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Genome-Size Variation in Switchgrass:
Examination of the Origin and Consequences of Aneuploidy

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Background

" From a practical standpoint, a varietal improvement program can be outlined more intelligently when the cytogenetical behavior of the species is understood."

W.M. Myers and Helen D. Hill (1940)

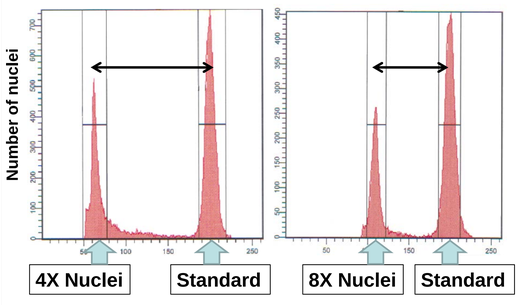
These words should be the mantra for all researchers, geneticists and breeders alike, who are currently studying the plant species that have been targeted for biofeedstock development, most of which have complex, polyploid genomes with limited prior research devoted to them. Here we use a combination of approaches, Including flow cytometry, classic cytology and molecular cytogenetics, to gain a better understanding of the extreme variation in chromosome numbers found in the model herbaceous biofeedstock species, Switchgrass (*Panicum virgatum* L.).

Knowledge of chromosome-number variation in our key germplasm will be critical for the interpretation of genetic marker data, genetic mapping and breeding efforts.

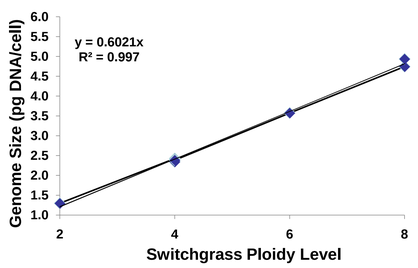
Flow Cytometry

Total DNA contents of propidium iodide (PI) stained nuclei isolated from leaf blade tissue were measured using flow cytometry. PI is a DNA-intercalating dye that fluoresces, producing an emission signal that is detected by a laser-equipped flow cytometer. The protocol for preparation of nuclei evolved from the basic technique described in Arumuganathan and Earle (1991).

In each sample, the plant nuclear DNA content (picograms) was determined by taking the ratio of the G_0/G_1 peak mean and the peak mean for the standard (3X trout nuclei from Biosure® (http://www.biosure.com/)) and multiplying by 7.8, the published DNA content for 3X trout nuclei. In the histograms of PI fluorescence below, the peaks on the left are nuclei from a 4X Cave-In-Rock plant and an 8X Cave-In-Rock plant, the peaks on the right are from the standard (3X trout nuclei).



Linear relationship between ploidy and flow-cytometric measurement of genome size.



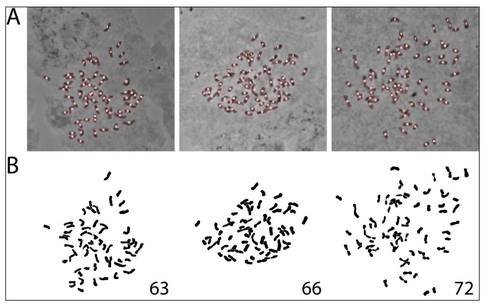
Cytology

Root tips for cytogenetics were harvested multiple times over the course of six months from greenhouse-grown plants, following the protocol described on the KSU Wheat Genetics & Genomics Resources Center Website-- http://www.k-state.edu/wgrc/Protocols/labbook.html.

Images of metaphase chromosome spreads were examined by three people using two methods: manual counts and automated counts using ImageJ software (see below). Non-concordant counts were re-examined, and only unambiguous counts were used in the analysis (136 out of 180 examined).

Fluorescence in situ hybridization (FISH) analysis was performed using clone pTa71 from wheat, a 9 kb EcoRI fragment of the 18S-25S rDNA genes that will hybridize to nucleolus organizing regions (NORs). See website above for further details about the FISH protocol. Images were analyzed from four tetraploids and seven octoploids.

Variation in chromosome number counts within a single root tip of "Cave-In-Rock"



A) Raw images of acetocarmine-stained root tip chromosome spreads from three cells. Final chromosome counting traces have been overlaid (red).
B) Threshold images generated from raw images (in A) were used to automate counting with the Analyze Particle function of ImageJ (NIH, Bethesda, MD).

