POLYPLOIDY EVOLUTION IN *SPARTINA PECTINATA* L.: NEOPOLYPLOID FORMATION AND CYTOGEOGRAPHIC DISTRIBUTION

BY

SU MIN KIM

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

Advisor:

Assistant Professor D.K. Lee

ABSTRACT

The next generation of bioenergy crops will probably be grown on marginal lands. Prairie cordgrass (*Spartina pectinata* Link) is well suited to marginal land that is not well-suited for conventional crop production. Prairie cordgrass is a tall (1-3 m), rhizomatous, C4 perennial grass, native to North America and tolerant of environmental stresses such as salinity and water fluctuations. Developing prairie cordgrass as an energy crop requires genomic information such as genomic size and ploidy level. This species is well known as a polyploid species comprising three ploidy levels of tetraploid (2n = 40), hexaploids (2n = 60), and octaploids (2n = 80) with base chromosome number of x = 10. By using flow cytometry, cytogeographic distribution of prairie cordgrass has been investigated throughout U.S. Across sampling areas, the tetraploid populations extend from the East North Central to the New England regions in U.S., while the octaploid cytotypes were mostly distributed in the west North Central regions; overlapped regions of tetraploids and octaploids were found in both the west North Central (IA and KS) and west South Central (KS) regions. The hexaploid cytotype was found in one mixed population (4x + 6x) occurring in Illinois. Polyploids often possess novel traits, such as changes in flowering time, cell size, and biomass. An increase in polyploidy resulted in a greater variability of morphological expression in mixed population (4x + 6x) occurring in Illinois. Substantial differences in the flowering time, stomatal size, and aboveground biomass were observed between tetraploids and hexaploids. The presence of ploidy mixtures in natural populations of prairie cordgrass offers unique opportunities for studying the formation and establishment of polyploidy under natural conditions considered as an ultimate step in plant evolution.

To my family and friends

ACKNOWLEDGMENT

This project would not have been possible without the support of many people. Many thanks to my advisor, D.K. Lee, for allowing me to work in his lab and develop molecular skills. Also, his patience and thorough mentoring has helped me grow into a good researcher and he has encouraged me to continue in the research field. I also thank Professor A. Lane Rayburn, who offered guidance for my project in most time and allowed me to work in his lab to finish my projects. Without his big help, I would not have found what I wanted to do for my future research. Thanks to Professor Thomas Voigt, who always believed in my research and provided me with financial means to complete this project. Thanks to Allen, for helping me to get all plant materials and lab supplies for my research. I also thank Joe, Justine, Santanu, Ron, Josephine, and Kalan for helping me. And finally, thanks to my parents who endured this long process with me, always offering support and love.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Global energy consumption will increase by 40% with over two decades escalating the use of conventional energy resources, such as coal, petroleum and natural gas (OECD/IEA, 2009).Petroleum-based fuels have limited reserves that are concentrated in the hands of few capricious nations. In addition, the production and utilization of fossil fuels leads to environmental burdens such as smog, acid rain, global warming, and air toxic. The scarcity of known petroleum reserves and causes of environmental problems increase the attractiveness of bio-renewable energy resources, known as biofuel. Biofuels, liquid fuels produced from biorenewable resources, have the potential to replace petroleum as a source of transportation fuels, and by so doing, address many of the problems associated with petroleum-based fuels (Brown, 2003).

Biorenewable sources includes virgin plant products such as grains, grass, and wood, and processed plant material including paper, manure, and other plant-based wastes and residuals. The U.S. Department of Energy (DOE) has selected perennial plants (e.g. switchgrass, willow, and poplar) for future development as energy crops (USDA, 2011). The perennial crops were selected for efficient and fast growing, solar energy collection, and ease of growing, harvesting, and processing. The U.S. Department of Agriculture (USDA) and the DOE have promoted the sustainable production of biomass crops for energy due to both the recent increase in gasoline prices in the U.S. and the international scientific consensus that reducing greenhouse-gas emission is necessary in order to mitigate climate change (Perlack et a,. 2005). To be sustainable energy crops, plants should be produced with lower life-cycle greenhouse gas emissions than

traditional fossil fuels and with minimum competition with food productions. Perennial energy crops gown on degraded lands and/or marginal crop-production land can produce adequate quantities for our future energy needs (Tilman et al., 2009).

Prairie cordgrass (*Spartina pectinata* Link) has been recently gained attentions as a species suitable for the production of bioenergy on marginal land, and possesses the co-benefit of water/soil conservation (Skinner et al., 2009). Prairie cordgrass is a tall (1-3 m), rhizomatous, warm-season perennial native of North America and is predominantly found in lower, poorly drained soils along roadsides, ditches, steams, marshes, wet meadows, and potholes where soils are too wet for other grain, forage, and biofuel crops (Hitchcock, 1950; Mobberley, 1956; Stubbendieck et al., 1982; USDA NRCS, 2002; Barkworth et al., 2007). Skinner et al. (2009) reported that prairie cordgrass shows flooding tolerance with the greatest root growth and the highest survival rate among four other warm season species of switchgrass, Indiangrass, big bluestem, and eastern gamagrass under waterlogged condition. Prairie cordgrass is also well adapted in various climatic and environmental stresses including cold, salinity, and droughty soils (Weaver, 1954; Mobberley, 1956; Long, 1975; Potter et al., 1995; Montemayor et al., 2008; Boe et al., 2009; Kim et al., 2011a). Under drought and salinity conditions, prairie cordgrass has produced comparable biomass yields to switchgrass cultivars with high frequency of reproductive tillers (Boe et al., 2009; Kim et al., 2011a). The combination of salt and drought tolerances within this species results in a wide geographic distribution throughout the eastern coast and inland marshes of the Middle West to Alberta in North America (Canada and United States) (Barkworth et al., 2007).

Despite these promising features, growing and processing prairie cordgrass for bioenergy production is still in its infancy. Breeding improvement programs generally conduct wild

selections of plants that occur in a wide range of geography, climate, and soils, and planted wild selections in local nursery to identify the best material with which to establish programs and to test for the genetic control of economically important traits such as growth, form, and diseases resistances. Prairie cordgrass is known as polyploid species that can have more potentially more genetic variations than parental ancestors, such as an increase of cell size and gene expression, changes in physiology, morphology, and ecological tolerance arise due to natural selection acting on random mutation (Levin, 2002; Ramsey and Schemske, 2002; Lumaret, 1988; Adams and Wendel, 2005). More detailed genetic studies on prairie cordgrass, therefore, are needed to establish breeding programs for a more efficient bioenergy crops.

Both allopolyploids and autopolyploids have been documented in the genus *Spartina* (Marchant, 1967; Raybould et al., 1991; Baumel et al., 2001) which belongs to the sub-family Chloridoideae (Mobberley, 1956), a well-supported monophyletic lineage (Hsiao et al., 1999). The basic chromosome number of the genus *Spartina* is x = 10. The chromosome numbers of fifteen *Spartina* spp. investigated to date, range from tetraploid (2n = 40), hexaploid (2n = 60 - 62), octaploid (2n = 80), to dodecaploid (2n = 120, 122, 124), with possible aneuploidy existing as well (Marchant 1968ab; Reeder 1977). Among the well-documented natural allopolyploids within *Spartina* spp., a distinctive example of interspecific hybridization is *Spartina anglica* which originated in England. The hybridization between *S. altinaflora* (2n=60) and the native British species *S. maritima* (2n = 60) led to a new F_1 hybrid species, *S. x townsendii* (2n = 62). This resulted in the new fertile allopolyploid species, *S. anglica* (2n = 120, 122, 124), through spontaneous chromosome doubling (Marchant, 1968ab; Groves and Groves, 1980). The creation of the new fertile allopolyploid species, *S. anglica*, has revealed that **polyploidization** occurs during the evolutionary process of natural populations. Unlike allopolyploid *Spartina* species,

autopolyploid prairie cordgrass has not been well studied, and there are no reports focused on the putative evolutionary history of autopolyploid in this species.

Prairie cordgrass is autopolyploid species, comprising three ploidy levels of tetraploid (2n = 40) and octaploids (2n = 80) (Church, 1940; Marchant, 1968ab). They arise spontaneously in nature as result of a failure of cell division during the mitotic and meiosis stages or as a result of some condition in the natural environment (Appels et al., 1998; Otto and Whitton, 2000). Attempts have been made to delineate the geographic distribution of tetra- and octaploid cytotypes of prairie cordgrass in United States (Reeder, 1977). Given the fact that there are now three cytotypes identified and that only a limited number of locales have been sampled with respect to prairie cordgrass populations (Marchant, 1968ab; Reeder, 1977), a more thorough investigation is required to determine for geographic distributions of the tetraploid, hexaploid and octaploid cytotypes of prairie cordgrass in U.S.

A survey of cytotype variation and distribution of prairie cordgrass is crucial for a better understanding of the evolutionary process of polyploids involved in its origin, as well as the history of contemporary distribution patterns of cytotype variations across U.S. Knowledge about the extent of ploidy levels will provide important information for prairie cordgrass breeding program with respect to long- term improvement in biomass yield performance. The present study explores the distribution of genome size in natural populations of dominant prairie grass. Flow cytometry (Rayburn et al., 2005) is well known as a useful method in the assessment of genome size of ploidy level variation and distribution, and detailed within populations screening (Michaelson et al., 1991; Suda et al., 2007; Hufft Kao, 2008). When multiple cytotypes occur within the same species, and in same locality (Baack, 2004; Mraz, 2008; Hijmans, 2007), opportunities to examine the interaction between cytotypes, and to understand the evolutionary

process governing origins and establishment of polyploids in natural populations exist (Ramsey and Schmske 1998). Our objectives were: (1) to determine the genome size variation that exists in prairie cordgrass; (2) to determine the cytogeographic distribution of prairie cordgrass, more precisely defining the tetra- , hexa-, and octaploid cytotypes locations; and (3) to examine in detail the morphological differences between tetraploids and hexaploids, showing any possible relationships with the genetic change.

CHAPTER 2

GENOME SIZE AND CHROMOSOME ANALYSIS IN PRAIRIE CORDGRASS[1]

ABSTRACT

The next generation of bioenergy crops will be grown on marginal lands. Prairie cordgrass (*Spartina pectinata* Link) is well suited to marginal land because of its tolerance to stresses such as salinity and water fluctuations. Developing prairie cordgrass as an energy crop requires genomic information such as genomic size and ploidy level. The 2C nuclear genome size of 11 prairie cordgrass populations, originating from Illinois, North Dakota, and South Dakota, were determined by flow cytometry using somatic G1 nuclei, and the ploidy variation confirmed by counting the chromosome number. One population from each of North Dakota and South Dakota, as well as the Red River, had genome sizes of 3.1 pg of DNA per G1 somatic nucleus whereas; the majority of the eight populations from Illinois had a genome size of 1.6 pg. One plant from one of the Illinois populations had an estimated genome size of 2.3 pg. These genome sizes of 3.1 and 1.6 pg, along with their respective chromosome numbers of 80 and 40 indicate octoploid and tetraploid plants. The chromosome number of 60 observed in the plant with 2.3 pg indicated that this plant was hexaploid. This study provides the first estimates of genome size for prairie cordgrass and is also the first to report observed hexaploidy in this species. This genomic information provides critical knowledge for designing efficient breeding schemes for plant improvement.

[1] Reprinted, with permission, from S Kim, A.L.Rayburn, and D.K.Lee, 2010. Genome size and chromosome analysis in prairie cordgrass. 50:2277-2282. This article is reprinted with the permission of the publisher and is available from https://www.crops.org and using DOI: 10.2135/cropsci2010.03.0140

INTRODUCTION

The success of the world's bio-industry largely depends on sustainable biomass feedstock production and cost-effective processes for conversion of that feedstock into biofuels. Sustainable feedstock production includes all of the steps required to produce large quantities of high quality biomass. An important issue for sustainable biomass feedstock systems is to maximize production in given land resources without significant land-use changes (United Nations, 2007; Simmons et al., 2008). Energy crop diversification at the landscape level will be an important factor for the sustainable cellulosic biomass feedstock and biofuel production system (Jordan and Warner, 2010). Dedicated perennial energy crops will provide excellent opportunities for environmentally friendly biomass feedstock production for sustainable biofuel industries. To be highly successful, biofuel species selected should have minimal competition for land area with the prevalent commodity food crops (Simmons et al., 2008).

The next generation of bioenergy crops will be grown on marginal lands, which are not suitable for conventional food crops (Biomass Research and Development Board, 2006; Simmons et al., 2008; Gopalakrishnan et al., 2009). Prairie cordgrass is a strong candidate for production of bioenergy on marginal lands. The plant is a tall (1– 3 m), robust, sod-forming warm-season (C_4) grass that can reproduce both sexually by seeds and asexually by rhizomes. Prairie cordgrass is predominantly found in lower, poorly drained soils along roadsides, ditches, streams, marshes, wet meadows, and potholes throughout Canada to 60° N latitude and throughout the Northeast, Great Lakes, and Midwest States, as well as most other states in the continental United States (Hitchcock, 1950; Mobberley, 1956; Stubbendieck et al., 1982; USDA NRCS, 2002; Barkworth et al., 2007).

