St. Augustinegrass Cytogenetics

Stenotaphrum secundatum

Luellen Swayzer

Introduction

St. Augustinegrass (*Stenotaphrum secundatum* [Walt.]) Kuntze is a perennial robust warm season turfgrass widely adapted to humid regions of the world. The species is primarily of tropical origin and native to sandy beach ridges, fringes of swamps and lagoons, salty and fresh marshes (Busey, 2003). St. Augustinegrass is common in the Carolinas to Florida, along the Gulf Coast, and in southern and central California (Duble, 2015).

The species is characterized by its coarse texture and spread by stolons or "runners" (**Fig. 2**). It has good salt tolerance and relatively low maintenance requirements and medium to superior shade tolerance, an especially valuable trait for home lawns, cemeteries, parks, and other spaces where trees are present (Waltz and Griffin, 2013). St. Augustinegrass is primarily used as a lawn turf because it does not tolerate traffic as well as some other warm season species **(Fig. 1)**.

The *Stenotaphrum* genus is comprised of seven species. The base chromosome number of *S. secundatum* is x = 9 and diploid, triploid, tetraploid, hexaploid ranging from 2n = 2x = 18 to 2n = 6x = 54 have all been identified (Milla-Lewis et al., 2013). Aneuploids (2n = 28-32) have also been reported in cultivars, however the extent of aneuploidy in the genus has not been fully assessed. The composition of the genome is not fully understood but it has been hypnotized to be allopolyploid (Busey, 2003). The first comprehensive assessment of genetic diversity at the molecular and cytological level among public sources of *Stenotaphrum* germplasm used flow cytometry, chromosome counts, and amplified fragment length polymorphic (AFLP) markers to determine the extent of variations in the germplasm and establish ploidy levels. Mean 2C nuclear DNA content ranged from 1.11 to 2.41, and although all five ploidy levels were found there was a lack of agreement between ploidy level as shown by flow cytometry and actual chromosome number (Milla-Lewis, 2013). There was a clear positive correlation between ploidy level and number and AFLP bands scored (Milla-Lewis et al., 2013).

Historically breeding efforts of St. Augustinegrass cultivars have focused on diploid genotypes, but as more genetic diversity is desired other ploidy levels are being assessed. Abiotic resistances and increased water use efficiency have been observed in polyploid genotypes (Milla-Lewis et al., 2013). The benefits of polyploids over diploids have been exemplified in several turfgrass and forage grass species. Bahiagrass (*Paspalum notatum* Flugge) cultivar 'Argentine' is a tetraploid and has wider leaves and faster regrowth compared to diploid cultivar 'Pensacola' (Schwartz et al., 2013). In the *Stenotaphrum* species polyploids are usually not morphologically different but there do have variations in resistance to south chinch bug *Blissus insularis* Barber and gray leaf spot *Magnaporthe grisea* (Milla-Lewis et al., 2013).

Though polyploid germplasm is available, the induction of polyploid has been used in turfgrasses, as well as other plant species to generate variation and sterility. Polyploidy induction has been used in zoysiagrass (*Zoysia* Willd.) through colchicine treatments to yield variation (Schwartz et al. 2013). Five putative octaploids and one cytochimera were identified and with further self- and cross pollination hexaploids genotypes were identified. Induced plants showed variation in stomata lengths, pollen grain diameter, sterility, and leaf blade morphology (Schwartz et al., 2013a). In bermudagrass (*Cynodon dactylon*), the tetraploid genotype was crossed with diploid African bermudagrass (*Cynodon transvaalensis*) to produce clonal triploid bermudagrass cultivars that has improved turfgrass quality and mostly sterile seeds. Sterility is useful in turfgrass because it provides cultivar uniformity for turf managers, and genetic purity for sod producers and plant energy resources are not used for seed development (Schwartz et al., 2013b).

In efforts to increase genetic variation, facilitate interspecific hybridization, and to create sterile lines St. Augustinegrass cultivar 'Raleigh' was subjected to varying levels of colchicine (Carabjal et al., unpublished data, 2017). 'Raleigh' (2n = 18) is a cultivar that was developed at North Carolina State University in the early 1980s and is known for its cold tolerance. From this experiment, tetraploid and diploid lines were generated and will be further evaluated to determine their value in improving St. Augustinegrass (Carabjal et al., unpublished data, 2017).

Figure 1: St. Augustinegrass homelawn in Florida

Figure 2: St. Augustinegrass stolons

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Plant Material

In 2003, 1500 mature 'Raleigh' St. Augustinegrass seeds (2n = 18) where collected and exposed to varying concentrations of colchicine to induce chromosome doubling. Concentrations included 0.025%, 0.05%, 0.I%, and 0.2% colchicine w/v with varying exposure times. Final plants recovery consisted of a population of 405 individuals. Flow cytometry determination identified the derived individuals to be diploids and tetraploids. Individual DSA-16016 (**Fig. 3**) was generated from self-pollination of DSA 13005, which was generated from 0.025% colchicine for 48 hours (Carbajal et al., unpublished data, 2017).

