Effect of Polyplodization on Pollen Viability and Inflorescence Morphology in Prairie Cordgrass

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Introduction

Prairie cordgrass is a tall (1-3 m), robust, sod-forming, perennial C_4 grass that can reproduce both sexually and asexually by rhizomes. Due to its high tolerance of environmental stresses, this species has recently gained attention as a species suitable for production as a bioenergy feedstock on marginal land with a co-benefit of water and soil conservation. Prairie cordgrass is also a multiple cytotype species having three cytotypes: tetra-, hexa-, and octoploids. In previous studies, we observed two recently discovered distinct hexaploid cytotypes. One was observed as a seed collected from a single tetraploid population and the other was from a mixed-ploidy population co-occurring with tetraploids in a single location in Illinois. Establishment of neopolyploids may have resulted from various degrees of morphological traits (e.g. cell size and reproductive abilities) which could have contributed to a competitive advantage over their progenitors. The combination of these factors makes the two types of hexaploid prairie cordgrass excellent model crops for the study and exploitation of ploidy variability.

Objective

To examine the morphology, pollen characteristics and seed set between the parental cytotype and the neo-cytotype which could influence adaptation and competition of neohexaploids.

Materials and Method

Plant Materials

In this study, two recently discovered distinct hexaploid cytotypes were sampled. One (99C; Fig. 1A) was observed as a seed from a single tetraploid population collected in the southwestern portion of Illinois. The other hexaploid population was collected from a mixed ploidy population co-occurring with tetraploids in a single location in Illinois (1036x; Fig. 1B). In June 2012, the fresh rhizomes of 8 populations (4 tetra- and 4 hexaploids) were transplanted in Urbana, IL. The experiment was a complete randomized block design with three replicates.

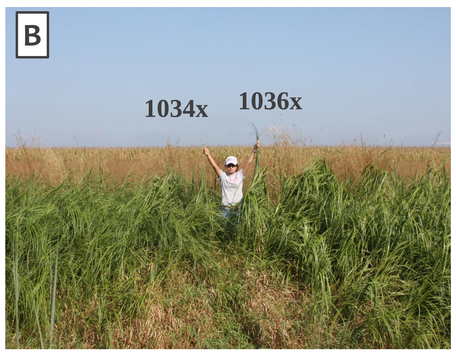

Figure 1. Two distinct types of hexaploid prairie cordgrass: (A) 99C derived from seed source of a single tetraploid population and (B) 1036x grown in mixed ploidy population with tetraploids.

Materials and Method

Pollen Characterization

Pollen characterization was determined using a 1% acetocarmine staining method. Pollen grains were stained and examined under a microscope.

Leaf Morphology

Leaf morphology was determined by measuring the length of the 1st (flag leaf) and 5th leaves below the peduncles.

Lodging Data

Lodging score was determined by visually rating each plot at maturity using a scale of 1 (no lodging) to 5 (severe lodging).

Inflorescence Morphology and Seed Set

The following four inflorescence morphological traits were measured: spikes panicle^{-1}, spike weight panicle^{-1}, spike length, spikelets spike^{-1}. For seed set analysis, seed weight and the presence or absence of a caryopsis were measured.

Results

Pollen Characteristics

- Pollen size increased as the ploidy levels increased.
- Viability of pollen grains estimated by staining with acetocarmine showed differences in both content and shape of normal pollen grains versus abnormal ones.
- Large variations were found in the degree of pollen grain contents among populations.

Pop.	Pollen Size (μm)
99A4x	32.19
99C6x	34.036
1034x	29.56
1036x	35.354
1094x	30.28
MID6x	39.109
MBB4x	31.314
MBB6x	34.32
P-value	0.0005

Table 1. Mean of pollen size estimated by pollen grain diameter in 8 different prairie cordgrass populations.

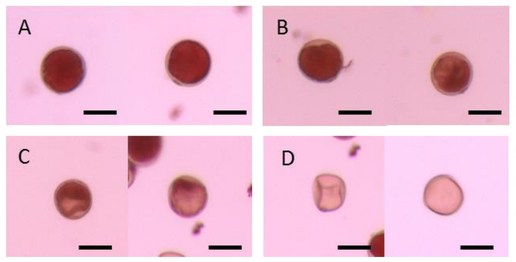

Figure 2. Micrographs showing the different pollen grain contents: (A) 90-100 % of contents; (B) 50-90 % of contents; (C) 10-50 % of contents; (D) 0-10 % of contents. Black bar indicates 25 μm.

