zoysiagrasses were treated with colchicine to induce chromosome doubling. This effort resulted in the creation of octaploid ($8x$) genotypes, and with further self- and cross-pollination, pentaploid ($5x$), hexaploid ($6x$), and septaploid ($7x$) genotypes were produced. Several stages of meiosis were observed in order to estimate the fertility levels of each of these ploidy levels. In anaphase I, 4x plants had 10% abnormal events while 5x, 6x, 7x, and 8x had 40%, 28.8%, 65.4% and 9.3%, respectively. Pollen stainability results showed 90%, 70%, and 95% good pollen for 4x, 5x, and 8x plants, respectively. These results suggest that fertility is greatly reduced in 5x and 7x genotypes. Further evaluation of these genotypes is needed to determine turfgrass quality and performance in the field, and if there is a measurable decrease in the number of segregating seedlings in these unique polyploids over time.

Introduction

Zoysiagrass (*Zoysia spp*. Willd.) is a perennial warm season turfgrass adapted to tropical and southern temperate regions of the world (Chandra et al., 2017). In the United States, zoysiagrasses are primarily used on golf courses, lawns, roadsides, and commercial landscapes in the southern region and upward into the transition zone (Kimball et al., 2012). They are considered a low input turf species because they provide a high quality turf at a lower maintenance than other turfgrass species (Murray and Morris, 1988). The most commonly used species in the turfgrass industry are *Zoysia japonica, Zoysia matrella*, and *Zoysia pacifica* previously known as *Zoysia tenuifolia* (Chandra et al., 2017; Turgeon, 2011). All species can create interspecific crosses and all have been characterized as allotetraploids (2n= 4x = 40) (Forbes, 1952).

Since the introduction of *Zoysia spp.* to the United States over 125 years ago, almost 50 improved cultivars have been released (Patton et al., 2017). Turfgrass breeders are still working to overcome the species shortcomings such as slow growth rate, winter injury, slow seed establishment, and susceptibility to various biotic stressors. There are also efforts in zoysiagrass as well as other warm season turfgrass species to produce sterile cultivars. Turfgrass sterility would provide cultivar uniformity, genetic purity, and energy would not be used for seed development (Schwartz, et al., 2013a).

In order to produce new cultivars, breeders need genotypic and phenotypic variation. For many years, plant breeders have used mutation breeding to widen the variation in their germplasms. During the past eighty years, mutation breeding has led to over 3000 plant varieties (FAO/IAEA, 2017). A database of plant varieties derived from mutations is accessible on the FAO/IAEA Mutant Variety Database and it is acknowledged that database is far from complete.

Frequently information about how varieties are obtained is not published, so it is estimated that even more varieties have been developed from mutation breeding (Schouten and Jacobsen, 2007). Colchicine is a chemical mutagen well known for its polyploidizing capacity that has been used to induce variation in plant species (Semeniuk and Arisumi, 1968). Colchicine induces polyploidy by binding to tubulin dimers that cause the disruption of microtubule formation preventing the migration of chromatids to the correct poles at anaphase (Pornchuti et al., 2015). The main components of the spindle apparatus are microtubules and the spindle is necessary to equally divide the chromosomes into the daughter cells.

Irregular pairing of chromosomes during meiosis can lead to sterility in plants. Chromosome doubling has led to development of seedless varieties such as citrus (Oluk et al., 2017), bananas (Sardos 2016), and watermelons (Sugiyama and Morishita, 1999). In watermelon, tetraploids were developed and then crossed with diploids lines resulting in triploid watermelons with non-viable seeds (Wang et al., 2016). Seedless varieties have been proven successful products in horticulture and the turfgrass industry as well. Hybrid bermudagrass is a cross between *Cynodon dactylon* (2n = 4x = 36) and *Cynodon transvaalensis* (2n = 2x = 18) resulting in a sterile triploid (2n = 3x= 27) (Hanna et al., 2013). In diploid centipedegrass *Eremochloa ophiuroides* (2n = 2x = 18), colchicine was used to induced tetraploid plants, and with further breeding efforts triploid plants were identified (Schwartz et al., 2013b).

Polyploids with odd ploidy levels such as 3x, 5x, and 7x have difficulty successfully complete meiosis due to the genetic imbalance (Wang et al., 2016) Unequal meiotic division can result unequal chromosome segregation and chromosome elimination, and therefore a high level of sterility in gametes (Tel-Zur et al., 2005). The knowledge about chromosome behavior during meiosis is an essential step to developing superior varieties. One observable abnormality that

can result in non-viable gametes is anaphase lag and the presence of micronuclei. Micronuclei are tiny nuclei that form outside of the main nucleus as a result of a lagging chromosome or a fragment of a chromosome (Potapova and Gorbsky, 2017).

In 2009, long term breeding efforts to increase the variation of the University of Georgia's zoysiagrass germplasm was initiated by inducing polyploidy using colchicine. This effort resulted in hexaploid (6x) and octaploid (8x) genotypes and with subsequent crossing resulted in tetraploid, pentaploid, and septaploid genotypes. Previous observations have shown tetraploid, hexaploid, and octaploid genotypes being fertile and pentaploid and septaploid to be partially sterile (Schwartz et al., 2013a). The objectives of this study were to 1) confirm ploidy level of colchicine altered octaploids genotypes and derived tetraploids, pentaploids, hexaploids, and septaploids. 2) Evaluate the chromosome behavior at meiosis of each ploidy level for abnormalities.

Materials and Methods

Plant Material

Zoysiagrass materials evaluated in the research consisted of 10 genotypes of 4x (Fig. 3.1), 5x (Fig. 3.2), 6x (Fig. 3.3), 7x (Fig. 3.4), and colchicine-derived 8x plants from Schwartz et al., (2013c) and subsequent breeding. Plants were vegetatively maintained in a 15 cm diameter pot containing SunGro® Fafard 3B Mix/Metro-Mix 830 professional growing mix in a greenhouse at the University of Georgia in Athens, GA. Natural light was supplemented with artificial light from 400 watt high pressure sodium overhead lighting (Sylvania, Wilmington, MA) at 500 μmol m^{-2} s^{-1} photosynthetic photon flux in a 12-h day to approximate summer light intensity and photoperiod. Conditions in the greenhouse were maintained at day/night temperatures of 32/24°C. Plants were fertilized weekly (20 N-20 P_2O_5-20 K_2O) at a rate of 3.6 kg

N ha^{-1} (Masterblend International, Morris, IL). Plants were surface irrigated daily with tap water and were trimmed carefully bi-weekly by hand with scissors to avoid cutting emerging inflorescence.