An important characteristic of any bioenergy crop grown on marginal land is stress tolerance (Biomass Research and Development Board, 2006). Prairie cordgrass is recognized for having significant tolerance to salinity and water fluctuations. Montemayor et al. (2008) demonstrated that prairie cordgrass had high tolerance for the conditions of water logging, high salinity, and low pH. Evaluation of prairie cordgrass for wetland restoration has indicated its high potential for establishment of highly desirable native vegetation and high tolerance for variable water levels (Bonilla-Warford and Zedler, 2002). Prairie cordgrass is well adapted to soils that are wet throughout the growing season or frequently flooded in early season (e.g., Land Capability Class V). Although prairie cordgrass is a facultative wetland species, predominantly found in wet prairies, it is well adapted to mesic prairies and on high ground along railroad rights-of-way in the Midwest (Mobberley, 1956). Potter et al. (1995) demonstrated that prairie cordgrass could be successfully established on well-drained arable soils in cool temperate eastern England. Mean biomass production of prairie cordgrass during the 9-yr study was 12.7 Mg ha^{-1} (Boe et al., 2009) and prairie cordgrass consistently produced more biomass than switchgrass (*Panicum virgatum* L.) in South Dakota (Boe and Lee, 2007). Madakadze et al. (1998) reported that prairie cordgrass was one of the most productive warm-season grasses in southwestern Quebec.

Even though prairie cordgrass has many positive aspects for biomass production and conservation (Potter et al., 1995; Madakadze et al., 1998; Bonilla-Warford and Zedler, 2002; Boe and Lee, 2007; Montemayor et al., 2008; Boe et al., 2009), currently limited information is available on the genetics of biomass production and other agronomic traits in this species. Boe and Lee (2007) reported significant variation for differences in biomass production (5.0–9.7 Mg ha^{-1}) among seven populations of prairie cordgrass collected from eastern South Dakota. These

populations were concluded as valuable sources of genetic diversity for breeding for biomass production in the northern Great Plains. Moncada et al. (2007), using AFLPs, found that prairie cordgrass populations from Minnesota had high levels of genetic diversity among and within populations. One factor suggested as contributing to this diversity is variation in ploidy level.

Chromosome analysis has revealed a variation in chromosome number in prairie cordgrass. Marchant (1963) reported that the basic chromosome number was 10, with a somatic chromosome number of 2n = 40. Church (1940) reported two types of prairie cordgrass; one type with 42 chromosomes and another with 84 chromosomes. Both types reproduce via normal sexual reproduction and asexual reproduction by rhizomes. The type with 42 chromosomes was from the eastern United States while the 84-chromosome type was from the Midwest. Reeder (1977) concluded that prairie cordgrass populations from Colorado, Nebraska, and Wyoming were octaploids, whereas populations from Canada and the eastern United States (including Illinois) were tetraploids and that the base number of prairie cordgrass was indeed 10 chromosomes. Critical to the successful use of prairie cordgrass germplasm is the accurate assessment of ploidy level in populations of interest.

Gathering genomic information of specific populations is a necessary first step in the breeding of high yielding cultivars of prairie cordgrass with local adaptation. The objectives of this study were to determine the genome size of prairie cordgrass population from South Dakota and Illinois. Upon observing any significant genome size variation, the chromosome number of the populations will be determined to confirm that ploidy variation is occurring. The information collected in this study will provide valuable new information required for both genomic analysis and plant breeding strategies.

MATERIALS AND METHODS

One cultivar and 10 populations of prairie cordgrass were analyzed for nuclear DNA content, leaf stomatal length, and chromosome number (Table 1). The Red River Natural Germplasm was obtained from a commercial seed source. Red River was developed by open pollination among vegetative propagules from several populations from east central Minnesota, northeastern South Dakota, and east central North Dakota in an isolated common garden nursery in North Dakota (Boe et al., 2009). For the 10 populations, seed was collected from single populations in each of central North Dakota and southeastern South Dakota, and eight populations in central Illinois. Seeds from each population were planted in the greenhouse, and seedlings of the 10 populations and Red River were transplanted to a field nursery in Urbana, IL in May 2009. The experiment was a randomized complete block design with three replications. Each replication consisted of single-row three-plant plots for each population.

For nuclear DNA content determinations, flow cytometric analysis using the protocol of Rayburn et al. (2005) was used. Briefly, actively growing, fresh leaf tissues (~1 cm^2 of each) from prairie cordgrass and maize, used as an internal standard, were co-chopped, and placed in a 15 mL beaker containing 10 mL extraction buffer and 200 μL 25% Triton X. The extraction buffer consisted of 13% (v/v) hexylene glycol, 10 mM Tris-HCl [pH 8.0], and 10 mM $MgCl_2$. The nuclear DNA content of the maize (W22 subpopulations) was calibrated at 5.14 pg using sorghum Pioneer hybrid 84G62 with 1.74 pg/2C nuclei (Rayburn et al., 2009). The tissue was homogenized using a tissue grinder for 10 s at 4500 rpm, and the samples were filtered through a 50 um CellTrics® disposable filter (Partec, GmbH) into a test tube. Samples were kept on ice throughout. Following filtration, samples were centrifuged for 15 min at 11,000 rpm at 4°C. The

supernatant was removed, and nuclei were resuspended in 300 µL of PI stain (3% w/v polyethylene glycol (PEG) 6000, 50 µg/mL PI, 180 units/mL RNase, 0.1% Triton X-100 in 4 mM citrate buffer). The solution was transferred to a 1.5 mL conical tube, and incubated for 20 min at 37°C. Following incubation, 300 µL of PI salt (3% PEG, 50µg/mL PI, 0.1% Triton X-100 in 400 mM NaCl) was added to each sample. Samples were then briefly vortexed, placed on ice, and stored at 4°C for at least 1 h.

The nuclei were analyzed using BD LSR II flow cytometer (BD Biosciences, San Jose, CA). The excitation wavelength was set at 488 nm. The emission filter was a 695/40 nm filter. We analyzed 30,000 nuclei per sample. The nuclei were gated on the basis of fluorescence integral vs. pulse width to exclude doublets. Small debris was excluded from the histogram based on low integral fluorescence, usually 1/10 of maize nuclei fluorescence. The resulting histograms were used for statistical analysis. The total 2C genome size reported in this study is the amount of nuclear DNA in somatic G1 nuclei of each prairie cordgrass population. Three plants were analyzed per population. Using Statistical Analysis Software version 9.1 (SAS 9.1), the general linear model (GLM) analysis was conducted and Fisher's protected least significant difference (LSD) tests were run to determine significance ($\alpha = 0.05$).

Epidermal impressions of leaf tissues were made according to Nelson et al. (2002). Cyanoacrylate glue was applied to a beveled glass microscope slide. The leaf tissue was firmly pressed onto the glue. After the glue had set, the tissue was removed. Epidermal imprints were obtained from the plants used in determining genome size above. Slides were stored in a cool dark area under low humidity until analyzed.

Slides were viewed using an Olympus CK2 inverted microscope using a 40X objective. Pictures were taken using an Olympus DP11 camera system. Five stomata were measured per

leaf, three leaves were examined per plant and three plants were analyzed per population. Statistical analysis was as described above.

Root tips were obtained for all 11 populations. Rhizomes were collected and placed in 15 cm round pots filled sand. The pots were placed in the greenhouse and the sand saturated daily to ensure good root growth. The day length was 14 h, with at temperature regime of 24 to 26°C. After the roots were at least 2 to 3 cm in length root tips were collected. Roots were cut from the rhizomes and soaked in ~15 mLs of 0.05% 8-hydroxyquinoline for approximately 2 h. The roots were then rinsed in ddH_2O for 5 min and stored in 3:1 ethanol/acetic acid. The roots were stored at room temperature for 4 d.

For chromosomal counts, fixed root tips were rinsed in ddH_2O, hydrolyzed in 5 N HCL for 45 min, and placed in Feulgen's stain for 2 h. Root tips were rinsed in ddH_2O and a drop of enzyme solution (0.2 g Cellulysin and 0.1 g Macerase in 10 mL 10 mM EDTA) was placed on the tip and then incubated for 45 min at room temperature. The enzyme solution was rinsed off with 45% acetic acid, a drop of 45% acetic acid was added to the root tip, a cover slip was placed over the tissue, and gently tapped with a dissecting needle to disperse all of the tissue. The slide was then flamed over an alcohol burner and direct pressure was applied to the slide. The slides were then viewed using Olympus BX61 microscope. Photographs of chromosome spreads were taken using an Olympus U-CMAD3 camera.

RESULTS

Three very distinct DNA content histograms were obtained from the prairie cordgrass populations (Fig. 1). All of the G1 nuclear peaks had a coefficient of variation of between 3 and

5%. Three populations were observed to have two peaks representing the G1 nuclei of both prairie cordgrass and maize, with the somatic G1 prairie cordgrass nuclei having a 2C genome size of around 3 pg, about 56% of the maize genome (Table2.1; Figure 2.1A). In eight of the prairie cordgrass populations, three distinct peaks were observed representing the G1 and G2 nuclei of prairie cordgrass and the G1 peak of maize, respectively (Figure 2.1B). The approximate 2C genome size of these populations was around 1.63 pg or ≈ 30% of the maize genome (Table 2.1). One plant in accession IL-99C had a histogram that was different from the previous histograms. This plant had two peaks representing the G1 nuclei of both prairie cordgrass and maize (Figure 2.1C). However, the prairie cordgrass G1 peak was ≈ 43% of the maize G1 nuclear peak resulting in a 2C genome size estimate of 2.3 pg for this particular plant (Table 1). Statistical analysis revealed significant variation in genome size of the prairie cordgrass populations (Table 2.1).

Upon chromosome analysis, a similar pattern was observed. The three populations that had a 2C genome of 3 pg of DNA were observed to have a chromosome number of 80 (Figure 2.2A; Table 2.1). The eight populations that had a 2C genome size of 1.63 pg had a chromosome count of 40. The lone plant with a 2C genome size of 2.3 pg had 60 chromosomes.

The results of the stomata analysis appeared much more complex. Statistical analysis revealed significant variation among the populations with the delineations of the groups not as distinct. The three populations with 3 pg of 2C nucleus were observed to have larger stomata than the eight remaining populations (Figure 2.3A and 2.3B; Table 2.1). However, unlike the genome size analysis, not all three of these populations were statistically significant from the populations with 1.63 pg of DNA. The response of the 2.3 pg plant was completely contrary to the genome size and chromosome data with instead of this plant having an intermediate stomata

size, the size of the stomata was numerically the lowest of the prairie cordgrass accessions although not statistically different.

DISCUSSION

With the exception of one plant, all of the plants analyzed in this study fell within a typical ploidy level. The three accessions that originated west of longitude 95° all had 80 chromosomes, large stomata, and 2C genome size of ≈3.1 pg. The eight accessions that originated east of longitude 95° had 40 chromosomes, smaller stomata, and 2C genome sizes of ≈1.6 pg. All of the results are consistent with the more western accessions having a higher ploidy level than the eastern originating lines. Given that the base number of x = 10 (Reeder, 1977), the 1.6 pg plants would be designated tetraploids (2*n* = 4x = 40 chromosomes), while the 3.1 pg plants would represent octaploid plants (2*n* = 8x = 80 chromosomes). These results agree with the hypothesis of Reeder (1977) that populations of prairie cordgrass have distinct chromosomal variants that appear to be delineated by the geographic origin of the populations. As indicated by Gonzalez-Hernandez et al. (2009), distinct ecotypes as defined by cytotype potentially exist in prairie cordgrass.

A third cytotype was also observed in the more eastern populations. One plant of IL-99 had a genome size of ≈ 2.3 pg, which was intermediate between the tetraploid and octaploid accessions. Upon chromosome analysis, this plant was observed to have 60 chromosomes. The stomata of this plant were not as large as those of the octaploid individuals. Given the genome size and chromosome count, this plant is a hexaploid individual (2*n* = 6x = 60). While the ploidy mechanism of this plant has yet to be determined, the seed of this plant was collected from a

natural growing Illinois population. Given that all of the plants from Illinois analyzed to date have been observed to be tetraploid, the likelihood that this plant is a result of a hybrid between a tetraploid and an octaploid is very low. The plant is very similar in phenotype to the tetraploid plants and in fact all the populations observed in this study whether tetraploid, hexaploid, or octaploid all were morphologically prairie cordgrass. However, there was one phenotypic variation between the tetraploid and hexaploid IL-99 plants. The hexaploid plant did not flower and thus produced no seed while all of the tetraploid and octaploid plants were observed to flower and set seed. Indications are that the hexaploid is sexually sterile, at least in the first year after transplanting reproduces by rhizomes. While initially the environmental impact of a sterile odd ploidy level plant may seem to be a nonentity in the evolution of a species, the perennial nature of this species may allow such plants to play a pivotal role in evolution and adaptation. A recent study has indicated that sterile odd ploidy plants of other species of *Spartina* can compete very well with fertile and invasive species of this genus under natural conditions (Renny-Byfield et al. 2010).