Chromosome visualization

The apical meristem of actively growing roots was excised from plants growing in commercial potting mix and sand in the early morning (**Fig. 4**). The roots were rinsed in cold water and placed in a microfuge tube with holes bored on the tops and a drop of water to humidify the tube before being treated with nitrous oxide at 200 psi for 4.5 hours (Kato, 1999). Root tips were fixed in freshly made Carnoy's solution for 30 min. Roots were washed 3 times with double-distilled water and each root was placed on a microscope in a humid chamber with 10 µL of enzyme solution (2% cellulase and 1% pectolyase Y-23 in citric buffer at 5.5 pH). The slides were incubated at 37°C for 2 hours. Once removed from incubation the excess enzyme was blotted away and 10 µL of 45% acetic acid was added to the root tip to stop enzymatic digestion. Each root tip was then macerated to produce a homogenous solution and a cover slip was placed on the slide. The slide was flipped over and squashed to remove air bubbles. The slides were placed in -80°C freezer for 24 h. A razor blade was used to pop off cover slips off the frozen slide and they were air-dried. Slides were stained and mounted with 10 µL of DAPI + Vectorshield. After 30 minutes the slides were viewed a with Zeiss AxioImager M2 epifluorescence microscope (Carl Zeiss Microscopy GmbH). Images were captured with Zeiss Axiovision Release 4.8 software.

Figure 3: DSA 16016, tetraploid line
Figure 4: Actively growing root

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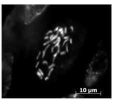
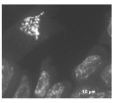
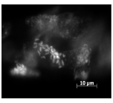
Chromosome Count

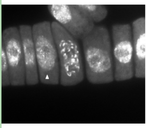


Estimated chromosome count for tetraploid St. Augustinegrass is 2n = 4x= 36



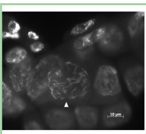


Stages of Mitosis



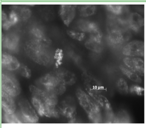
Interphase & Early Prophase

Chromosomes are fully uncondensed and as early prophase begins the chromosomes become visible



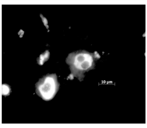
Prometaphase

The nuclear envelope disappears and the chromsomes are condensing



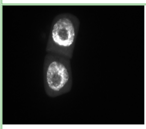
Metaphase

Chromosomes are fully condensed and are moving to the metaphase plate



Telophase

Daughter nuclei are formed and cytokinesis has started



Cytokinesis

The cell wall is fully formed and two daughter cells are formed

Discussion

- **Unambiguous counts were not found therefore more chromosome visualization is necessary to confirm the chromosome number. The estimation for this genotype is 2n = 4x = 36, however this is based on flow cytometry. Each chromosome count that was achieved did visualize 36 chromosomes.**
- **Anaphase was not found and this could be due to the mitotic index association with a pretreatment of nitrous oxide. Other pretreatments such as ice water have been found to have a higher mitotic index compared to other treatments therefore to observed mitosis in the future this method will be used.**

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References

Busey, P., 1980. St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. In: M.D. Casler and R.R. Duncan (eds.). Biology, Breeding, and Genetics of Turfgrasses, 309-330. John Wiley & Sons, Inc., Hoboken, NJ.

Duble, R. 2015. St. Augustinegrass. Texas AgriLife Extension. http://aggie-horticulture.tamu.edu/archives/parsons/turf/publications/staug.html (accessed 15 April 2017)

Kato, A. 1999. Air drying method using nitrous oxide for chromosomecounting in maize. Biotech. Histochem. 74:160–166.

Waltz, C. and B. Griffin. 2013. Popularity of St. Augustinegrass growing across Georgia. Media Newswire. College of Agricultural and Environmental Sciences, University of Georgia. http://www.caes.uga.edu/newswire/story.html?storyid=4692 (accessed 15 April 2017).

Milla-Lewis, S.R., M.C. Zuleta, G.A. Van Esbroeck, K.H. Quesenberry, and K.E. Kenworthy. 2013. Cytological and Molecular characterization of genetic diversity in *Stenotaphrum*. Crop Sci. 53:296-398

Schwartz, B. M.,R.N. Contreras, W.W. Hanna, S.A. Jackson. 2013a. Manipulating the Chromosome Number of Zoysiagrass. In ASA-CSSA-SSSA Abstracts 2013 Madison, WI.

Schwartz, B.M., K.R. Harris-Shultz, R.N. Contreras, C.S. Hans and S.A. Jackson. 2013b. Creation of Hexaploid and Octaploid Zoysiagrass Using Colchicine and Breeding. Crop Sci. 53: 2218-2224.

Images:
St. Augustinegrass Lawn: https://sodsolutions.com/grasses/sapphire-st-augustine/
St. Augustinegrass stolons: http://www.plantanswers.com/floratam_distinguish.htm

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