Cytology

Chromosome number and meiotic behavior was determined using pollen mother cells (PMC) squash methods described by Higgins et al., 2014. Immature inflorescences at the boot stage were fixed in 3:1 (v/v) ethanol:glacial acetic acid solution. After 1hr, inflorescences were transferred to fresh fixative for 48h. Inflorescences were then dissected and spikes are washed with citrate buffer by adding 500 µl and then removing (3 x 5 min). For cell wall digestion, spikes were incubated in a humidified chamber at 37°C for 75 min in an enzyme digestion medium consisting of 1% (w/v) cellulase, 1% (v/w) pectolyase in a working solution 0.01 M citrate buffer at pH 4.5. After incubation, cell wall enzyme solution was removed and replaced with cold (4° C) sterile water to stop enzymatic reaction. Five to ten spikes were placed on a slide with a small drop of water and then quickly macerated with a mounted needle. Then 7 µl 65% acetic acid was added to cells and places in a hot plate at 45° C for ~30s. Then 200 µl of 3:1 fixative was placed as a ring around the material and the slide was placed aside to dry. Once dry, slides were stained and mounted with 10 µl of Vectashield with DAPI (4',6-diamidino-2-phenylindole) (Vector Laboratories, Burlingame, CA). Slides were viewed using a Zeiss Axio Imager M2 epifluorescence microscope (Carl Zeiss Microscopy, Thornwood, NY). Photomicrographs were captured with attached Zeiss AxioCam MRc camera and analyzed with Zeiss Axiovision Release 4.8 software. For chromosome counts at each ploidy level, 10-15 cells were analyzed at metaphase I to determine the final chromosome number. For analysis of meiosis abnormality, 26- 77 cells at anaphase I were analyzed and classified as abnormal if

lagging chromosomes were present. Between 15-36 cells and 5-42 cells were analyzed for dyads and tetrads, respectfully, and classified as abnormal if micronuclei or triad formations were present.

Pollen Staining

The starch content of pollen grains from seedheads was estimated by staining pollen deposited onto microscope slides with 50 µl of 2% iodine-potassium iodide solution (Carolina Biological Supply Company, Burlington, NC) for 10 min before observation (Schwartz et al., 2013c). Percentage of pollen stainability was estimated according to methods of Kumar et al., 2014 by classifying 200-300 pollen grains within the field of view at 100X magnification as good (well-filled and uniformly stained cytoplasm) or bad (shivered/flaccid and poorly stained cytoplasm).

Results and Discussion

Chromosome counts

During the meiotic analyzes, each genotype (4x, 5*x*, 6*x*, 7*x*, and 8*x*) chromosome number was counted at metaphase I cells confirming the ploidy of tetraploid ($2n = 4x = 40$) (Fig. 3.5A), pentaploid ($2n = 5x = 50$) (Fig. 3.5B), hexaploid ($2n = 6x = 60$) (Fig. 3.6A), septaploid ($2n = 7x = 70$) (Fig. 3.6B), and octaploid ($2n = 8x = 80$) (Fig. 3.7) genotypes supporting the observations about the parent genotypes made by Schwartz et al., 2013c. This demonstrates that with colchicine treated seeds and subsequent breeding polyploid *Zoysia spp*. plants can be obtained. Initially only four putative M_0 octaploids and one cytochimera were identified and further self- and cross- pollinations led to the development of M_1 octaploid and M_1 hexaploid (Schwartz et al., 2013c). This study further verifies that inherited ploidy increases in induced plants were stable through the sexual process for multiple generations, however the pedigree of tetraploid

plants (Fig. 3.1) used in this study were initially reported to be pentaploids (Schwartz et al., 2013c). One possibility is this plants reverted back to initial ploidy level. Leher et al. (2008) found that in *Berberis thunbergii* treated with colchicine there was a low frequency of reversion from tetraploid back to diploid following a dormancy period. Ploidy reversions have also been seen in *Coix lacryma-jobi* (Venkateswarlu and Panuganti, 1975) and diploid bananas (Hamill et al., 1992). Another possibility is self-pollination of the tetraploid parent. Zoysiagrass species are self –fertile, but protogynous nature favors outcrossing, however in swards of turf self-pollination is possible (Forbes, 1952; Patton et al., 2017).

This study gives a better understanding of interploidy hybridization in *Zoysia spp.* Interploidy cross-pollinations between octaploid female parents and tetraploid male parent genotype resulted in a hexaploid progeny (Fig. 3.3), and cross-pollination between tetraploid female parent and hexaploid male parent result in pentaploid as did the reciprocal cross (Fig. 3.2), and hexaploid female parent and octaploid male parent cross-pollinations resulted in septaploid progeny (Fig. 3.4). Similar interploidy hybridization results have been seen in hydrangea (*Hydrangea paniculata*), where crosses between tetraploids and hexaploids produced pentaploid progeny (Beck and Ranney, 2014). The direction of the cross-pollination in interploidy plants seems to not have an effect on the progeny produced in these zoysiagrasses as shown in 4x-6x and 6x-4x pollinations. In *Arabidopsis thaliana* (2n = 2x = 10) as the female parent and *Arabidopsis arenosa* (2n = 4x = 32) as the male parent, the interploidy cross produced progeny with fruits with aborted seeds. However, the reciprocal cross failed to produce fruits, and the flowers collapsed one day after pollination exemplifying the direction of the cross can have an effect in other species. (Cisneros and Tel-Zur, 2012). Hybridization is a major force in plant evolution and is the cause of phenotypic and genotypic variation in many plants. The

hybrids assessed in this study provide variation to the zoysiagrass germplasm that was not previously available and can be used to produce improved turfgrasses.

Meiotic behavior in polyploid zoysiagrasses

The percentage of cells with abnormal meiotic behavior is shown in table 3.1. Tetraploid genotypes had 10% of cells at anaphase I that displayed lagging chromosomes (Table 3.1). Tetraploid genotypes had the lowest number of cells undergoing abnormal meiosis suggesting that this population would be fertile in conjunction with pollen stainability of 90%. The stainability of goof pollen is based on iodine potassium iodide detecting the starch content. Starch is an important marker of pollen grain development (Oliveira et al., 2015). This result is expected because tetraploid is the natural occurring ploidy level in the genus and successful hybridization between various tetraploids is the basis of all zoysiagrass breeding to date (Forbes, 1952; Patton et al., 2017). Initial cytogenetic observations in tetraploid *Z. japonica, Z. matrella,* and *Z. tenuifolia* species found that the plants were fertile and there were no meiotic irregularities such as lagging chromosomes or anaphase bridges observed and over 95% good pollen was formed and this is consistent with observations in this study (Fig. 3.8A) (Forbes, 1952). More recent cytological studies assessing cultivars and experimental lines in *Zoysia spp*., have shown that all plants were tetraploid, further exemplifying that variation exploited in zoysiagrass breeding has always been due to hybridization at the naturally occurring ploidy level due to the stability of the meiotic process (Harris-Schultz et al., 2014; Schwartz et al., 2010).