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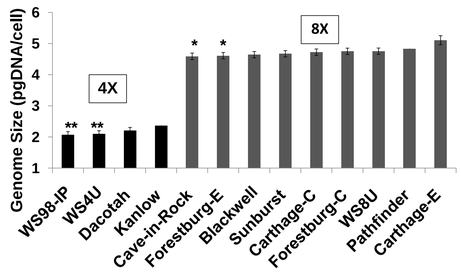
Genome size varies among and within switchgrass cultivars

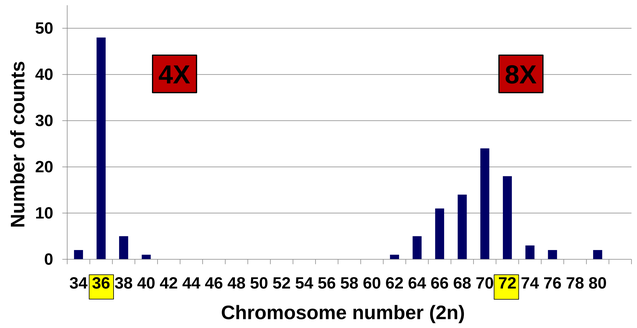


Genome-size Variation among switchgrass accessions:
Best linear unbiased estimates of genome sizes of cultivars within ploidy levels, with the significant block effect removed. Tetraploid (4X) cultivar estimates compared to Kanlow, octoploid (8X) cultivar estimates compared to Pathfinder (*=0.05; **=0.01).
[C=Casler germplasm; E=Ernst Conservation Seeds]

On average, there is an 11% difference in genome size between the largest and smallest genomes within a cultivar, indicating the existence of aneuploids.

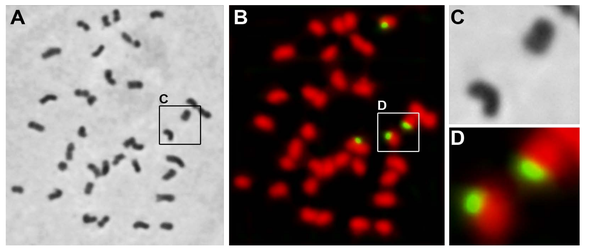
Aneuploidy is much more common in 8X than 4X Switchgrass

Tetraploid (4X)
Total counts = 56
Euploid [2*n*] = 36
% Aneuploid = 23.2
% 2*n*<36 = 53.8



Octoploid (8X)
Total counts = 80
Euploid [2*n*] = 72
% Aneuploid = 86.3
% 2*n*<72 = 89.9

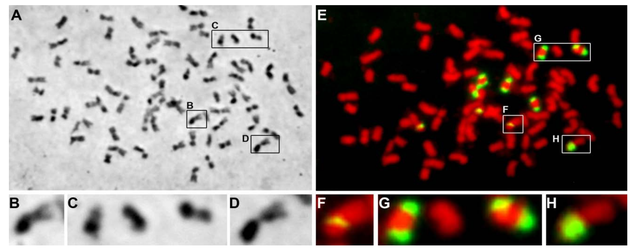
Greater genomic instability in 8X Switchgrass revealed by FISH



Representative Example of 4X Switchgrass

A) Root tip spread prepared from the cultivar Dacotah (2n=4x=36) and stained with acetocarmine.
B) The same cell as in A with chromosomes stained with DAPI (pseudocolored in red) and subjected to FISH with a NOR probe (pseudocolored in green).
C) Close-up of two chromosomes with telomeric NORs, stained with acetocarmine.
D) Same chromosomes as in C, alternate staining.

There are two pairs of pTa71 FISH sites in tetraploids, and NORs are located at the telomeres.



Representative Example of 8X Switchgrass

A) A root tip spread prepared from the cultivar Blackwell (2n=8x=72) and stained with acetocarmine.
E) The same spread in A with chromosomes stained with DAPI (pseudocolored in red) and subjected to FISH with a NOR probe (pseudocolored in green).
F) Interstitial NOR (located between the centromere and telomere.
G) NORs at both telomeres on an isochromosome.
H) Telomeric NOR.
[For comparison, B, C, and D show the acetocarmine- stained chromosomes from F, G, and H, respectively.]

There are three types of pTa71 FISH sites in octoploids.

Future Directions

- Examine variation in genome size in pollen:
  How common are unreduced and/or aneuploid gametes?
- Examine variation in embryo/endosperm ploidy in seeds:
  Are alternate reproductive pathways (such as apomixis) present?
- Examine the phenotypic consequences of ploidy variation for vegetative growth and survival in agronomic environments.