Chromosome Doubling of Prairie Cordgrass

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Introduction

Establishment of bioenergy crops propagated successfully on marginal land will be a practical way to reduce land competition with food crops, to increase energy security and to mitigate climate change. Marginal lands are defined as land associated with abiotic stress such as drought, salinity, and flood. These abiotic stresses are pivotal factors that reduce sustainable crop production. Identifying abiotic stress tolerance plants will play a key role in overcoming global demand for agricultural production. Polyploidy has been hypothesized to increase stress tolerance in plant. Prairie cordgrass (*Spartina pectinata* Link), native North America perennial grass, has been recognized as dedicated energy crop that has the ability to grow on marginal lands; prairie cordgrass provides a convenient model to study polyploids. To determine relationship between ploidy level and abiotic stress tolerance in prairie cordgrass, a reproducible protocol for chromosome doubling was established. Colchicine and oryzalin were used as doubling agents. Flow cytometry was used to select doubled plants. Chromosome doubling of prairie cordgrass is a means to establish different ploidy levels in the same genetic background and then evaluate them under various abiotic stress conditions.

Objective

- ❑ To induce doubling chromosome of prairie cordgrass
- ❑ To establish optimal conditions for doubling chromosome

Materials & Methods

Plant material /Colchicine treatment

Octaploid prairie cordgrass originated from South Dakota, PCG109, (*2n=8x=80*) was used in this experiment. 500 seeds were exposed to 0, 0.01, 0.1, 1, and 2% colchicine. Exposure times of 2, 4, 8, 12, and 24h were tested.

Flow cytometry analysis

The protocol of Rayburn *et al*. (2005) was used for Flow cytometry analysis. The isolated nuclei were analyzed using BD LSR II flow cytometer (BD Biosciences, San Jose, CA). 10,000 nuclei per sample were analyzed.

Measurement of Stomata size

Cyanoacrylate glue was applied to a microscope slide. The leaf tissue was firmly pressed onto the glue. After the glue had set, the tissue was removed. Slides were viewed using an Olympus CK2 inverted microscope using a 40X objective.

Results

Figure 1. The coexistence of different ploidy level in one PCG 109 plant. For reference pot diameter is 15cm. Arrows designate chimeric tillers.

Flow analysis

No completely doubled (16x) plants were found. However, we found two different ploidy levels (8x and 16x) within two tillers of one plant. These two chimeras coexist with six normal tillers (Figure1). Flow cytometric histograms of somatic nuclei stained with PI are observed in Figure 2. The bars represent the nuclei used to calculate the mean fluorescence of each peak.

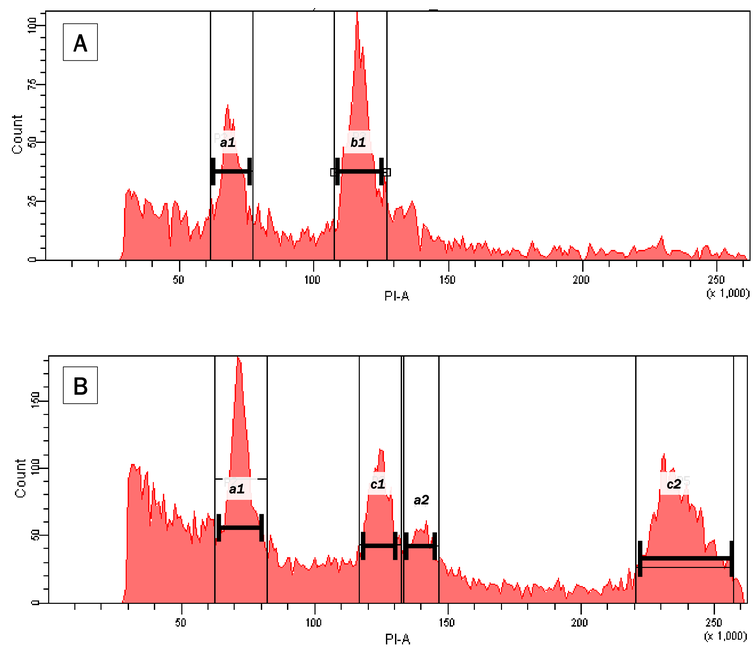

Figure 2. Flow cytometry histograms of nuclei from normal tiller, 8*x* ploidy level (A) and from a chimeric tiller (B), 8*x*-16*x* ploidy level. Nuclear peaks a1 and a2 represent sorghum peaks, G1 and G2 respectively. Peak b1 represents normal 8x tiller. Peaks c1 and c2 represent chimeric tiller peaks.

Table 1. Genome size and stomata size of chimera and control tillers used in this study

Line	2C Genome Size(pg)	Stomata Size (μm) ± S.D.
Chimera-1	3.00/6.03	29.2^{b}± 1.5
Chimera-2	3.12/5.72	29.7^{b}± 1.9
Control	3.04	31.2^{a}± 1.6

*Means followed by the same letter in a column are not significantly different at the 5% level by Duncan's multiple range test

Table 2. Proportion of cell types

Line	8x	16x
Chimera-1	35.1%	64.9%
Chimera-2	55.9%	44.1%
Control	100%	-

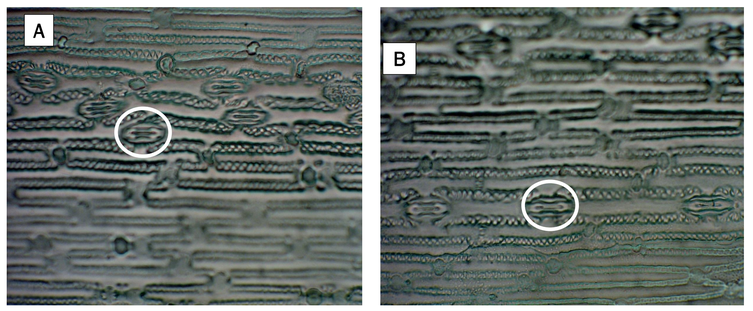

Figure 3. Leaf surface of chimera (A) and control (B). Average of stomata size for chimera and control is 29μm±0.2μm and 31μm ± 0.3μm, respectively.

Conclusion

Prairie cordgrass is a rhizomatous plant, consisting of multiple regeneration tillers. We observed different ploidy levels (chimera) within one plant. The differences between chimera and control tillers in stomata size may be a result of the coexistence of different ploidy level in one tiller thus disrupting normal growth and development. In addition, the conditions used in this study did not result in completely doubled plants and therefore new studies need to be initiated to find condition suitable for chromosome doubling in prairie cordgrass.

References

❑ Rayburn, A. L,. et al. (2005). Genome Size Analysis of Weedy Species. Crop Sci. 45: 2557-2562.

❑ Kim S, et al.(2010) Genome size and chromosome analyses in prairie cordgrass. Crop Sci. 50: 2277-2282