At anaphase I, pentaploid genotypes had 40% (Table 3.1) of cells exhibiting lagging chromosomes (Fig. 3.8B) and 60% dyads with micronuclei (Fig. 3.9) suggesting some degree of infertility. A study on the meiotic chromosome behavior of *Cenchrus cilaris* (Poaceae: Panicoideae) polyploids, found that when compared to tetraploid and hexaploid plants,

pentaploids had the largest number of cells with lagging chromosomes at anaphase I. They further postulated that this was due to an unbalanced polyploid level and the laggards were seen in later stages of meiosis as micronuclei (Visser et al., 1998). Of the tetrads observed in pentaploid genotypes, 57.7% (Table 3.1) of them made abnormal formations (Fig. 3.10B). Similar abnormalities during meiosis of pentaploid have been seen in other species such as rice (Watanabe, 1974) and *Brachiaria brizantha* (Gramineae) (Risso-Pascotto et al., 2003). The meiosis abnormalities and low pollen fertility are seen in pentaploid plants derived from 4x-6x cross-pollinations (Kumar et al., 2014), 3x-4x cross-pollinations (Carputo 2003), natural accessions (Kovarik et al., 2008), and the fusion of unreduced 4n pollen and haploid eggs. This may signify that regardless of method of conception, pentaploid plant abnormalities at meiosis are due to unbalanced chromosome numbers. Pentaploid genotypes and other odd ploidy levels are sometimes referred to genetic "dead ends" because of their inability to hybridize due to infertility (Carputo, 2003). Although *Zoysia spp*. pentaploid genotypes show reduced fertility, at a low rate there is a chance of producing viable pollen. Triploid plants like pentaploid plants have abnormalities during meiosis due to odd ploidy number and at low rates produce euploid gametes (Ramsey and Schemske, 1998). Since sterility is desired in turfgrass cultivar development, these pentaploids with evidence of reduced fertility may have value in the breeding process with continual efforts to decrease fertility.

Hexaploid genotypes exhibited 28.8% of abnormal cells during anaphase I (Table 3.1). Since there are an even number of chromosomes at the haploid stage (n=30), these plants are expected to have a higher level of normal meiotic cells than odd ploidy levels because homeologous chromosome are available for paring and balanced gametes are produced (Habashy et al., 2004). Tosun and Sagsoz (2003), studied meiosis in high and low yield hexaploid triticale

and found that 85.5 % of cells were undergoing normal meiosis without incidence of lagging chromosome in high yielding genotypes and 69.4% of plant in low yielding genotypes. This study demonstrated in triticale that hexaploid genotypes express some degree of fertility in relation to the incidence of normal or abnormal meiosis events. The *Zoysia spp*. hexaploids are likely to express similar results since they have been hybridized with tetraploid plants and produced pentaploids plants in this study.

Much like pentaploid genotypes, septaploid genotypes were expected to show high levels of infertility due to the unbalanced chromosome number (n = 35) during meiosis. Septaploid genotypes in this study had 65.4% of abnormal cells at anaphase I (Table 3.1). The high level of abnormality suggests that 7x genotypes make a good candidate for desired sterile genotypes. There is a lack of published information of septaploid level meiosis. One study on the hybridization between *Agropyton trachycaulum* and *A. intermedium* produced one septaploid hybrid that exhibited a reduction in stainable pollen (Napier and Walton, 1983).

Octaploid genotypes exhibited 9.3% of cells at anaphase I with abnormal meiotic activity and this is the lowest for all ploidy levels. A study on the meiotic analysis of *Astragalus cicer* octaploids found similar results with the percentages of abnormal cells at anaphase I ranging from 7.6% to 13.8% (Latterell and Townsend, 1993). They concluded the consequences of the abnormalities were negligible and the plants were fertile. Similar results are demonstrated in *Zoysia spp.* octaploid genotypes, as shown in Table 3.1, 8x-4x cross-pollination were used to create hexaploid and septaploid genotypes (Fig. 3.3). Pollen stainability results show that octaploid genotypes have 95% of good pollen (Table 3.1) and similar results were shown in *A. cicer* where >95% of pollen was stained (Latterell and Townsend, 1993).

Conclusion

The data presented herein confirm previous investigations of polyploid *Zoysia* genotypes and expand the understanding of pollen and seed fertility in grasses used at the University of Georgia Tifton Campus. Zoysiagrass seeds were initially treated with colchicine in efforts to increase genetic variation and produce an improved germplasm (Schwartz et al., 2013c). Various ploidy levels in species not only adds genetic variation but also gives breeders the opportunity to obtain sterile plants by exploiting the inability of meiosis to create successful gametes in the absence of balanced chromosome segregation (Comai, 2005). The success of sterile hybrid bermudagrass that produces higher quality turf, more pest resistances, denser turf, fewer seedheads, and finer texture has guided efforts in numerous other turfgrass species, and this body of work is an example of that (Hanna, 1986). Increasing the polyploidy level variations and conducting cross-pollinations, that lead to meiotic abnormalities increases the chances of producing a zoysiagrass with similar success to that of triploid bermudagrass. Tosun and Sagsoz (2003) demonstrated the consequences of meiotic abnormalities on floret infertility, embryo abortion, incompletely developed seeds, and aneuploidy (Tosun and Sagsoz, 2003). While complete infertility was not exhibited in the genotypes examined, pentaploid and septaploid plants displayed higher numbers of abnormal meiosis, suggesting reduced fertility. Further evaluation of these genotypes for turfgrass performance and morphological characteristics will determine their true value to the breeding program. Tetraploid, hexaploid, and octaploid genotypes are useful in this germplasm, as they provide means of successful hybridization that increases genetic variation. Another option to increase genetic variation and develop a sterile hybrid is the induction of a diploid zoysiagrass through pollen culture or intergeneric crossing (Mason, 2017).

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T**able 3.1.** Normal cells and abnormal cells (%) counted and the percentage of pollen good pollen stained

Ploidy Level	Anaphase I		Dyads		Tetrads		Pollen Stainability
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Good Pollen
4*x*	77	8 (**10%**)	32	2 (**6.25%**)	40	5 (**12.5%**)	90%
5x	64	26 (**40%**)	15	9 (**60%**)	26	15 (**57.7%**)	70%
6x	52	15 (**28.8%**)	*	*	42	11 (**26.2%**)	*
7x	26	17 (**65.4%**)	*	*	5	2 (**40%**)	*
8x	32	3 (**9.3%**)	36	4 (**11.1%**)	*	*	95%

***** = No data

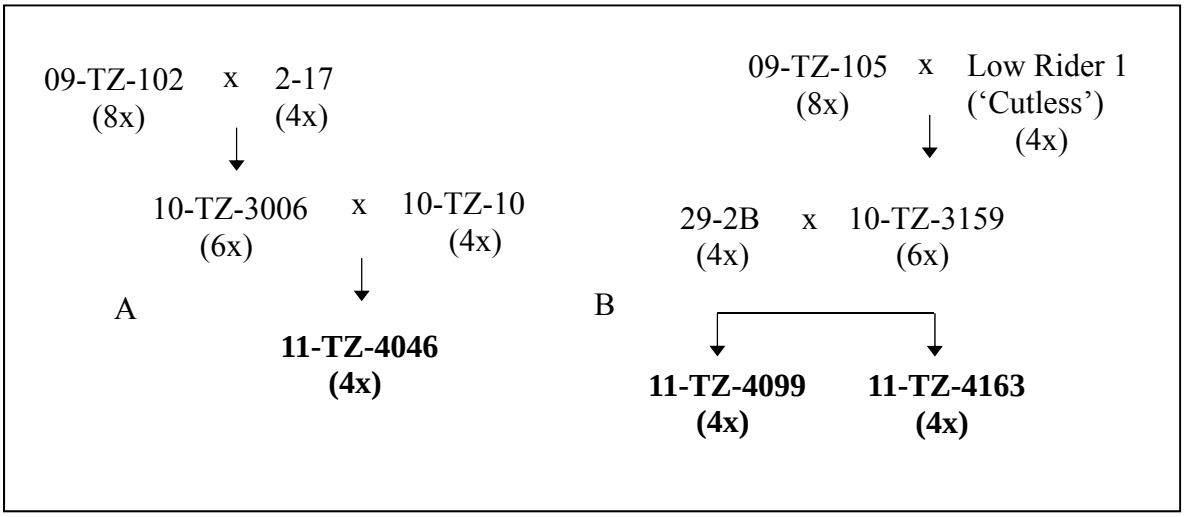

Figure 3.1. Pedigrees of *Zoysia spp.* tetraploid (2n = 4x = 40) plants analyzed in this study

