Cell size variance in switchgrass (*Panicum virgatum*) is related to ploidy, not ecotype

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BACKGROUND

- Polyploidy (genome duplication) is common in plants; increased genetic diversity is associated with faster growth rates³ and adaptation to climate⁴.
- Switchgrass is a species with variable ploidy and a large geographic/climatic range resulting in specialized ecotypes (Fig. 2) with remarkable morphological variance (Fig.1).
- We tested for differences in climatic niche and anatomy by ecotype and ploidy. We further related these relationships to biomass yield (adjusted for flowering time).

Hypothesis: Climate would drive divergence of ecotypes, but ploidy level would drive divergence of anatomical traits. Additionally, 8x variants would show shifts in anatomy that would enhance plant function.

METHODS

1. We used a common garden transplant experiment and selected 27 of each 4x and 8x individuals based on geographic proximity to control for climate (Fig. 2 maps; https://www.worldclim.org).

 Leaf hand sections were stained with TBO and imaged with an upright light microscope.
Measured bulliform, bundle sheath, mesophyll, chloroplast, xylem, and phloem cell

sizes with ImageJ software. 4. We used principal component analysis (PCA) to test for coordinated shifts in anatomical traits and tested for differences by ploidy and ecotype using nested analysis of variance (ANOVA).

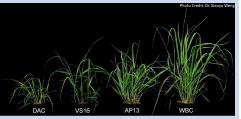


Figure 1: DAC and VS16 are examples of upland varieties; AP13 and WBC are examples of lowland varieties.

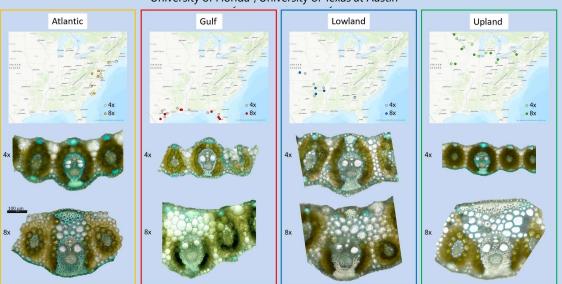


Figure 2: Switchgrass 4x and 8x ploidy locations plotted on maps with representative leaf cross-sections below for each ecotype: Atlantic, Gulf, Lowland, and Upland.

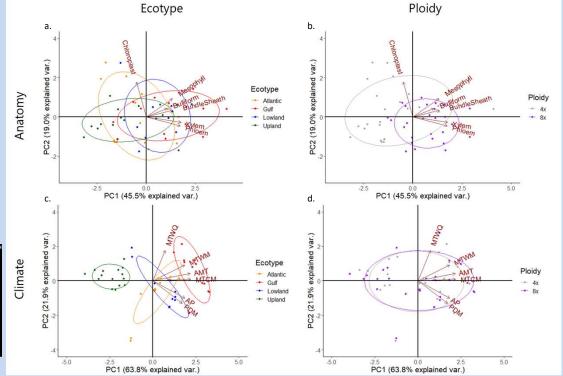


Figure 3: Principal component analysis of the first two axes (PC1 vs. PC2) for ecotypes and ploidy, based on anatomy (a and b) and climate (c and d). Ellipses indicate the conglomerate distribution of each ecotype or ploidy.

DISCUSSION

- Anatomical PC1 was driven by cell size across tissues, mainly vascular traits. PC2 was driven by chloroplast size. Climate PC1 was driven by temperature, while PC2 was driven by precipitation.
- 8x variants had larger cells (p=0.0012) and smaller chloroplasts (p=0.0245) than their 4x paired counterparts.
- The dominance of xylem and phloem in cell size variation suggests an increased hydraulic capacity with ploidy consistent with studies of stomatal conductance⁵.
- Decrease in chloroplasts size could confer an increase in photosynthetic rate due to an advantageous surface area to volume ratio⁷.
- Anatomical differences were likely not artifacts of experimental design with no differences in anatomy by ecotype (Fig. 3a) or climate of origin by ploidy (Fig. 3d).
- The anatomical differences likely had impacts at the whole-plant scale. Phenologically corrected biomass yield was different by ploidy (p=0.0326), but only within the Gulf ecotype. This suggests possible gene x environment interactions.

Takeaway: Polyploidization events can alter cellular anatomy with implications for broader scale plant performance.

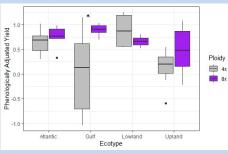


Figure 4: Boxplots of biomass yield for each ploidy level by ecotype. Asterisks above the Gulf boxplots indicate statistical differences based on Tukey analysis (p-value <0.05). Data were logarithmically adjusted to create normal distribution.