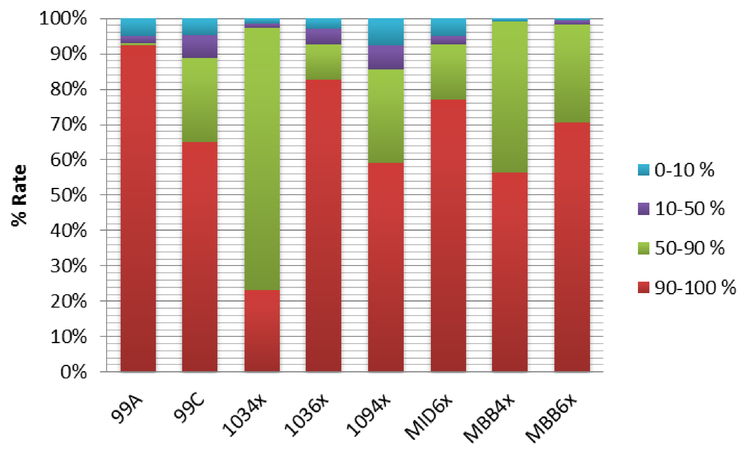

Figure 3. Percentage of pollen grain contents in 8 different prairie cordgrass populations

Results

Morphological Characteristics

Name	99A 4x	99C 6x	103 4x	103 6x	109 4x	MID 6x	MBB 4x	MBB 6x	*P*-value
Lodging (1-5)	3.67	1.33	5	3.33	3.33	1.33	3.33	1.66	0.004
1st Leaf (cm)	68.83	57.27	109.73	86.36	57.15	69.6	107.7	81.28	<0.0001
5th Leaf (cm)	110.49	86.995	113.41	121.54	102.87	106.17	118.11	111	<0.0001
Ratio 1st/5th	0.62	0.66	0.97	0.71	0.56	0.66	0.91	0.73	

Table 2. Mean of lodging score, length of 1st and 5th leaves, and ratio of 1st to 5th leaf length in 8 prairie cordgrass populations.(top)

Figure 4. A tetraploid population (1034x) showing a severe lodging problem. (left)

- Plants having severe lodging problems observed a higher ratio of 1st to 5th leaf length. (e.g. 1034x; Fig. 4).
- Lodging scores of tetraploids were significantly higher than lodging scores of hexaploids with an increased ratio of 1st to 5th leaf length.

Inflorescence Morphology and Seed Set

Plant	Spikes panicle^{-1} (no.)	Spike weight panicle^{-1} (g)	Spike Length (cm)	Spikelets spike^{-1} (no.)	1000 Seed Weight (g)	Seed set (%)
99A4x	14.38	1.38	8.38	47.33	1.66	58.10
99C6x	12.29	0.94	7.21	48.00	0.90	32.14
1034x	24.14	1.97	5.29	49.43	1.39	69.29
1036x	13.69	1.79	8.46	58.77	0.71	27.5
1094x	24.9	2.45	6.65	55.8	1.97	49.5
Mid6x	14.96	2.07	8.52	58.52	1.66	38.48
MBB4x	22.44	2.38	7.53	50.25	1.34	68.44
MBB6x	17.2	2.29	9.1	57.8	1.28	42.5
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 3. Mean and standard deviation of inflorescence morphological traits and seed set in 8 prairie cordgrass populations. Data has only been collected in 2012. Data for 2013 is still in process.

- Number of spikes per panicle for tetraploids, except for 99A, was significantly higher than for hexaploids. However, the spike length of the tetraploids was significantly shorter than that of the hexaploids.
- Tetraploids produced more seeds with caryopsis than hexaploids and seed weight of tetraploids was higher than hexaploids.

Conclusion

- No clear relationship between pollen viability and seed set was found possibly due to incomplete seed set data in 2013.
- Overall, hexaploids have more beneficial morphological characteristics for high biomass production, such as less lodging, but their reproductive ability is low as seen in low seed set as compared to tetraploids.
- This information will be of key importance in enhancing the breeding system of higher ploidy prairie cordgrass.