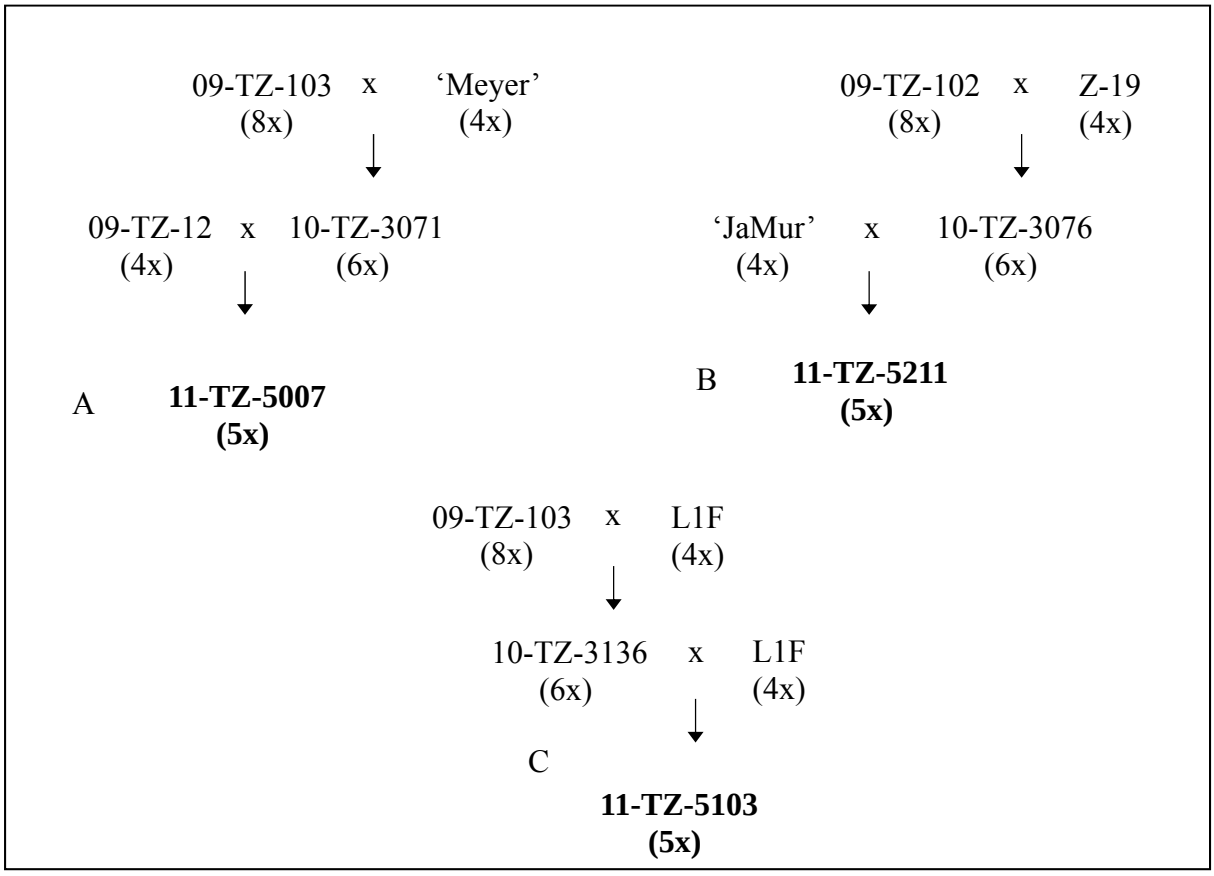

Figure 3.2. Pedigrees of *Zoysia spp.* pentaploid (2n = 5x = 50) plants analyzed in this study

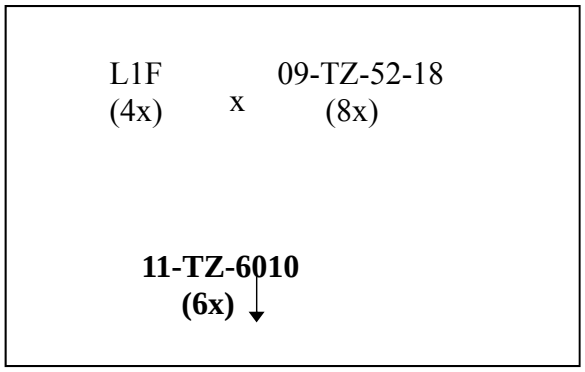

Figure 3.3. Pedigree of *Zoysia spp.* hexaploid (2n = 6x = 60) plant analyzed in this study

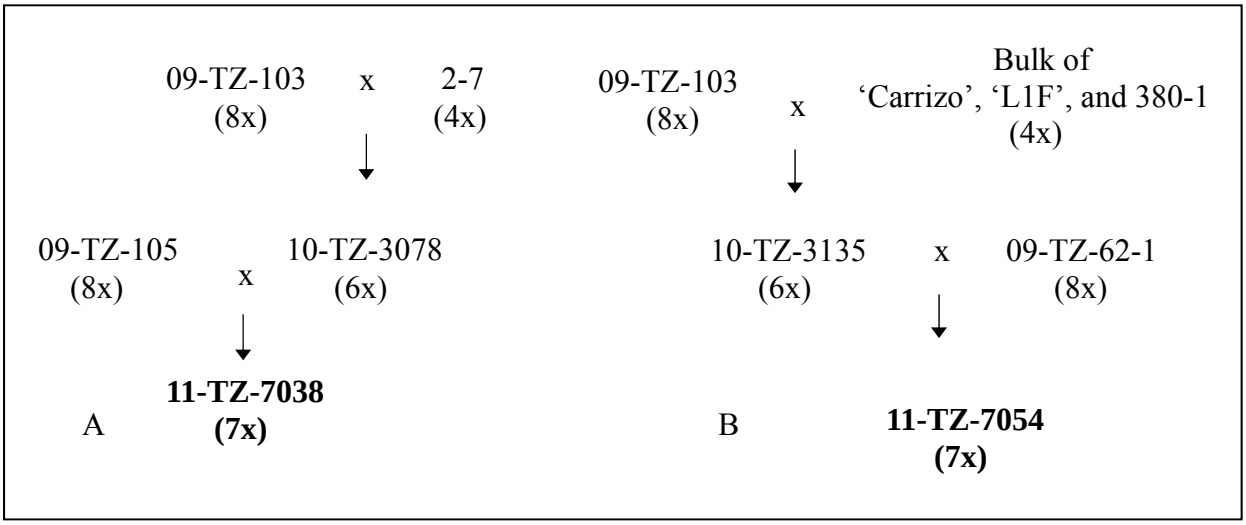

Figure 3.4. Pedigrees of *Zoysia spp.* septaploid (2n = 7x = 70) plants analyzed in this study