To realize the full potential of prairie cordgrass as a biofuel, a basic understanding of its genome is critical. Given the amount of ploidy variation observed in this species it is critical to establish the ploidy level of any population being analyzed. For instance, if one is trying to establish a molecular genetic map or under gene expression studies, the ploidy level of the population is crucial (Rousseau-Gueutin et al., 2008; Auger et al., 2005). The ploidy and genome size data presented in this study will provide the prairie cordgrass genome, the first step toward improving the biomass potential of this important species. However, the genome sizes reported in this, and more importantly, in every study estimating genome size by flow cytometry, should be considered estimates and not absolute amounts. While flow cytometry has become

increasingly popular for determining genome sizes in plant species, mechanism of the fluorochrome used (Shapiro, 1995), plant compounds present in the cells from which the nuclei are isolated (Dolezel and Bartos, 2005) and differential chromatin compaction (Freeman and Rayburn, 2004) are just a few of the parameters that can bias genome size estimates. Flow cytometry therefore has limitations that preclude the certainty of any genome size estimates to the absolute picograms of DNA that exist in the plant nucleus of interest.

FIGURES AND TABLES

Table 2.1 Geographical location, accession number, genome size, chromosome number, ploidy level, and stomata size of the prairie cordgrass populations used in this study.

Line	2C Genome size (pg) ± SD	Chromosome number (2n)	Ploidy level (x = 10)	1Cx genome size, Mb†	Stomata size, µm	Location
Red River	3.05 ± 0.08	80	8x	373	40.886	Natural germplasm
SD	3.06 ± 0.04	80	8x	374	38.133	42°50'51.81" N, 97°04'50.52" W
D	3.08 ± 0.1	80	8x	377	37.644	47°27'26.70" N, 99°05'02.59" W
IL-98	1.61 ± 0.02	–	4x	394	35.111	39°47'31.08" N, 88°26'53.52" W
IL-99	1.59 ± 0.03	40	4x	389	31.517	39°44'59.76" N, 88°42'02.76" W
IL-100	1.64 ± 0.03	40	4x	401	31.755	39°40'23.70" N, 89°09'19.68" W
IL-101	1.69 ± 0.03	40	4x	413	34.022	40°06'21.08" N, 88°09'10.14" W
IL-102	1.66 ± 0.05	–	4x	406	34.333	40°03'54.87" N, 88°14'18.78" W
IL-104	1.68 ± 0.03	–	4x	410	32.144	40°10'44.70" N, 88°44'31.44" W
IL-105	1.61 ± 0.04	40	4x	394	33.666	40°54'41.40" N, 87°56'35.82" W
IL-106	1.59 ± 0.02	–	4x	389	35.711	40°39'24.06" N, 88°01'11.76" W
IL-99C	2.34 ± -	60	6x	381	32.000	39°44'59.76" N, 88°42'02.76" W
	LSD = 0.09				LSD = 3.50	

† Conversion–978 Mb = 1 pg according to Dolzel et al. (2003).

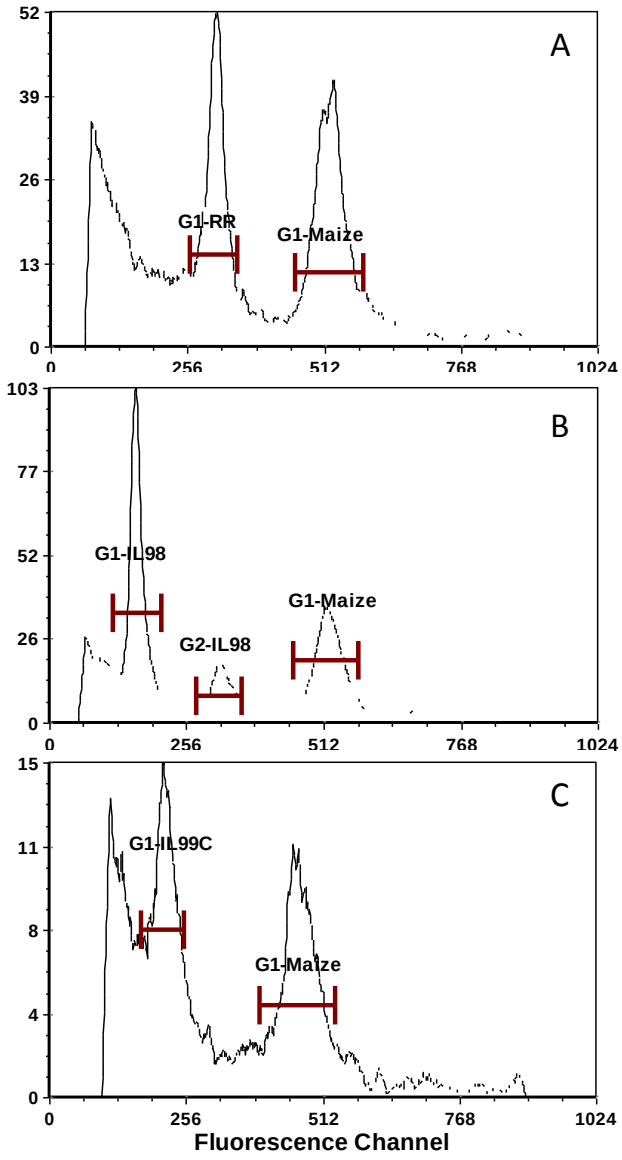

Figure 2.1 Flow Histograms of Prairie cordgrass somatic nuclei stained with PI. The bars represent the nuclei used to calculate the mean fluorescence of each peak. (A) G1 somatic nuclei of Red River, 8x ploidy level. (B) G1 somatic nuclei of Il-98, an Illinois population, 4x level; (C) G1 somatic nuclei of IL-99C, an Illinois population, 6x ploidy level.

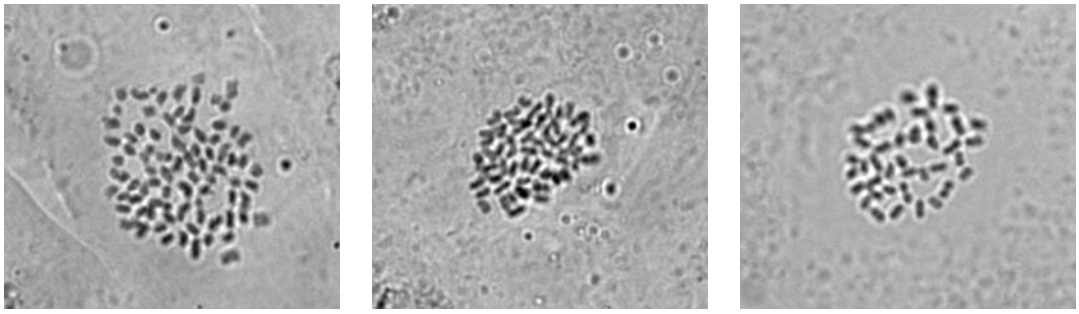

Figure 2.2 Chromosome spreads from root tips of Prairie cordgrass. (A) Red River, 2*n* = 8x = 80 chromosomes. (B) IL-99C, 2*n* = 6x = 60 chromosomes. (C) IL-100, 2*n* = 4x = 40 chromosomes. Bars, 5.0 µm.

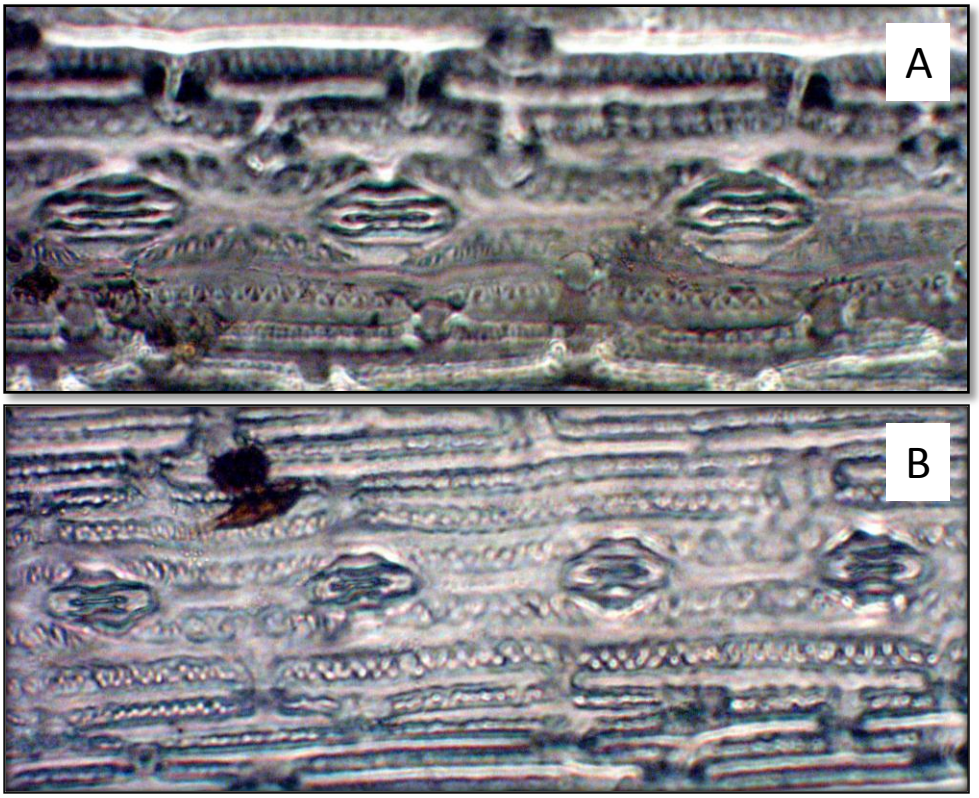

Figure 2.3 Stomata of Prairie cordgrass as observed from epidermal impressions. Octaploid plants such as (A) Red River tend to have much larger stomata than the tetraploid plants such as (B) IL-99A. Bars, 50.0µm.

CHAPTER 3

CYTOGEOGRAPHIC DISTRIBUTION AND GENOME SIZE VARIATION IN PRAIRIE CORDGRASS (*SPARTINA PECTINATA* L.)[2]

ABSTRACT

Prairie cordgrass plants (*Spartina pectinata* Bosc ex Link.*)* were examined from 61 locations representing the geographic distribution of prairie cordgrass in the U.S. Using flow cytometry, the genome size of 183 individual plants of prairie cordgrass was determined and the chromosome counts were obtained. Three distinct ploidy levels were observed: tetraploid ($\bar{x}$ = 1.56 pg; 2n = 4x = 40), hexaploid ($\bar{x}$ = 2.33 pg; 2n = 6x = 60), and octoploid ($\bar{x}$ = 3.06 pg; 2n = 8x = 80). In the sampled areas, the tetraploid populations extended from the East North Central to the New England regions of the U.S., while the octoploid cytotypes were mostly distributed in the West North Central regions. Populations of the tetraploids and octoploids were found in close proximity in the West North Central (IA and KS) and the West South Central (OK) regions. The hexaploid cytotype was found in one mixed population (4x + 6x) occurring in Illinois. No statistically significant intraploidy genome size variation was found in the tetraploid populations, while significant intraploidy genome size variation was found in the octoploid populations. This study precisely defined the geographic distribution of cytotypes in prairie cordgrass throughout the different regions of the U.S. These results provide critical genome size and ploidy

[2] Reprinted, with permission, from S Kim, A.L.Rayburn, and D.K.Lee, 2011.Cytogeograhic Distribution and Genome Size Variation in Prairie Cordgrass (*Spartina Pectinata* Link). Summited to Plant Molecular Biology Reporter

distribution information needed to design efficient breeding schemes for high yielding cultivars of prairie cordgrass with local adaption.

INTRODUCTION

Prairie cordgrass (*Spartina pectinata* Bosc ex Link.*)* is a tall (1-3 m), rhizomatous, perennial, and a native warm-season grass to North America. It has recently gained attention as a species suitable for the production as a bioenergy feedstock on marginal land with a co-benefit of water and soil conservation (Bonilla-Warford and Zedler, 2002; Boe and Lee, 2007, Boe et al., 2009; Gonzalez-Hernandez et al., 2009; Skinner et al., 2009). The plant is predominantly found in lower, poorly drained soils along road sides, ditches, streams, marshes, wet meadows, and potholes where soils are overly saturated for other grain, forage or biofuel crops (Hitchcock, 1950; Mobberley 1956; Stubbendieck et al., 1982; USDA NRCS, 2002; Barkworth et al., 2007). Skinner et al. (2009) reported that prairie cordgrass showed the greatest root growth and the highest survival rate among the warm-season species of switchgrass, indiangrass, big bluestem, and eastern gamagrass under waterlogged conditions. Prairie cordgrass is also well adapted to various abiotic stresses including cold, water saturation, and saline soils (Weaver 1954; Mobberley, 1956; Long et al., 1975; Potter et al., 1995; Boe and Lee, 2007; Montemayor et al., 2008; Boe et al., 2009; Kim et al., 2011). The combination of salt, waterlogged, and other abiotic stress tolerances within this species has resulted in a wide geographic distribution in North America throughout the eastern coast and inland marshes of the Midwestern United States to Alberta, Canada (Barkworth et al., 2007).

Prairie cordgrass is well known as a polyploid species comprised of two ploidy levels, tetraploids (2n = 40) and octoploids (2n = 80) with a basic chromosome number of x = 10 (Church, 1940; Marchant, 1968 ab). Previous attempts have been made to delineate the geographic distribution of tetraploid and octoploid cytotypes of prairie cordgrass in the United States (Reeder, 1977). Recently, Kim et al. (2010) reported the existence of a single hexaploid plant (2n = 60) grown from seed collected in central Illinois. Given the fact that there are now three cytotypes identified and that only a limited number of locales have been sampled with respect to prairie cordgrass populations (Marchant, 1968 ab; Reeder, 1977), a more thorough investigation is now required determining the geographic distributions of the now three cytotypes of prairie cordgrass in the U.S.

