NATURALLY OCCURRING STABLE 8X AND CHIMERICAL 4X/8X ZOYSIAGRASS GENOTYPES REVEALED BY FLOW CYTOMETRY

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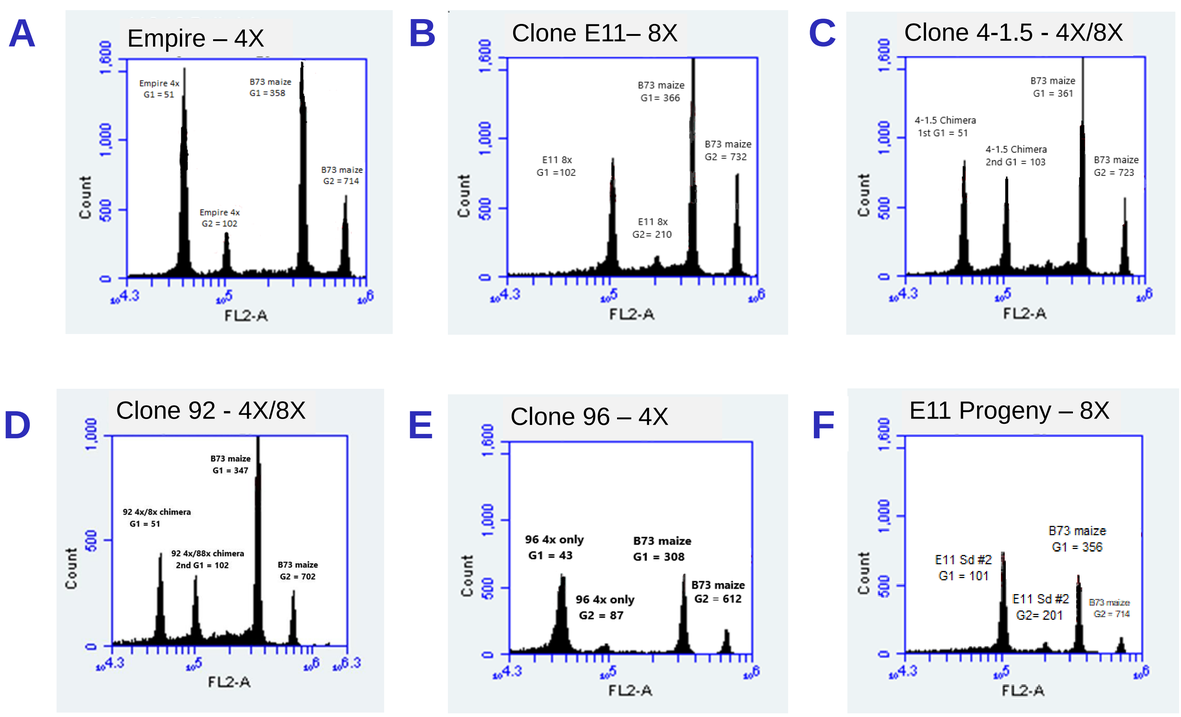
Background/ Rationale

- Use of zoysiagrass (*Zoysia* Willd.) as a turfgrass is increasing in warmer climatic zones.
- *Zoysia* spp. are tetraploids, although recently both induced and naturally occurring octaploids have been reported.
- Variable ploidy levels in a turfgrass can have advantages, e.g. sterile hybrids between ploidy levels and changes in texture; or disadvantages, e.g. crossing barriers and genetic instability.
- Flow cytometry has been widely used in turfgrass breeding programs as an aid in determining ploidy.
- The objectives of this research were to: (1) Determine ploidy levels and morphology of a mutant 8x 'Empire' clone (E11), other clones collected in the same field, and progeny of E11 and (2) Determine ploidy of a sample of progeny from E11.

Materials & Methods

- In 2014 a mutant 8x variant (E11) was identified in a 930 m^2 block of Empire zoysiagrass initially planted in 2005.
- In an attempt to identify additional ploidy variation, more clones were collected from the field with samples taken on 3.05 m centers.
- Flow cytometry measurements were determined on pots established from single node cuttings from plugs taken from the field block.
- The ploidy level and internal chimerical ploidy variability of E11, other clones collected in the same field block, a known Empire clone, and progeny of E11 were evaluated by flow cytometry.
- Known standards of sorghum or maize were used to calculate 2c DNA values.
- Morphological differences in leaf lengths, leaf widths, internode length, stem diameter, and raceme lengths were measured for the genotypes studies..

Figure 1. Flow cytometry histograms of [A] Empire (4X), [B] E11 (8X), [C] Clone 4-1.5 (4X/8X chimera), (D) Clone 92 (4X/8X) chimera, [E] Clone 96 (4x only), & [F] E11 Seedling #2 (8X)



Results

- E11 showed a consistent G1 peak 2x the value of Empire.
- Both of these clones had a "normal" G2 peak that consistently had 20% or fewer events than the G1 peak.
- Several clones sampled across the field showed a "G1 peak" at the value of Empire and a second "G1 peak" equivalent to E11, with both peaks having similar cell event counts.
  - These flow cytometry results remained consistent through frequent mowing for more than one year.
  - These results strongly suggest the presence of a periclinal L1/L2 chimera in some of the sampled clones.
- Flow cytometry evaluations of ploidy of 10 seedling progeny from open pollination of E11 show that all progeny were octaploid.
- Leaf width, stem diameter and raceme length were larger for E11 compared to Empire, Meyer, the chimeric entries and 4x clone 96.
- Leaf length of E11 was shorter than Meyer and similar to Empire, 4x clone 96 and the chimeric entries.

Summary & Future Research

- Research is underway to evaluate seedling progeny from the "chimerical clones" and E11 to determine ploidy stability through a sexual seedling generation.
- Differences among the variant clones indicate the possibility of differing doubling events and/or sorting of L1/L2 layers.
- The cause of these ploidy changes is unknown. It has been speculated that dinitroaniline herbicides (commonly used in turfgrasses) and also known to be mitotic poisons might be the source of these ploidy changes.
- Breeding behavior of these clones needs to be investigated.
- The potential to use the octaploid E11 clone in crosses with tetraploid zoysiagrasses to produce sterile hexaploids is interesting.

Table 1. Ploidy and morphological characteristics of tetraploid, octaploid, and chimeric 4x/8x zoysiagrasses.

Entry	Ploidy	Leaf Length	Leaf Width	Internode Length	Stem Diameter	Raceme Length
		- cm -	- mm -	- cm -	- mm -	- cm -
Empire	4x	13.4	3.4	3.8	1.42	3.4
Meyer	4x	16.4	3.3	3.5	1.28	.
E11	8x	11.5	5.8	3.9	1.9	4.3
4-1.5	4x/8x	12.7	4.1	3.7	1.54	3.7
92	4x/8x	13.5	4.1	4.1	1.47	4.0
93	4x/8x	14.3	3.8	4.3	1.62	3.7
96	4x	14.1	3.6	3.8	1.68	3.8
lsd.		2.9	0.7	1.1 (NS)	0.16	0.3