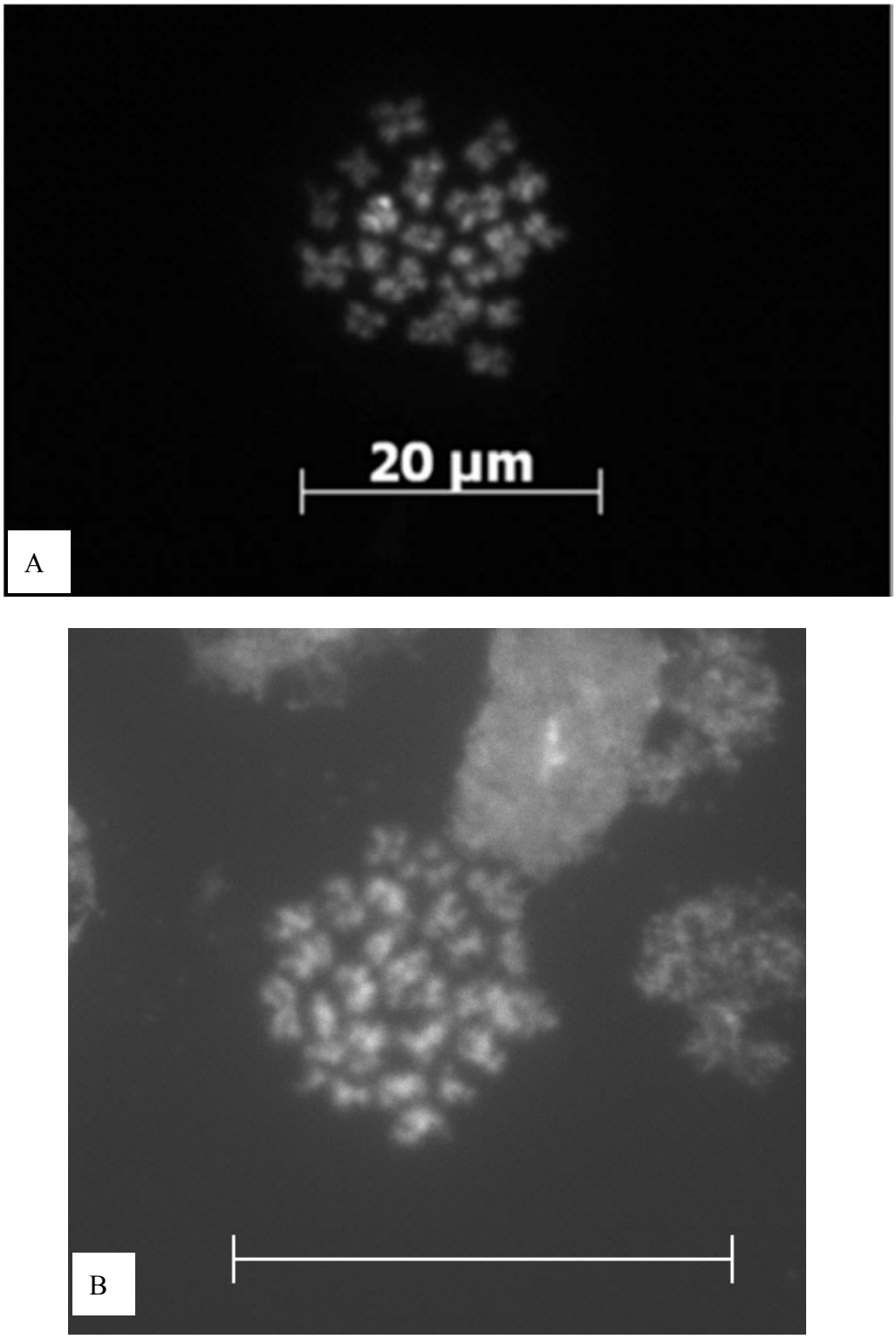

Figure 3.5. Photomicrographs of *Zoysia spp*. pollen mother cells taken at 100X magnification. (A) Tetraploid (2n = 4x = 40). (B) Pentaploid (2n = 5x = 50). Scale bar = 20 µm