A survey of the cytotype variation and the distribution of prairie cordgrass are crucial for a better understanding of the evolutionary process of polyploids, as well as the contemporary distribution patterns of the cytotype variations across the U.S. Knowledge of the extent of the ploidy levels will provide important information for prairie cordgrass breeding programs with respect to long-term improvement in biomass yield performance. This study explored the distribution of genome size in natural populations of dominant prairie cordgrass. Flow cytometry was used (Rayburn et al., 2005, 2009), because it is well known as a useful method in the assessment of genome size of ploidy level variation and distribution, and detailed within population screening (Michaelson et al., 1991; Suda et al., 2007b; Huff Kao, 2008). Our objectives were: (1) to determine the cytogeographic distribution of prairie cordgrass, more precisely defining the tetraploid, hexaploid, and octoploid cytotype locations; (2) to determine if any intraploidy genome size variation in prairie cordgrass.

MATERIALS AND METHODS

Plant material

Between 2009 and 2010, a total of 60 prairie cordgrass populations (*Spartina pectinata* Bosc ex Link.*)* were sampled from locations that covered five geographic regions of the U.S. (U.S. Census Bureau, 2011): New England (Maine, New Hampshire, Massachusetts, Connecticut), Middle Atlantic (New Jersey, New York), East North Central (Indiana, Wisconsin, Illinois), West North Central (Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, Kansas), and West South Central (Oklahoma) (Fig. 1). Rhizomes or seeds (when possible) were collected throughout each location to represent the population. Two populations originating from New Hampshire and New York were obtained as fresh rhizomes from the Plant Material Center of USDA-NRCS (New York). Plants were cultivated in 11 cm x 11 cm pots filled with Sunshine SB300 Universal soil (American Plant Product & Service, OK) and maintained under greenhouse conditions (16 h photoperiod at temperature of 24-26 °C).

Analysis of DNA content

In this study, the relative ploidy levels were determined by flow cytometry for all plant samples and were confirmed by counting chromosome numbers with representative plants in each ploidy level, according to methods described by Kim et al. (2010). PI staining method was used for genome size estimation of three plants from each population which are presented in Table 1. For confirming the intraploidy result, the ploidy levels of four populations, a total of nine plants per population, of octoploid prairie cordgrass were reassessed using flow cytometry. After flow cytometry, chromosome counts were obtained from actively growing roots tips

collected from three random plants of three populations per ploidy level, except for the hexaploid cytotype. For the hexaploid cytotype, root tips were obtained from three random plants of one population.

Statistics

All statistical analyses were done using SAS 9.2 (SAS Institute Inc., Cary, NY, USA). Mixed-model ANOVA was used to test the significant difference between two ploidy levels in DNA content and 1 Cx genome size. *P*-values were reported for all parameters measured.

RESULT AND DISCUSSION

Cytotype variation was found in the prairie cordgrass plants examined from the locations throughout the Midwest and the Northeast regions of the U.S. (Fig. 3.1). The population averages of 2C genome size, chromosome number, and monoploid DNA amount (1Cx) are listed in Table 1. Three different DNA ploidy levels were revealed in this study: tetraploid, hexaploid, and octoploid cytotypes. No intraploidy significant genome size variation was observed in the tetraploid populations (range 1.52 -1.63 pg); while a significant intraspecific genome size variation was observed in the octoploid populations (range 2.97-3.15 pg). An additional set of samples were tested and confirmed the significant variation between the highest and lowest genome sizes of octoploid ($P = 0.0002$; Table 3.2). There is no consistent pattern of intraspecific genome size variation found along the geographic distribution range of octoploid populations. The mechanisms for DNA variation are not clearly understood but may be associated with natural selection to reduce the nucleotypic effects of increased DNA amounts (Bennett, 1987;

Carvalho et al., 2010; Shilman et al., 2010; Bao-Quan et al., 2011; Kong et al., 2011). Despite varied genome size among populations of the same ploidy level, the variation did not confuse the classification of the ploidy level for individuals because the variation within the ploidy level was much smaller than the variation between the ploidy levels. The chromosome numbers were estimated for three individuals from each of seven populations (one hexaploid and three from each tetraploid and octoploid populations), confirming the presence of tetraploid (2n = 4x = 40), hexaploid (2n = 6x = 60), and octoploid (2n = 8x = 80) cytotypes, given that the base number of x=10 (Marchant 1968 ab). No additional ploidy levels or aneuploid counts were found.

The monoploid DNA amount (1Cx) was also significantly different among ploidy levels (P = 0.0002): tetraploid (383 Mb), hexaploid (381 Mb), and octoploid (375 Mb) cytotypes. The 1Cx tended to decrease with increasing ploidy levels, with the tetraploid 1Cx having about 8 Mb more DNA than the octoploid 1Cx. According to Bennett (1987) and Leitch et al. (2004), the loss of DNA in polyploids or genome downsizing is a widespread phenomenon occurring in many polyploidy species such as *Hieracium bauhinii* Besser (mouse-ear hawkwee) (Suda et al., 2007a) *Ranunculus parnassifolius* L. (buttercup) (Cires et al., 2010), and *Cardamine Yezoensis* Maxim.(yamahatazao) (Marhold et al., 2010). The reduced genome size following polyploid formation varies depending on factors such as the DNA amount in the diploid, the life cycle strategy of the species and the ecological environment.

In this study, the most frequent ploidy levels were tetraploids (2n = 4x = 40, 38 populations) and octoploids (2n = 8x =80, 23 populations) (Fig.3.1). The tetraploids essentially occupied almost all of the central U.S. regions with colonization extending to the New England (Maine, Massachusetts, Connecticut, and New Hampshire) and the Middle Atlantic (New Jersey and New York) regions (e.g. W 60° to 75° and N 40° to 50°). The octoploid populations, in

contrast, extended to the West North Central (Minnesota, North Dakota, South Dakota, and Nebraska) region (e.g. W 94° to100° and N 40° to 50°). This corresponds with the limited data published by Reeder (1977), who reported that populations from Illinois were tetraploids, while populations from Colorado, Nebraska, and Wyoming were octoploids. However, according to our detailed cytogeographic data, tetraploid and octoploid populations were found within 50 miles of each other in: (1) Iowa (12 populations, 8 tetraploid and 4 octoploid); (2) Kansas (8 populations, 2 tetraploid and 6 octoploid); and (3) Oklahoma (4 populations, 2 tetraploid and 2 octoploid). There is a slight through incomplete pattern within each of the state with the majority of the tetraploid populations residing east of the octoploid populations. Tetraploid and octoploid cytotypes have been found in close proximity in other plant species (Jersàkovà et al., 2010; Tràvnícek et al., 2011). According to Krahulcová et al. (2000) and Levin (2002), the overlapped regions may reflect the regions of origin of higher ploidy populations formed from lower ploidy. A similar distribution pattern was observed in *Chamerion angustifolium (L).* Holub (fireweed) species, where the intermediate altitudes of diploids and tetraploid populations overlapped (Mosquin and Small, 1971; Husband and Schemske, 1998). Such overlaps may indicate ecosystem changes of adaptive significance. The intermediate attitude has been hypothesized to be the region of origin of the higher ploidy populations adapted to lower elevators from the diploid progenitors adapted to higher elevator.

The octoploid populations were completely homogeneous with no mixing of cytotypes. This was also the case with the tetraploid cytotype excluding the one region where the hexaploid cytotype was found growing. The hexaploid population was therefore found in a heterogeneous setting with the tetraploid. Unlike the original hexaploid plant (Kim et al., 2010), the hexaploids in this study are a stable new cytotype, appear to be competitive with the establishing tetraploid

cytotypes, and have reproductive success for both flowering and rhizomes. The success of neohexaploid stabilization may be related to a higher fitness in local environmental conditions as seen in other plant species (Van Dijk et al., 1992; Tràvnícek et al., 2011).

CONCLUSION

This study is the most comprehensive report on prairie cordgrass cytotypes collected from various locations throughout the U.S. to date. Based on their nuclear DNA contents and chromosome counts, the plants were classified into three different ploidy levels: tetraploid (2n = 40), hexaploid (2n = 60), and octoploid (2n = 80) cytotypes. The monoploid DNA amount (1Cx) variation of the octoploid cytotype was smaller in comparison to the hexaploid and tetraploid cytotypes. With respect to the ctytogeography of prairie cordgrass, the tetraploid populations extend from the East North Central to the New England regions of the U.S., while the octoploid cytotypes were mostly distributed in the West North Central region. Overlapping regions of tetraploids and octoploids were found in the West North Central (IA and KS) and West South Central (OK) regions. One mixed population of tetraploids and hexaploids was found at a single location in Illinois. These results contribute to our understanding of the potential relationship between ploidy levels and the geographical distribution of prairie cordgrass. Knowledge of the geographical distribution of various ploidy levels is important in the evolutionary study of this species and is essential to the development of future breeding programs for high yielding cultivars of prairie cordgrass adapted to specific locales.

FIGURES AND TABLES

Table 3.1 Illinois Accession number and location, number of samples analyzed (N), year analyzed, mean 2C genome size (± SD), chromosome number, ploidy level, and 1Cx genome size of prairie cordgrass populations.

Illinois Accession Number[a]	N	Year	2C Genome size (pg) ± SD	Chromosome number (2n)	Ploidy level (x=10)	1Cx genome size Mb[b]
Maine[1]						
23101	6	2010	1.63 ± 0.01	40	4x	399
23104	5	2010	1.61 ± 0.03	40	4x	394
New Hampshire[1]						
9041805	3	2011	1.54 ± 0.04	40	4x	377
Massachusetts[1]						
25101	3	2011	1.6 ± 0.031	40	4x	391
Connecticut[1]						
09101	1	2011	1.56 ± NA	40	4x	381
New York[2]						
9046803	3	2010	1.58 ± 0.07	40	4x	386
New Jersey[2]						
34101	3	2011	1.58 ± 0.03	40	4x	386
Indiana[3]						
18101	1	2010	1.52 ± NA	40	4x	372
Wisconsin[3]						
55103	3	2011	1.57 ± 0.02	40	4x	384
55104	3	2010	1.59 ± 0.03	40	4x	389
55105	3	2010	1.59 ± 0.03	40	4x	389
Illinois[3]						
17115	2	2010	1.53 ± 0.02	40	4x	374
17116	2	2010	2.33 ± 0.04	60	6x	380
17118	2	2010	1.56 ± 0.05	40	4x	381
17119	3	2010	1.52 ± 0.05	40	4x	372
17122	2	2011	1.57 ± 0.00	40	4x	384
17124	2	2011	1.52 ± 0.01	40	4x	372
17125	2	2011	1.58 ± 0.02	40	4x	386
17126	2	2011	1.55 ± 0.01	40	4x	379
17127	2	2011	1.53 ± 0.00	40	4x	374
17128	2	2011	1.56 ± 0.03	40	4x	381
17129	2	2011	1.63 ± 0.04	40	4x	399

Table 3.1 (cont.)

Illinois Accession Number[a]	N	Year	2C Genome size (pg) ± SD	Chromosome number (2n)	Ploidy level (x=10)	1Cx genome size Mb[b]
17130	2	2011	1.56 ± 0.01	40	4x	381
17132	2	2011	1.56 ± 0.01	40	4x	381
Minnesota[4]						
27106	3	2010	3.04 ± 0.10	80	8x	372
27108	3	2010	3.07 ± 0.05	80	8x	375
Iowa[4]						
19101	3	2010	1.56 ± 0.01	40	4x	381
19102	3	2010	1.57 ± 0.06	40	4x	384
19103	3	2010	1.55 ± 0.04	40	4x	379
19104	3	2010	1.57 ± 0.01	40	4x	384
19105	3	2010	1.54 ± 0.06	40	4x	377
19106	6	2010	3.01 ± 0.10	80	8x	368
19107	3	2010	3.11 ± 0.05	80	8x	380
19108	3	2010	3.04 ± 0.04	80	8x	372
19109	1	2010	1.54 ± 0.00	40	4x	377
19110	3	2010	1.60 ± 0.03	40	4x	391
19111	2	2010	1.59 ± 0.01	40	4x	389
19112	1	2010	3.15 ± NA	80	8x	385
Missouri[4]						
29101	3	2010	1.54 ± 0.02	40	4x	377
29104	3	2010	1.52 ± 0.04	40	4x	372
29106	3	2010	1.60 ± 0.03	40	4x	391
North Dakota[4]						
38101	3	2011	3.15 ± 0.14	80	8x	385
South Dakota[4]						
46105	3	2010	3.08 ± 0.05	80	8x	377
46108	3	2010	3.00 ± 0.05	80	8x	367
46109	6	2010	3.01 ± 0.05	80	8x	368
Nebraska[4]						
31101	2	2011	3.15 ± 0.01	80	8x	385
31102	2	2011	3.12 ± 0.11	80	8x	381
31103	2	2011	3.11 ± 0.01	80	8x	380
31104	2	2011	3.10 ± 0.09	80	8x	379
Kansas[4]						
20101	4	2010	3.02 ± 0.03	80	8x	369
20102	3	2010	1.52 ± 0.04	40	4x	372

Table 3.1 (cont.)