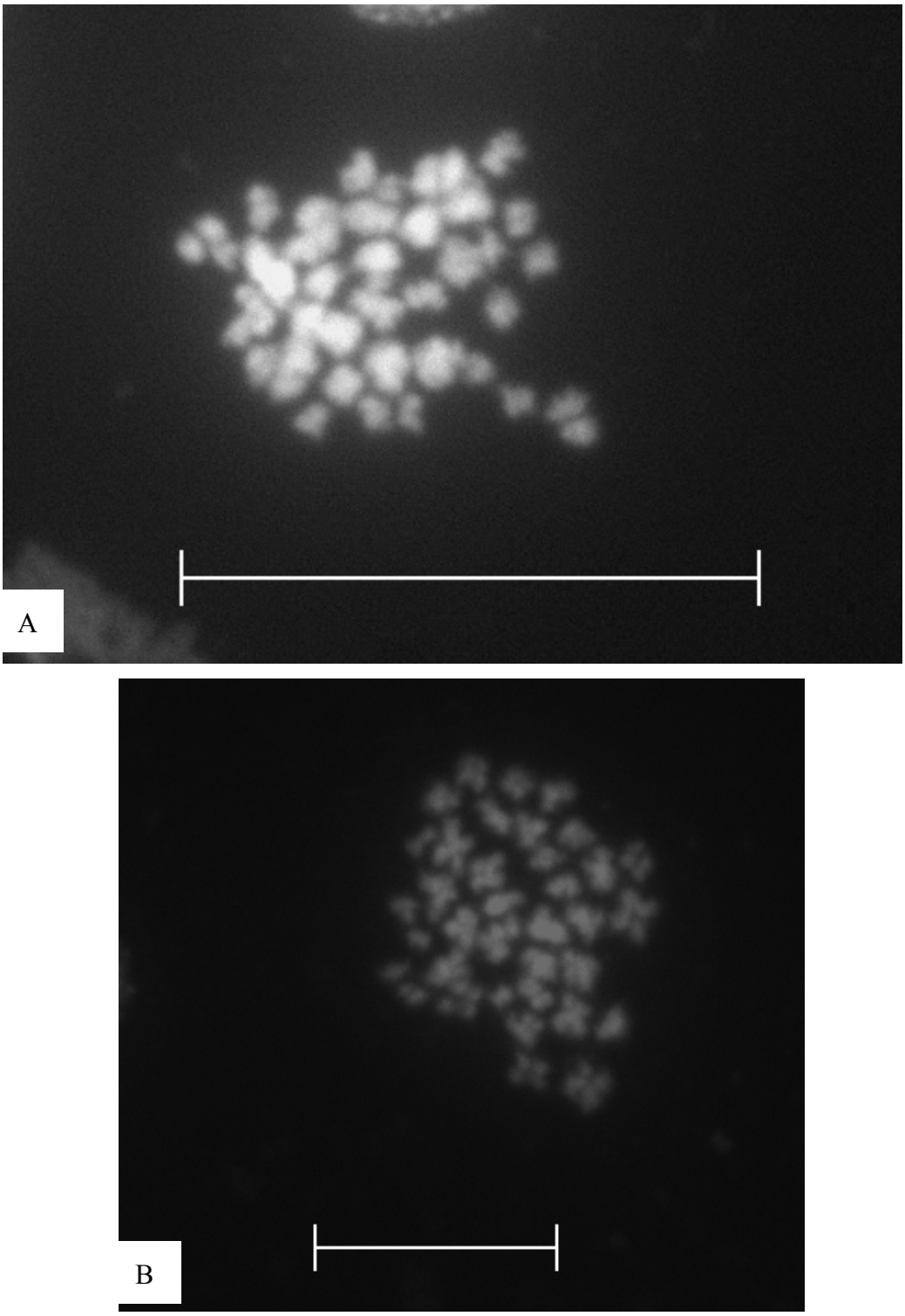

Figure 3.6. Photomicrographs of *Zoysia spp*. pollen mother cells taken at 100X magnification. (A) Hexaploid (2n = 6x = 60). (B) Septaploid (2n = 7x = 70). Scale bar = 20 μm

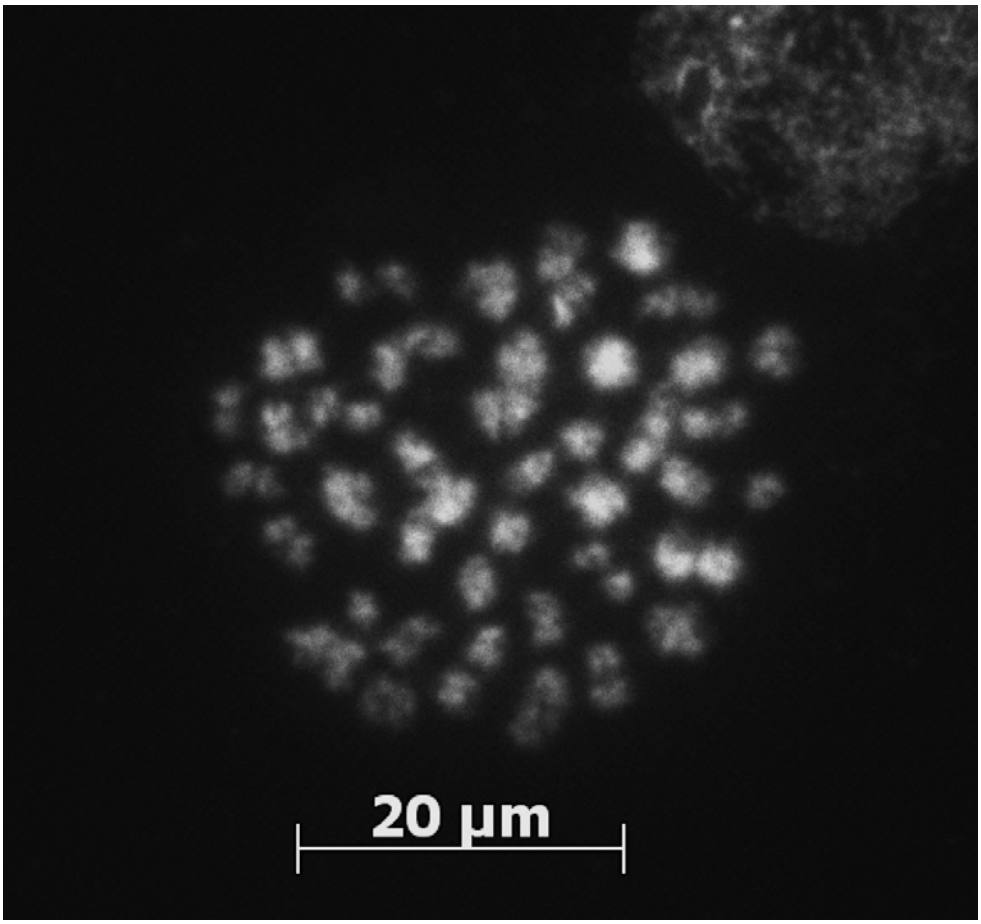

Figure 3.7. Photomicrographs of *Zoysia spp*. pollen mother cells taken at 100X magnification. Octaploid (2n = 8x = 80). Scale bar = 20 µm