Illinois Accession	N	Year	2C Genome size	Chromosome	Ploidy level	1Cx genome size
20103	4	2010	3.13 ± 0.08	80	8x	383
20104	3	2010	2.98 ± 0.05	80	8x	364
20105	3	2010	1.58 ± 0.03	40	4x	386
20106	3	2010	2.97 ± 0.01	80	8x	363
20107	3	2010	3.10 ± 0.01	80	8x	379
20110	2	2010	3.10 ± 0.02	80	8x	379
Oklahoma[5]						
40101	6	2010	1.54 ± 0.03	40	4x	377
40102	5	2010	1.53 ± 0.01	40	4x	374
40103	3	2010	2.98 ± 0.11	80	8x	364
40104	8	2010	3.15 ± 0.08	80	8x	385
LSD			0.18			15

[a]Subscript number is based on the map of U.S. Census Bureau (2011), the study was divided into five regions: 1, New England; 2, Middle Atlantic; 3, East North Central; 4, West North Central; 5, West South Central.
[b]Conversion: 978 Mb = 1pg according to Dolezel et al. (2003).
NA, not applicable

Table 3.2 Mean 2C and 1Cx genome size (± SD) of the highest and lowest genome size groups within octaploid populations.

Population	2C Genome size (pg) ± SD	1Cx genome size Mb[†]
38101	3.24 ± 0.08	396
40104	3.18 ± 0.09	389
20106	3.11 ± 0.09	380
46109	3.06 ± 0.05	374
LSD	0.08	9

[†]Conversion-978 Mb = 1pg according to Dolezel et al. (2003).

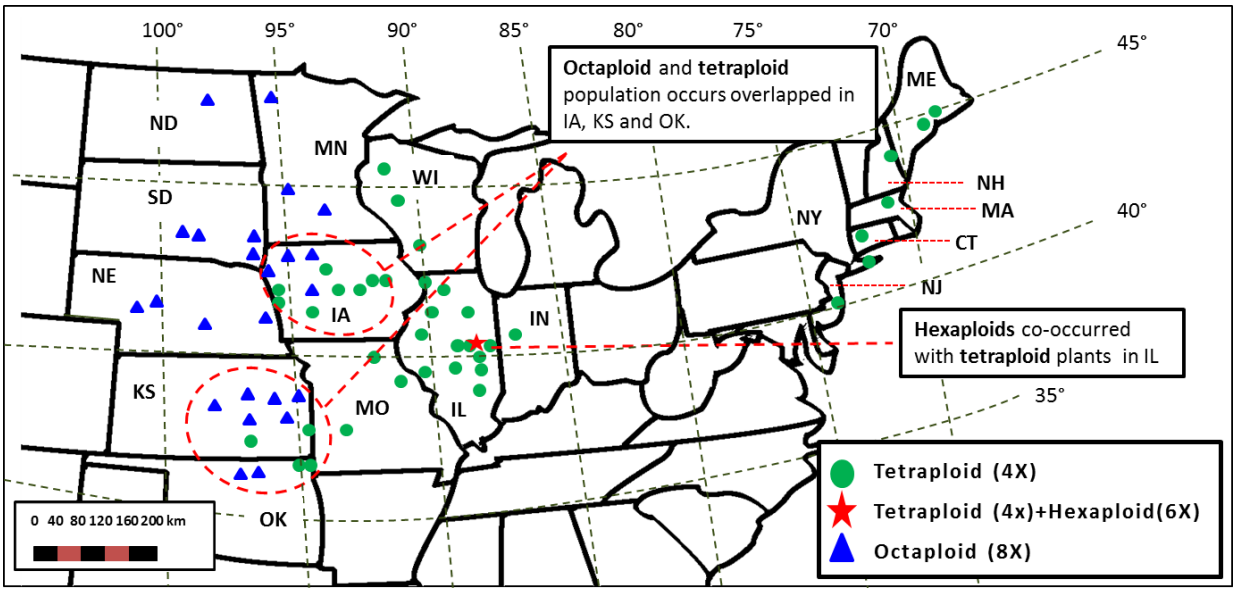

Figure 3.1 Geographic distribution of polyploidy in prairie cordgrass (*Spartina Pectinata L.*). Circles, star, and triangle represent tetraploid (4x), mixed tetraploid and hexaploid (4x+ 6x), and octaploid (8x) populations, respectively.

\

CHAPTER 4

NEOPOLYPLOIDY IN SPARTINA PECTINATA: 1. MORPHOLOGICAL ANALYSIS OF TETRAPLOID AND HEXAPLOID PLANTS IN A MIXED NATURAL POPULATION[3]

ABSTRACT

Prairie cordgrass has been reported as a multi-polyploidy species having three cytotypes: tetra- (2n = 4x = 40), hexa- (2n = 6x =60), and octaploid (2n = 8x = 80). A mixed-ploidy population comprising tetraploids and hexaploids was recently found at a single location in Illinois. However, adaptation and morphological differences between tetra- and hexaploids occurring in natural condition as well as the contact zones of these cytotypes have yet to be determined. In this study, the cytotypes of 147 individuals of prairie cordgrass collected across the contact zone (4x + 6x) were determined by flow cytometry using somatic G1 nuclei, and the results were confirmed by chromosome counts. Nineteen morphological characteristics were compared between the cytotypes. Tetra- and hexaploid plants have 2C genome sizes of 1.57 and 2.36 pg with chromosome counts of 40 and 60, respectively. This increase in polyploidy resulted in a greater variability of morphological expression in Illinois prairie cordgrass. Substantial differences in the flowering time, stomatal size, and plant morphological characteristics were observed between tetra- and hexaploids. The results indicate that the increasing of ploidy level in prairie cordgrass resulted in increased plant size in ploidy mixtures. The recent event of ploidy

[3] Reprinted, with permission, from S Kim, A.L.Rayburn, Arid Boe and D.K.Lee, 2011. Neopolyploidy in Spartina pectinata: 1. Morphological Analysis of Tetraploid and Hexaploid Plants in a Mixed Natural Population. Summited to Plant Systematics and Evolution

mixtures in prairie cordgrass natural populations offers unique opportunities for studying the formation and establishment of neopolyploidy.

INTRODUCTION

Polyploidy, defined as the possession of three or more sets of chromosomes, is one of the most distinctive and widespread characteristics of speciation in angiosperms (Otto, 2007; Parisod et al., 2010). The frequency of polyploidy in plants has been estimated to be 30% (Stebbins, 1971) to 80% (Goldblatt, 1980; Leitch and Bennett, 1997; Soltis et al., 2009). Most angiosperm species show signs of polyploids in their ancestries, and many polyploids are perennial plants that have an asexual mode of reproduction (Grant, 1981; Appels et al., 1998). Polyploid individuals arise spontaneously in nature as result of a failure of cell division during mitosis or meiosis stages (Appels et al., 1998; Otto and Whitton, 2000). Polyploids can be successfully isolated from their diploid ancestors. An increase of cell size and gene expression, changes in physiology, morphology, and ecological tolerance arise due to natural selection acting on random mutation (Levin, 2002; Ramsey and Schemske, 2002; Lumaret, 1988; Adams and Wendel, 2005; Soltis, 2005).

Polyploids are classified into two major categories: allopolyploids and autopolyploids. Allopolyploids result from interspecific hybridization between two different species (Doyle et al., 2008), whereas autopolyploids arise from the multiplication of chromosome sets within a single fertile species (Cain, 1944). Autopolyploidy has historically been considered less important and less frequent than allopolyploidy in natural populations of angiosperms (Soltis et al. 2007). However, molecular studies have revealed that genome duplication is not rare (Ramsey and

Schemske, 1998), suggesting that natural autopolyploids are more common than previously thought (Kron et al., 2007; Parisod et al., 2010). When multiple cytotypes occur within the same species at one location (Baack, 2004; Mràz et al., 2008; Hijmans et al., 2007; Suda et al., 2007; Tràvnícek et al., 2011ab), opportunities to examine the interaction between cytotypes, and elucidate the evolutionary process governing origins and the establishment of polyploids in natural populations exist (Ramsey and Schemske, 1998).

Both allopolyploids and autopolyploids have been documented in the genus *Spartina* (Marchant, 1967; Raybould et al., 1991; Baumel et al., 2001; Fortune et al., 2007, 2008) which belongs to the subfamily Chloridoideae (Mobberley, 1956), a well-supported monophyletic lineage (Hsiao et al., 1999). The basic chromosome number of the genus *Spartina* is x = 10. The chromosome numbers of fifteen *Spartina spp.* investigated, to date, range from tetraploid (2n = 40), hexaploid (2n = 60 - 62), octaploid (2n = 80), to dodecaploid (2n = 120, 122, 124), with possible aneuploidy existing as well (Marchant, 1968; Reeder, 1977). No diploid has been reported in this genus. Among the well documented natural allopolyploids within *Spartina* spp., an example of allopolyplodization is *Spartina anglica* which originated in England. The hybridization between *Spartina alterniflora* (2n = 62) and the native British species *Spartina maritima* (2n = 60) led to a new F_1 hybrid species, *Spartina x townsendii* (2n = 62). This resulted in the new fertile allopolyploid species, *Spartina anglica* (2n = 120, 122, 124), through spontaneous chromosome doubling (Marchant, 1968; Groves and Groves, 1980). The creation of the new fertile allopolyploid species, *Spartina anglica,* has revealed that polyploidization occurs during the evolutionary process of natural populations. Unlike allopolyploids, autopolyploids in the *Spartina* species have not been well studied, and there are no reports focused on the putative evolutionary history of autopolyploid in the genus *Spartina.*

Prairie cordgrass (*Spartina pectinata* Link), reported to have multiple cytotypes, including tetraploid (2n = 4x = 40), hexaploid (2n = 6x = 60), and octoploid (2n = 8x = 80) (Kim et al. 2010 and In press), offers a convenient model to study intraspecific autopolyploids. Prairie cordgrass is distributed throughout the eastern coast and inland marshes of the Midwest United States to Alberta, Canada (Barkworth et al., 2007). It is a tall (1 to 3 meters), robust, sod-forming, warm-season (C_4) grass that can reproduce both sexually by seeds and asexually by rhizomes. Due to its great viability of growing on flooded, saturated or water-logged soils (Weaver, 1954; Montemayor, 2008), this species is commonly found and widely distributed in marshes, sloughs, and flood plains (Mobberley, 1956). Tetraploid populations extend from the East North Central to the New England regions of the U.S., while the octoploid cytotypes are mostly distributed in the West North Central region (Kim et al. In press). The most frequently observed level is octaploid followed by tetraploid (Reeder, 1977). In contrast, a hexaploid prairie cordgrass seeds harvest from a tetraploid plant is very rare. The first hexaploid prairie cordgrass was found in seedlings grown from seeds harvested from a tetraploid plant (Kim et al., 2010). Unlike the hexaploid prairie cordgrass previously found, a more recent hexaploid was discovered as inhabiting a heterogeneous setting with tetraploid plants at one location in Illinois (Kim et al., In press). According to Petit et al. (1999), this location can be referred to as a primary origin which is a consequence of the emergence of a neopolyploid within a progenitor population.

Hybrid zones that involve two different cytotypes with different ploidy levels play a critical role in studying the ecology and the evolution of morphological and life-history traits (Stebbins, 1950; Petit et al., 1996). The recent discovery of neohexaploid in prairie cordgrass provides a rare opportunity to exam morphologic traits between a parental cytotype and the neo-cytotype that could influence adaptation and competition of neohexaploid. In this study, we have

used cytogenetic approaches to examine the spatial distribution of the different ploidy levels across a contact zone (4x + 6x) in prairie cordgrass and to document, if any, the morphological variability due to the polyploid formation and intraspecific evolution.

MATERIALS AND METHODS

Plant material and germplasm collection

Variations in ploidy levels and spatial distribution were estimated for 147 individual rhizomes. The rhizomes were randomly collected from 39 locales representing distinct clusters of plants occurring alongside a stream bank extending over a linear distance of 2.8 Km in Champaign County, Illinois, USA (Fig. 4.1). A Conservation Reserve Program (CRP) filter strip of thirty meters wide was established between the edge of the agricultural land and the stream bank 15 years ago. Switchgrass (*Panicum virgatum*), big bluestem (*Andropogon gerardii*), and Indiangrass (*Sorghastrum nutans*) were sown on tilled seedbed of the CRP filter strip, which was originally a part of agricultural production land. Thus the reclamation of agricultural land represents a disturbed habitat. The stream collects tile water from both sides of the agricultural land starting on the south end of the field area. Above ground biomass in the CRP land has also been burned every three years. Approximately five years ago, the CRP land went through additional disturbance with dredging of the bottom of stream. During these disturbances, prairie cordgrass, a native species in this area of Illinois, appears to have colonized the CRP strip. A cluster is identified as a group of clones with similar phenotypes (Fig. 4.2). The diameter of an individual cluster varied, and prairie cordgrass of each cluster was growing on the edge of the field and extending down on the sides of the stream bank, depending on the size of individual

cluster. The intervals between clusters also varied, ranging from 0 to 10 m. Four locations having both tetraploid and hexaploid clusters within a 1 m distance between ploidy levels were randomly selected and plant samples from these four locations were used for morphological description (Fig.4.1, 4.2). In each of the four locations, ten rhizomes from each ploidy level were randomly collected for chromosome counts. The rhizomes were transplanted in 15 cm round pots filled with potting soil and cultivated in a controlled environment greenhouse (16-h photoperiod at 24°C-26°C) at the University of Illinois at Urbana-Champaign, USA.

Ploidy level analysis

The nuclear DNA contents of the 147 individuals, 2 to 8 randomly selected plants per cluster, were estimated using flow cytometric measurement. Fresh young stems were randomly sampled under natural field conditions and prepared by the method described in Rayburn et al. (2005). An analysis of relative DNA content was performed with a BD LSR II flow cytometry (BD Biosciences, San Jose, CA) equipped with 5-W argon ion laser excitation at 488 nm in the Flow Cytometry Laboratory, Biotechnology Center at University of Illinois at Urbana-Champaign, USA. Stem tissues (~2 cm^2 of each) from both the sample and the reference internal standard, maize (*W22* subpopulations, 2C = 5.14 pg), were co-chopped with a razor blade in a 15 ml beaker containing 10 ml of extraction buffer and 200 µl of 25% Triton X. The extraction buffer consisted of 13% (v/v) hexylene glycol, 10 mM Tris-HCl [pH 8.0], and 10 mM $MgCl_2$. After the tissue was ground using a tissue grinder, the samples were filtered through a 50 um CellTrics® disposable filter (Partec, GmbH) into a test tube. The nuclei were isolated through centrifugation for 15 minutes at 11,000 rpm at 4 °C. The nuclei were stained with propidium iodide (PI) staining, and incubated for 20 minutes at 37 °C. Following incubation, 300 µl of PI

salt was added to each sample. The samples were then briefly vortexed, placed on ice, and stored at 4 °C for 1 hour. An average of about 30,000 nuclei per sample was analyzed. The relative DNA content (the DNA index) was calculated using the relative fluorescence of the sample divided by the relative fluorescence of the standard.

The chromosome counts were based on the somatic G1 nuclei in the fresh, active root tips collected from 24 plants grown in the greenhouse. Chromosome preparation was made according to the cell squashing technique described by Kim et al. (2010). The collected root tips were pretreated with ~15 ml of 0.05% 8-hydroxyquinoline for approximately 2 hours and fixed at room temperature for 4 days in a 3:1 ratio of ethanol to acetic acid. The fixed root tips were rinsed in ddH_2O, hydrolyzed in 5 N HCL for 45 minutes, and stained with Feulgen's reagent for 2 hours. The root tips were then rinsed again with ddH_2O and soaked in an enzyme solution (0.2g Cellulysin and 0.1g Macerase in 10 ml of 10 mM EDTA) for 45 minutes. One drop of 1% acetocarmine was added to the root tips before squashing. A cover slip was placed over the tissue and gently tapped with a dissection needle to disperse all of the tissue. The cells were viewed with an Olympus BX61 microscope and documented with an Olympus U-CMAD3 camera.

Flowering time

Date of first flowering was defined as the emergence of inflorescence from the sheath of the uppermost leaf (flag leaf) (Somers and Grant 1981) and the flowering dates were observed and recorded every 3 days from July 2010 to September 2010. The mean flowering time was estimated as the average flowering date for the observed clusters within the ploidy level and expressed in Julian days (natural days from January 1st).

Shoot and inflorescence morphology and above ground biomass collection

To determine the differences in morphological characteristics and the above ground biomass between ploidy levels, plant samples of each cytotype were collected from four locations (Fig. 1). After the completion of stem elongation with the appearance of inflorescences, the 1st (the flag leaf) and 5th leaves below the peduncle were sampled. For each location, three plants per each ploidy were analyzed. Stomata length and density, and prickle density were measured using an environmental scanning electron microscopy (ESEM) (FEI-XL30, Philips Electron Optics, Eindhoven, The Netherlands) located in the Beckman Institute at the University of Illinois at Urbana-Champaign, USA. Ten micrographs with 1 mm^2 areas from each replicate leaf were made on both sides of the abaxial and adaxial leaves for counting the number of stomata and prickle, respectively (Fig. 4.5, 4.6). For stomata length, fifteen stomata were measured on the abaxial leaf side per replicate leaf. Another set of the 1st and 5th leaves below the peduncle were collected from 10 randomly selected tillers from each ploidy level at each location. The leaf length and width were immediately measured. Finally, the leaves were dried at 60°C for 72 hours to a constant weight, and the final leaf weight was measured.

At the end of October 2010, two of the 0.2 m^2 sub-plots from each ploidy level within a location were harvested by excising all tillers at the surface of the soil using a rice knife. The above ground vegetation obtained from each sub-plot was dried at 60°C for 72 hours to a constant weight; and the dry weight of the total biomass of each plot was converted to Mg DM ha^{-1}. After drying, the number of tillers was determined for each plot. From sub-samples of the 10 tillers selected at random, the number of leaves per tiller and the shoot length (the base of the plant to the tip of the tallest leaf) were measured. Mass tiller^{-1} was determined by the plot's above ground biomass dry weight divided by the number of tillers for each plot.

While harvesting above ground biomass, ten reproductive tillers were randomly collected for each ploidy level within a given location. The number of spikes was counted per replicate plant. Thirty spikes for each cluster were analyzed for spike length and number of spikelets per spike. Spikelet length, total length of upper glume, and awn length (the length from the distal end of the upper glume to the distal tip of the awn) was measured from 10 spikelets per cluster (Fig. 7). Four replicates of the weights of 1000 caryopses were also measured per cluster.

To test the viability of the seeds, a germination test was conducted on 80 caryopses from each cluster using a germination chamber (VWR international, Cornelius, Oregon, USA) at 15°C dark/ 30°C light with a 16 hour photoperiod (light photon flux density: 25 µmol photon $m^{-2}s^{-1}$, 380-680 nm). The caryopses were surface sterilized with a 10% solution of commercial bleach for 10 minutes and then washed 3 times with distilled water. Four replicates of 20 caryopses each were used, and the caryopses were sown in 10 cm diameter petri dishes, on two folds of white seed germination blotter paper (Anchor, St. Paul, MN) imbibed with 12 mL of distilled water. The petri dishes were arranged in a completely randomize block design, and the germinated seeds (when the radicle had started to grow) were counted at 1 day intervals for 7 days. The germination percentage was calculated by dividing the number of germinated seeds by the total number of seeds.

Statistics

All statistical analyses were carried out using SAS 9.2 (SAS Institute Inc., Cary, NY, USA). Mixed-model ANOVA was used to test the significant difference between the two ploidy levels in DNA content, 1Cx genome size, and the flowering time expressed in Julian days. The ploidy level was considered a fixed effect, and each location was considered a random effect.

The above ground biomass and the shoot morphological traits among the selected clusters in four locations were analyzed in the mixed-model ANOVA, where ploidy level was considered a fixed effect, and the site was considered a random effect. Due to a limited number of inflorescences for tetraploids and hexaploids in two locations, only two locations were used for the statistical analyses of the inflorescence morphological traits. *P*-values were reported for all the parameters measured.

RESULT

Ploidy level analysis

The DNA-ploidy levels were determined for 147 individuals sampled in 39 natural clusters of prairie cordgrass found at the study site (Table 1). A representative flow histogram is shown in Figure 3. The coefficient of variation for all of the G1 nuclear peaks ranged between 3 and 5%. Based on their nuclear DNA contents, the plants were classified into two different ploidy levels and their chromosome numbers were analyzed from a selected number of each cluster (Fig. 4). This confirmed the existence of tetraploid (2n = 40) and hexaploid (2n = 60) cytotypes. No octaploids cytotypes were observed in this study. The 2C genome size of tetraploid and hexaploid prairie cordgrass is around 1.57 pg, about 31 % of the maize genome, and 2.36 pg, about 46 % of the maize genome, respectively. Statistical analysis revealed a significant difference in the genome size of tetraploid and hexaploid prairie cordgrass ($P < 0.001$), whereas the monoploid DNA amount (1Cx ≈ 385 Mb) was similar between ploidy levels ($P = 0.8163$) (Table 1). The field distribution of cytotypes, estimated using flow cytometric analysis, was reflected in the morphological variability of the field (Fig. 2). Tetraploid (2n = 4x=

40) and hexaploid (2n = 6x = 60) cytotypes were found in 21 (75 plants) clusters and 18 (72 plants) clusters, respectively (Fig. 1).

Flowering time

Table 1 shows the mean flowering dates in 2010 for the tetraploids and hexaploids cytotypes over all study ranges, respectively. A difference in the timing of the emergence of inflorescences between tetraploid and hexaploid was observed ($P = 0.0377$), with the mean flowering time on July 22nd (JD = 203) for tetraploids and Aug 2nd (JD = 214) for hexaploids. The earliest flowering plants found in the tetraploid and hexaploid populations had flowering dates on July 13th and July 23rd, respectively. Most tetraploid plants, about 71% of the total, emerged their first panicles by July 21st, whereas the flowering times of hexaploid plants varied between July 23rd and August 10th.

Seed germination

Overall germination rates in this study were low due to fungi growing on the surface of the caryopsis. A difference was observed between ploidy levels for seed germination ($P = 0.0529$), with the hexaploids having a lower germination rate than the tetraploid plants.

Morphological analyses

The results of the analysis of variance on the morphological characteristics are seen in Tables 2 and 3. Upon comparisons of the mean values between the tetraploid and hexaploid plants a trend was observed. Traits such as stomata size, mass per tiller, and 5th leaf blade length were observed to have a definitive increase in the hexaploids as compared to the tetraploids ($P =$

0.0004, 0.01, and 0.02, respectively). Other traits such as spikelets per spike, above ground biomass, shoot length and 5th leaf blade width were also observed to have an increase in the hexaploid plants as compared to the tetraploid plants albeit not as definitive as the previous characteristics (P = 0.07, 0.14, 0.09, and 0.09, respectively). In the remaining morphological characteristics measured (with the exception of 1000 caryopsis weight), a numerical increase was observed in the hexaploid plants as compared to the tetraploid plants, however the P values were observed to be very high with respect to these characteristics (see Tables 2, 3).

DISCUSSION

Observed genome size and chromosome numbers of polyploid prairie cordgrass plants collected from this study area fell within the expected genome size and chromosome numbers. According to Marchant (1968), the basic chromosome number of prairie cordgrass is 10. The ≈ 1.56 pg plants are therefore tetraploids (2n = 4x = 40 chromosome), whereas the ≈ 2.35 pg plants are hexaploids (2n = 6x= 60 chromosomes).This first hexaploid prairie cordgrass detected was from one seed collected from natural Illinois populations genetically isolated and located 70 km southwest from the current study location (Kim et al. 2010, In press). This hexaploid cytotype was not found in nature and appears to have reduced fitness as compared to the tetraploid plants collector from the same area (data not shown). The existence of hexaploid prairie cordgrass in the area in this study was demonstrated in Kim et al. (In press). One possible explanation on the formation of the neohexaploid cytotype may arise spontaneously as a result of recombination of an unreduced gamete (40 chromosomes) with a normal reduced gamete (20 chromosomes). This appears to be the most common mechanism for the co-occurrence of different ploidy levels in

natural populations (Ramsey and Schemske 1998; Krahulcová and Krahulec, 2000; Levin, 2002). Since no octaploid cytotype was observed in Illinois region (Kim et al. In press), this hexaploid appears to be a neoploid arising out of an indigenous tetraploid population.

The neohexaploid plants observed in this study were found to be competitive with tetraploids and become successfully established in nature. The neohexaploids appear to colonize the areas representing the most disturbed habitats which are frequently burned and dredged areas of the established CRP grass land, while tetraploids remain on or near the stream banks representing less disturbed habitats. This observation is in agreement with the hypothesis that disturbed habitats provide environmental conditions necessary for neopolyploid establishment (Ramsey, 2011). Moreover, in this study, morphological differences were observed between tetraploid and hexaploid plants. Since all the samples were grown in the same natural environment, limited to a small area, variability in morphological characteristics appear to be associated with ploidy levels. Polyploidization appears to be a key factor in growth, performance, and adaptability of polyploid species in nature (Cain, 1944; Lewis, 1976; Hancock and Bringhurst, 1981; Ranney, 2006).

Polyploids often possess novel traits, such as changes in flowering time, cell size, and biomass, which may allow new polyploids either to successfully establish within their ancestors or to establish a new population system by replacing their ancestors (Levin, 1983; Felber, 1991; Thompson and Lumaret, 1992). These changes, according to Müntzing (1936), can be strongly correlated with higher chromosome numbers. In the present study, the flowering time and the morphological characteristics of hexaploids were compared with those of tetraploids. This information was used to determine characteristics that varied with respect to ploidy to help elucidate the mechanism of polyploid establishment in prairie cordgrass.

By analyzing the cytotypes in the same natural location, the confounding effects of environmental factors, such as light, which can cause variance in flowering time (Seneca, 1974), were minimized. The results for the initial flowering time showed the date of hexaploids was about 11 days later than of tetraploids, and there was almost no overlap in the flowering time of these two cytotypes. A similar phenological pattern had been reported for the flowering times of *Chamerion angustifolium* which occurred approximately 7 days earlier in diploids than tetraploids (Husband and Schemske, 2000). This is also supported by Bose and Choudhury (1962) who demonstrated that higher DNA content may be associated with slower growth and a later flowering time. Many other studies have also shown higher polyploid plants flower later than their progenitors (Ramsey and Schemske, 1998). Moreover, Pires et al. (2004) found that polyplodization can cause genomic rearrangement which can lead to divergence in plant morphology by late flowering time induction.