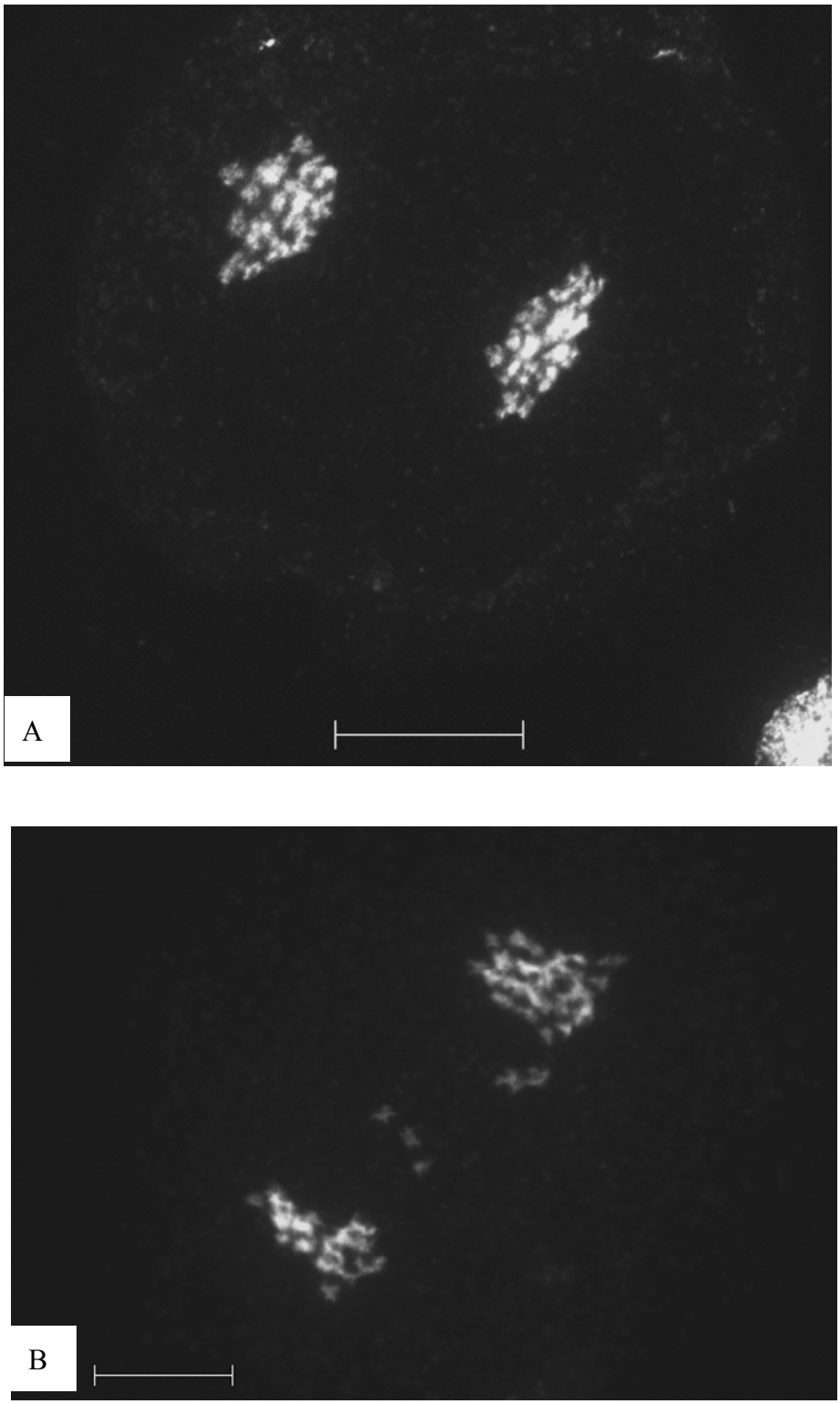

Figure 3.8. Photomicrographs of *Zoysia spp*. pollen mother cells during anaphase I taken at 63X magnification. (A) Normal anaphase I. (B) Abnormal anaphase I with lagging chromosomes. Scale bar = 20 μm

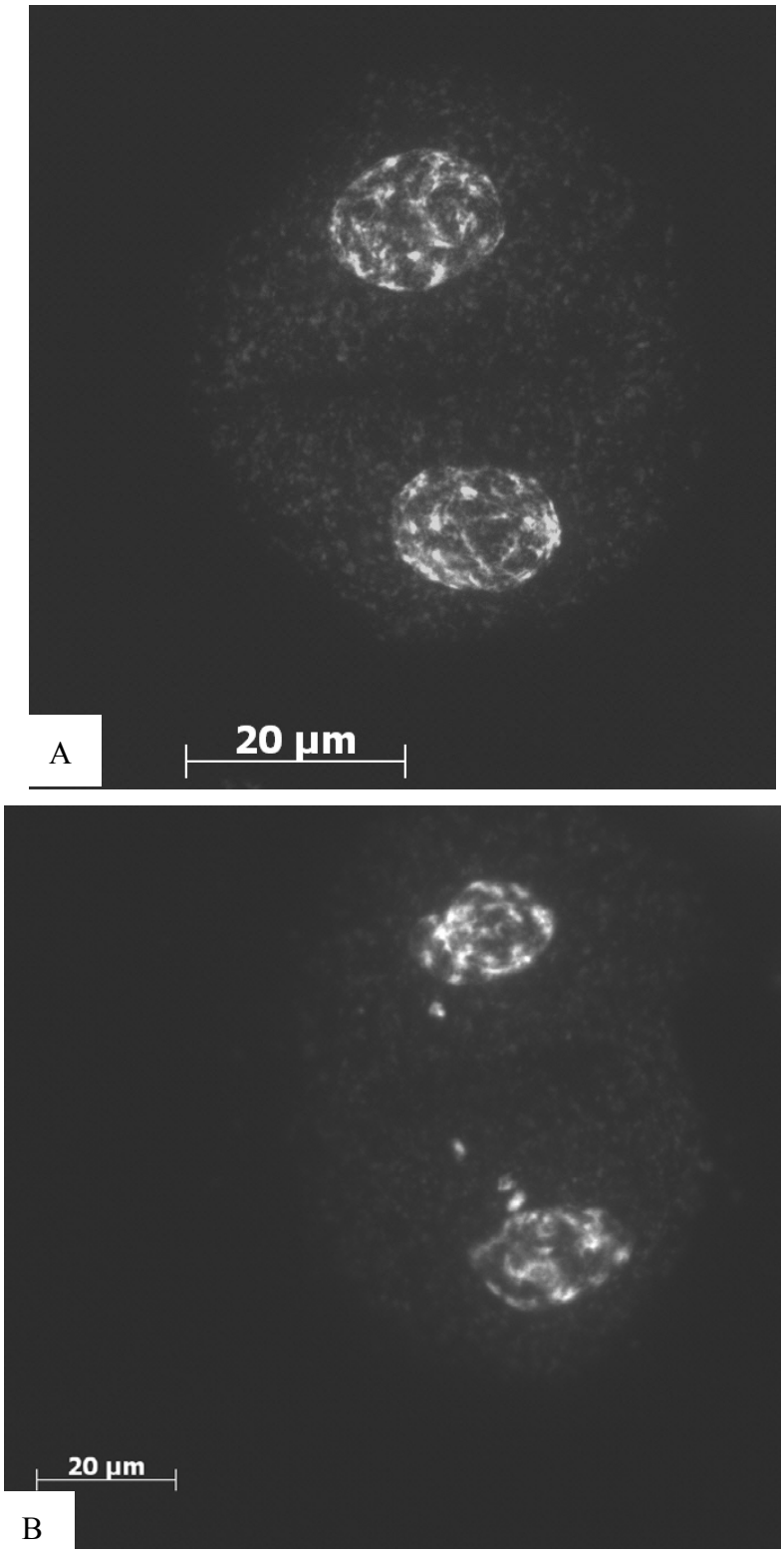

Figure 3.9. Photomicrographs of *Zoysia spp*. dyads taken at 63X magnification. (A) Normal dyad (B) Abnormal dyad with micronuclei. . Scale bar = 20 µm