Stomatal size previously has been used as a useful parameter to distinguish ploidy level because cell size generally increases with an increase of nuclear DNA content (Müntzing, 1936; Cavalier-Smith, 1978; Mishra, 1997; Gregory, 2001; Thomson et al., 2002). A correlation between stomata size and ploidy level in prairie cordgrass had been previously established, with the stomata size in octaploid prairie cordgrass being larger than that of tetraploids (Kim et al., 2010). Our study confirmed this correlation showing that the stomata size of hexaploids was 1.3 times bigger than that of tetraploids. This result is in the same range observed in the tetraploids of the *Malus* genotype which was 1.4 times higher than that of the diploid genotype (Korban et al., 2009). A large change in the genome size was influence the stomatal density. In the study, for example, the stomata in hexaploids were larger but less dense when compared to the tetraploids. Changes in stomatal density associated with increased ploidy levels has also been reported in

many other species (e.g. *Triticosecale*, Sapra et al., 1975; *Medicago sativa*, Setter et al., 1978; *Festuca arundinacea*, Byryne et al., 1981).

The germination rate in hexaploid prairie cordgrass seeds was low and delayed when compared with tetraploid seeds. Differences in germination rate and speed have also been observed in other polyploids (Levin, 1983; Hoya et al., 2007). Similar results were observed by Soliman (1980) who report that autotetraploid seeds of *Glycine wightii* showed slower germination rate than diploid seeds and Nilsson (1950) who reported that the autotetraploid seeds of *Lycopersicon esculentum* showed lower germination rate than diploid seeds. This reduced rate could be due to disruption in meiosis that results in reduced fertility and viable seed in the hexaploid type. If such a disruption in meiosis occurred this disruption could also explain the reduction of 1000 caryopsis weight and development of abnormal embryos and endosperm. This could also be due to an increase of the embryos and endosperm ratio, resulting from the hexaploid plant crossing with a tetraploid plant. In addition, the hexaploids may be more susceptible to fungal infect, due to the low seed viability, decreased seed germination, or to genetic susceptibility. Further experiments are underway to determine the mechanism of the reduced germination.

The diversity of the inflorescence morphological characteristics between ploidy levels has been observed in number of spikelet. The number of spikelets in hexaploid (976 spikelets per panicle) was higher than that of tetraploids (731 spikelets per panicle). This result agrees with the hypothesis of Shitsukawa et al. (2009) who estimated that the number of spikelet increases with the ploidy levels in wheat. Furthermore, the difference in morphological characteristics between ploidy levels, according to Souer et al. (1998), is derived from the differences in the behavior of

meristematic cells which reflected to the ploidy levels and the degree of heterozygosity caused by numerous mutations.

Differences in overall above ground morphologies between ploidy levels were observed, which are very common in polyploid plants (Stebbins, 1971; Bennett, 1987; Warner and Edwards, 1993). Overall, hexaploid plants showed higher values for morphological charateristics than those of tetraploids plants. These results are in agreement with Giles (1942) who reported the plant size (e.g. height of plant, leaf length and width) of *Cuthbertia graminea* increased with ploidy level. This increase in plant growth variables may have contributed to the 1.5 times higher above ground biomass observed in hexaploids. Similar results have also been found for *Phlox drummondii* (Garbutt and Bazzaz, 1983), *Dactylis glomerata* (Bretagnolle and Lumaret, 1995), and ginseng (Kim et al., 2004). Interesting, tiller densities per unit area were less in the hexaploids than in the tetraploids. However, the mass per tiller was so much higher that hexaploids showed an increase in total biomass. A negative correlation between tiller density and mass had also been observed in *Festuca arundinacea* Schreb.(Nelson et al., 1977). The hexaploid plants in this study was observed to have novel morphological variability not found in original tetraploid populations that could result in the neohexaploid being more fit in its present environment that its progenitor tetraploid species. Such increases in fitness of neopolyploid populations have been observed in other plant species (Petit et al., 1996; Ramsey, 2011).

CONCLUSION

A mixed-population of tetraploid (2C = 1.56 pg; 2n = 4x= 40) and hexaploid (2C = 2.35 pg; 2n = 6x =60) cytotypes of prairie cordgrass was found, thriving in a natural environment in

Illinois. The CRP land provided a disturbed habitat in which the production of novel morphological traits by neopolyploidy could be exploited during the colonization of prairie cordgrass. The result of this study reveals that neopolyploid results in an increase in the morphological variations in the prairie cordgrass population. This was seen in an increase in the range of stomatal cell size, biomass, and flowering time etc. These neohexaploids, co-occurring with tetraploids in the same area, will be useful in examining polyploidy evolution and comparing the fitness and evolution of neohexaploids with tetraploids in a natural setting.

FIGURES AND TABLES

Table 4.1 Means, standard deviations (SD), and *P*- values for genome size, chromosome number, and number of plants analyzed of the prairie cordgrass populations used in this study.

	Tetraploid		Hexaploid		
Trait	Mean	SD	Mean	SD	P-value
2C Genome size (pg)	1.57	0.05	2.36	0.07	<0.0001
1 Cx genome size, Mb[†]	385	11.8	386	10.92	0.8163
Chromosome number (2n)	40	0	60	0	-
Flowering time (JD)	203	5.72	214	13.34	0.0377

[†] Conversion-978 MB= 1 pg according to Dolesel et al. (2003).

Table 4.2 Means, standard deviation (SD), and Means, standard deviations (SD), and *P*-values for leaf and inflorescence morphological characteristics of tetraploid and hexaploid prairie cordgrass.

	Tetraploid		Hexaploid		
Trait	Mean	SD	Mean	SD	P-value
Stomata size (µm)	31	3.35	40.9	5.46	0.0004
Densities of Stomata (mm^{-2})	78.9	69.6	57.4	35.01	0.2428
Densities of Prickle (mm^{-2})	2.5	6.75	3.8	13.45	0.4757
1000 - caryopses weight (g)	1.9	0.36	1.6	0.81	0.6173
Spikes $stem^{-1}$ (no.)	14.5	2.69	15.4	2.21	0.5661
Spikelets $spike^{-1}$ (no.)	50.4	11.7	63.4	10.6	0.0738
Spike length (mm)	78.7	13.4	102	23.25	0.4872
Spikelet length (mm)	18.2	3.08	20.6	1.86	0.6058
Awn length (mm)	5.9	2.81	6.6	1.89	0.7184
Seed germination (%)	50.8	12	30.8	7.35	0.0529

Table 4.3 Means, standard deviations (SD), and *P*-values for aboveground biomass and morphological characteristics of tetraploid and hexaploid prairie cordgrass.

	Tetraploid		Hexaploid		
Trait	Mean	SD	Mean	SD	P-value
Aboveground (Mg DM ha^{-1})	23.1	6.48	33.1	11.95	0.1422
Tillers m^{-2}(no.)	297	84.9	196	43.44	0.1530
Mass $tiller^{-1}$ (g)	8.47	3.36	16.9	5.42	0.0100
Leaves $tiller^{-1}$ (no.)	7.7	1.9	8.97	1.47	0.2564
Shoot length (m)	1.9	0.23	2.06	0.21	0.0918
Leaf blade length (cm)					
1st leaf	81.8	18.7	90.8	18.97	0.2644
5th leaf	115	8.33	128	8.51	0.0182
Leaf blade width (mm)					
1st leaf	8.9	1.81	12.6	2.13	0.0283
5th leaf	12.8	1.39	16.6	2.76	0.0852
Leaf blade weight (g)					
1st leaf	0.6	0.29	0.75	0.34	0.4459
5th leaf	1.2	0.17	1.65	0.38	0.2304

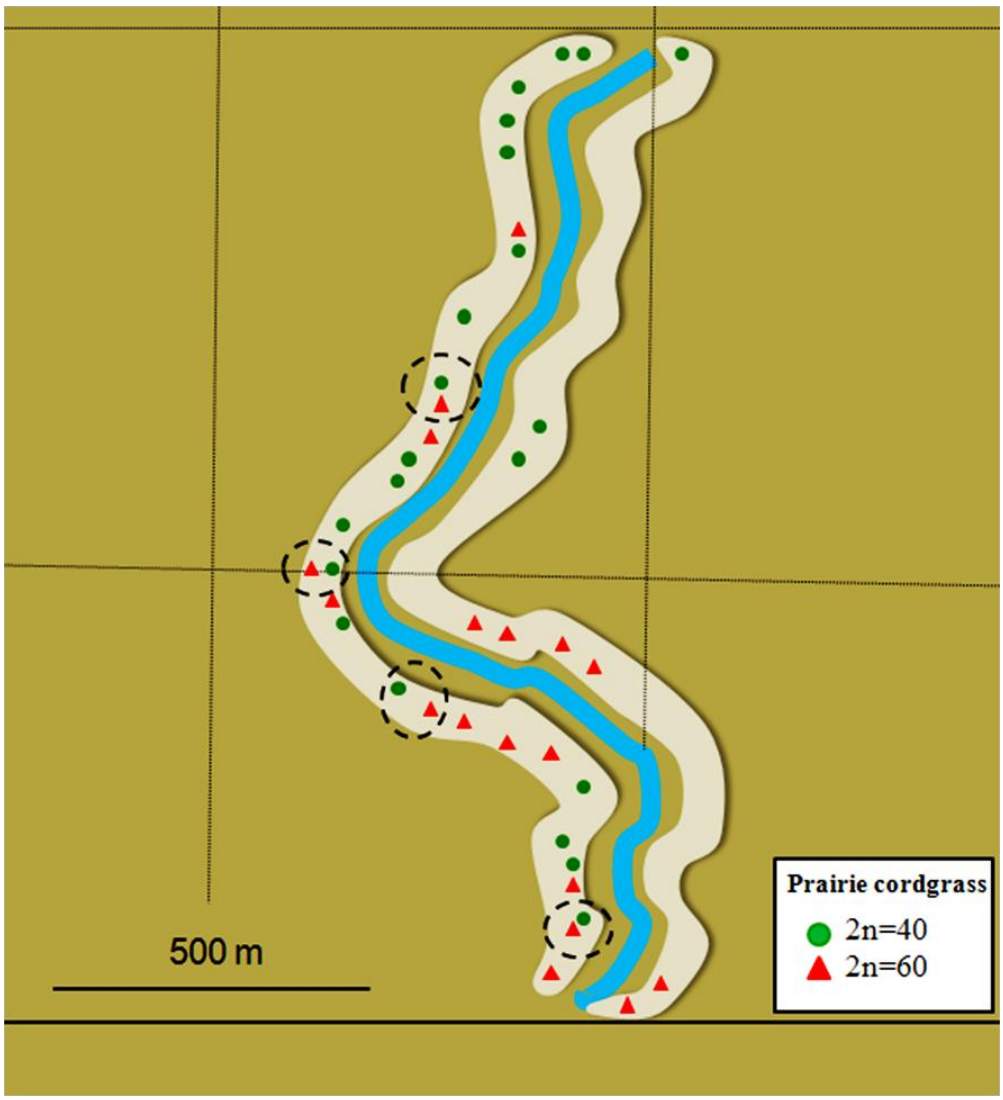

Figure 4.1 Distribution of the mixed-ploidy clusters of prairie cordgrass throughout the stream in Illinois; tetraploid and hexaploid clusters are indicated by green circle symbol and red triangle symbol, respectively; clusters in circled dots were used for chromosome counting, aboveground biomass and morphological characteristics.