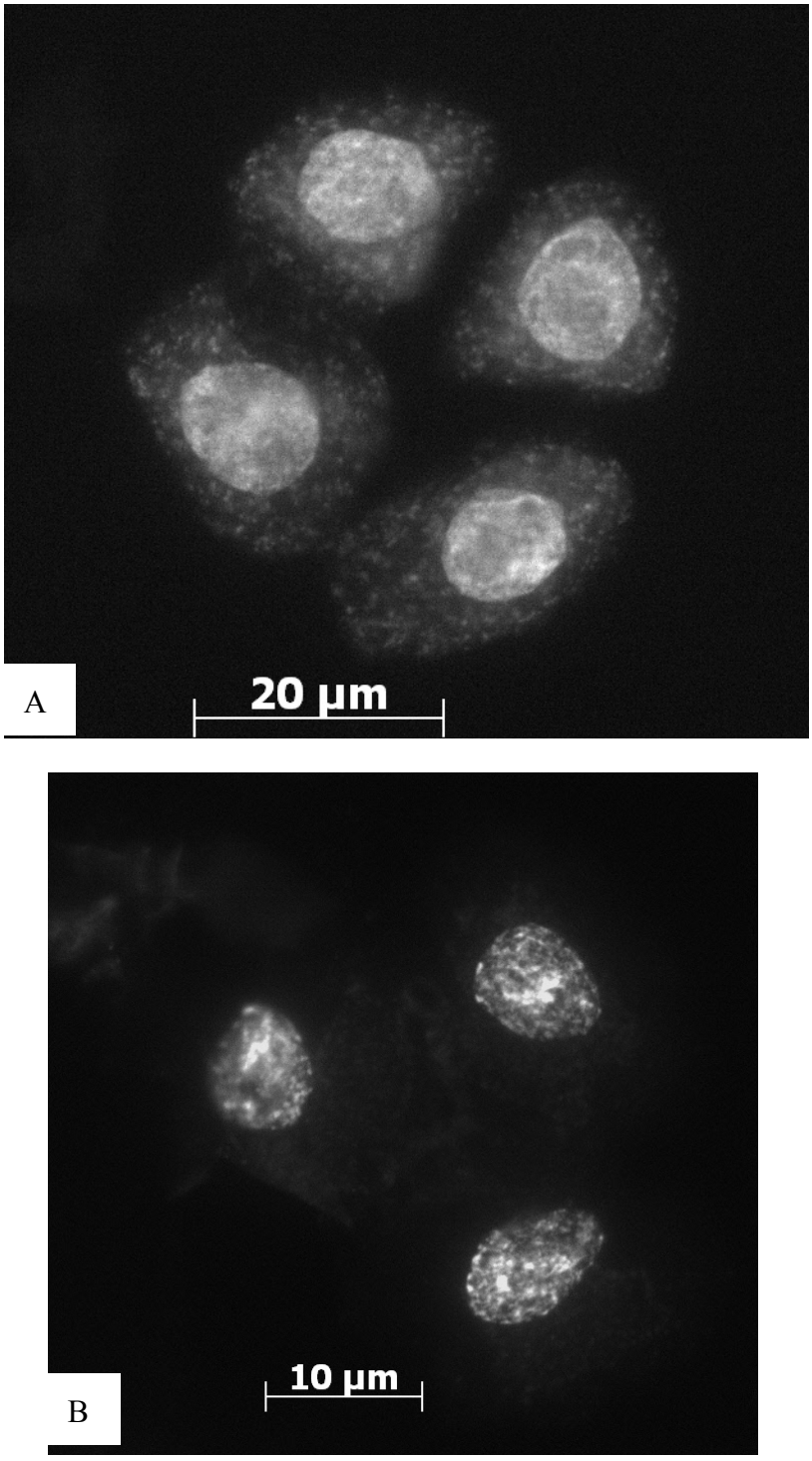

Figure 3.10. Photomicrographs of *Zoysia spp*. tetrad taken at 63X magnification. (A) Normal tetrad (B) Abnormal triad formation. . Scale bar = 10 and 20 µm

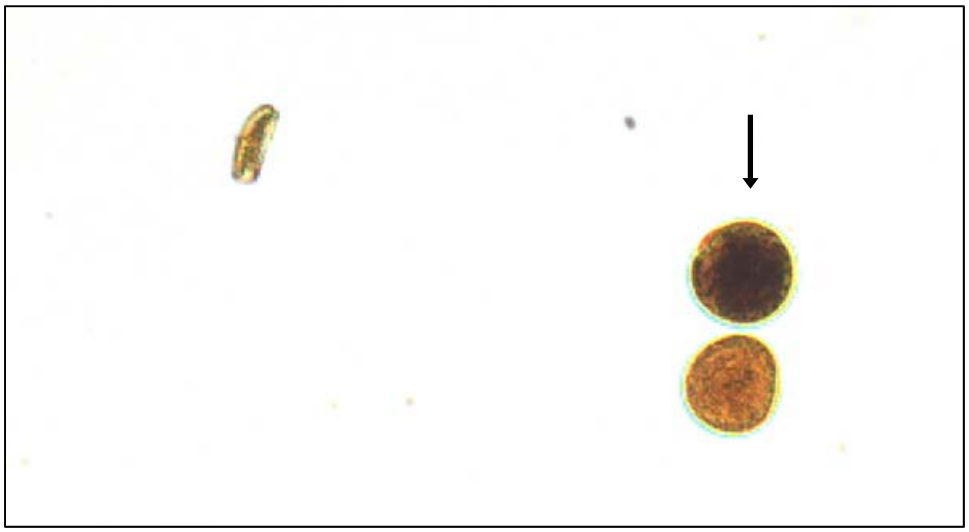

Figure 3.11. Photomicrographs of *Zoysia spp*. pollen taken at 100X magnification. Arrow indicating good pollen. Bad pollen and shivered pollen indicated by lighter color and shape.

CHAPTER 4

CONCLUSIONS

Utilizing phenotypic and genotypic variations that deviate from cultivars on the market is the principle of modern plant breeding. Variation can lead to improved cultivars that help turfgrass practitioners better and more efficiently manage turfgrass. The experiments described and the results presented provide information about how individual plant variability can be overlooked when evaluating centipedegrasses on the population level, and how chromosome doubling and interploidy crossing can alter pollen and seed fertility in polyploid zoysiagrasses.

The results presented in Chapter 2 show that genotype variance was not significant for leaf width, leaf length, internode length, green color mid and late season, green plot coverage mid and late season and inflorescence density mid-season. The results also showed that broad-sense heritability estimates were very low or null. These findings suggest that these populations do not vary from each other and that improvement through breeding and the development of superior hybrids is not possible. But, the measurements of individual plants within each population indicate that there was a wide range of variation for all traits analyzed. Similar means across all genotypes suggest that in the environments these populations were selected in, these traits were most advantageous or have no bearing on survival. In order for centipedegrass breeders to utilize the variation within each population, individual plants with large deviations from the mean values should be selected. Cultivar development using a recurrent selection scheme could allow a breeder to hybridize plants with varying morphology to shift future populations. These results demonstrate that in centipedegrass, mass selection based on

persistence in a particular environment may not be effective for developing novel cultivars with distinct morphological traits evaluated in this study.

The results from Chapter 3 demonstrate that in zoysiagrass it is possible to use colchicine mutations to induce ploidy variation and further hybridization to induce additional ploidy variation. Cytological results also displayed that there are varying frequencies of meiotic abnormalities and pollen stainability that have been correlated with sterility in other plant species. Tetraploid, hexaploid, and octaploid zoysiagrasses showed less meiotic abnormalities compared to pentaploid and septaploid genotypes. These results were expected since normal meiosis is depended on successful chromosome pairing, which is less frequent in odd ploidy numbers greater than the tetraploid level. Triploid plants are an example of how interploidy hybridization can lead to sterility that can be useful in turfgrass cultivar selection. The most successful bermudagrass cultivars have been triploid. Sterility in turfgrass is beneficial because sexual recombination in seed production would decrease the uniformity in a stand of turfgrass. Currently, all zoysiagrass cultivars are fertile tetraploids and the development of a sterile cultivar would have the same benefits as sterile bermudagrass.

The information obtained from these studies will help turfgrass breeders utilize individual plant variation in centipedegrass populations and polyploidy variation in zoysiagrass. Further studies are needed to determine if individual plant selection and hybridization based on morphological variation can be used to develop new, distinct centipedegrass cultivars. Only years of evaluation of turfgrass performance for both new centipedegrass populations and new polyploid zoysiagrasses in multiple environments will determine the value of these efforts for future cultivar development.