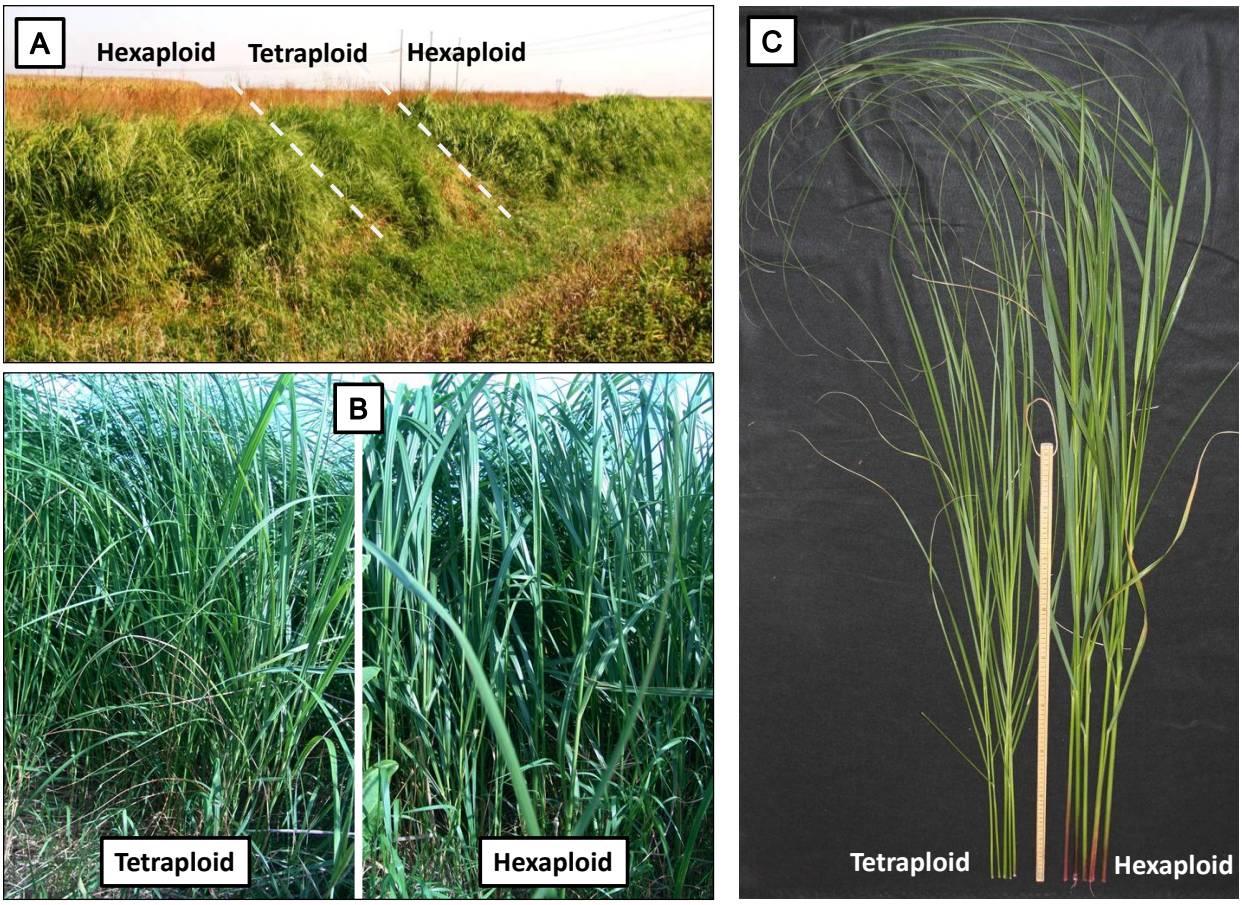

Figure 4.2 Pictures of hexaploid and tetraploid prairie cordgrass. A: View of prairie cordgrass distributed along the stream; white dot lines indicated the clear boundary between hexaploid (2n = 6x) and tetraploid (2n = 4x); B: Close-up view of hexaploid and tetraploids prairie cordgrass clusters; C: Side-by-side comparison of tillers of tetraploid and hexaploid prairie cordgrass.

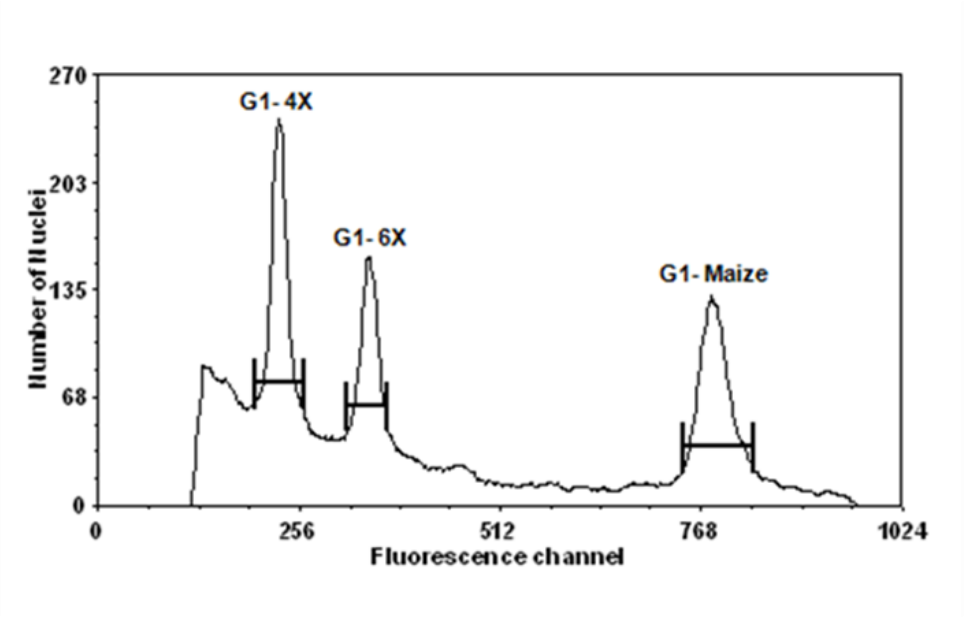

Figure 4.3 Flow Histograms of relative DNA content of mixed somatic nuclei of the 4x and 6x cytotypes stained with PI. The bars represent the nuclei from tetraploid (2n = 40) and hexaploid (2n = 60) plants used to calculate the mean fluorescence of each peak represented tetraploid and hexaploid of prairie cordgrass.

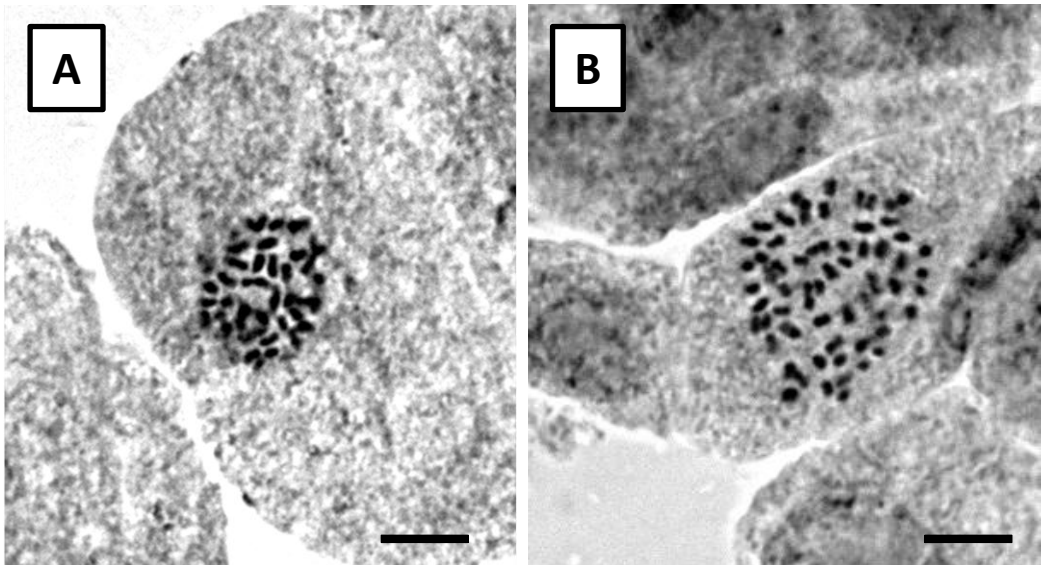

Figure 4.4 Chromosome spreads from root tips of prairie cordgrass. A, tetraploidy, 2n = 4x = 40 chromosomes; B, hexaploidy, 2n = 6x = 60 chromosomes. (Bar = 5.0 μm).

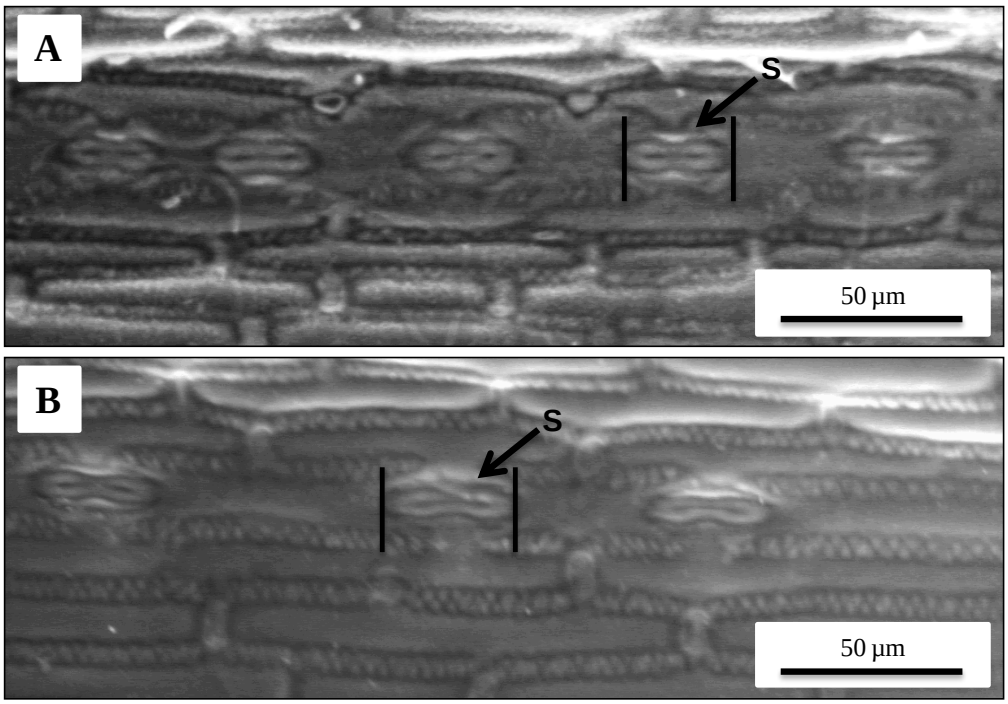

Figure 4.5 Environmental scanning electron micrographs showing abaxial surface of the leaf blade of prairie cordgrass. A, tetraploid; B, hexaploid; S, stomata (Bar = 50 µm). The stomata length measured with the distance between two black bars.

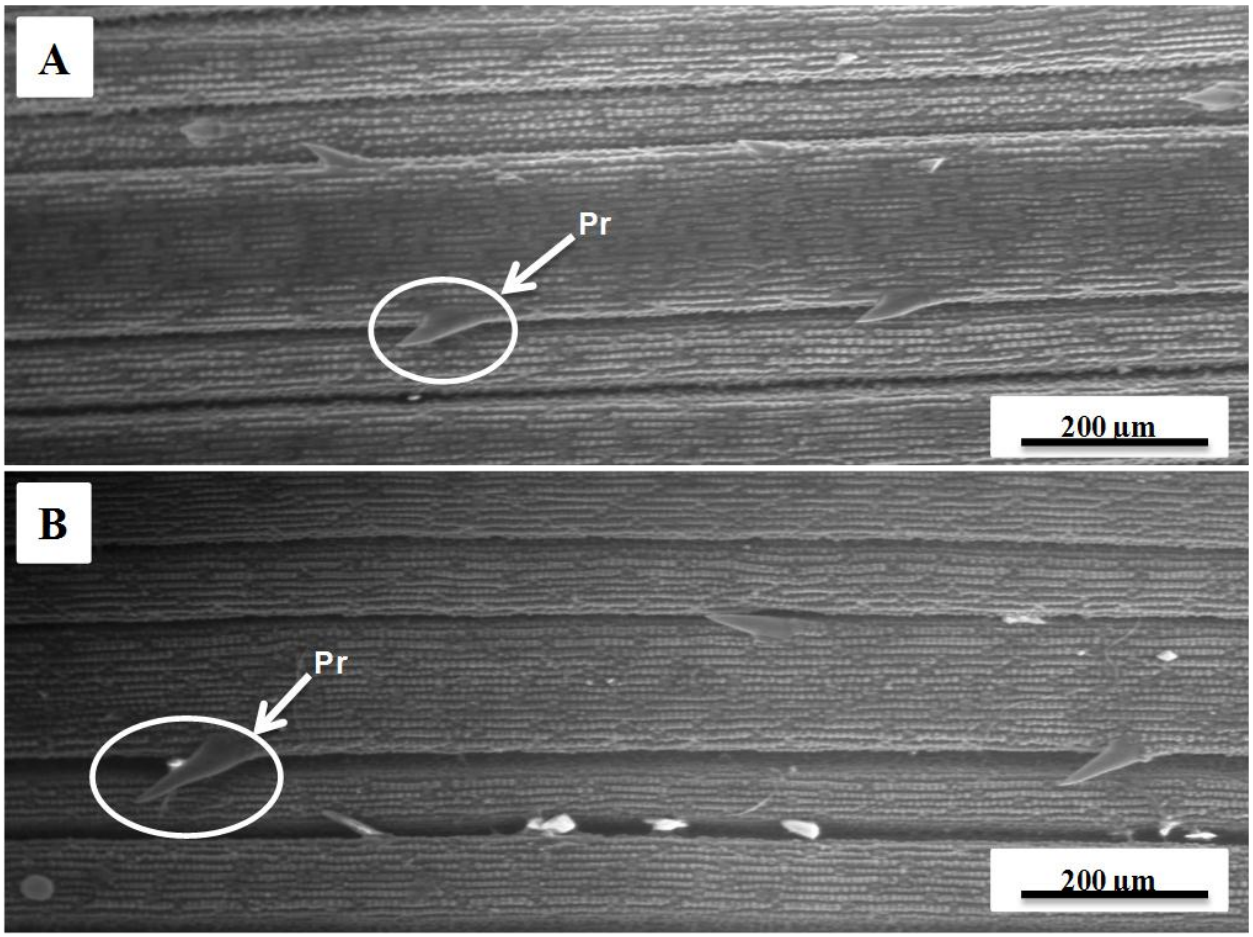

Figure 4.6 Environmental scanning electron micrographs showing adaxial surface of the leaf blade of prairie cordgrass. A, tetraploid; B, hexaploid; Pr, prickle (Bar = 200 µm).

.

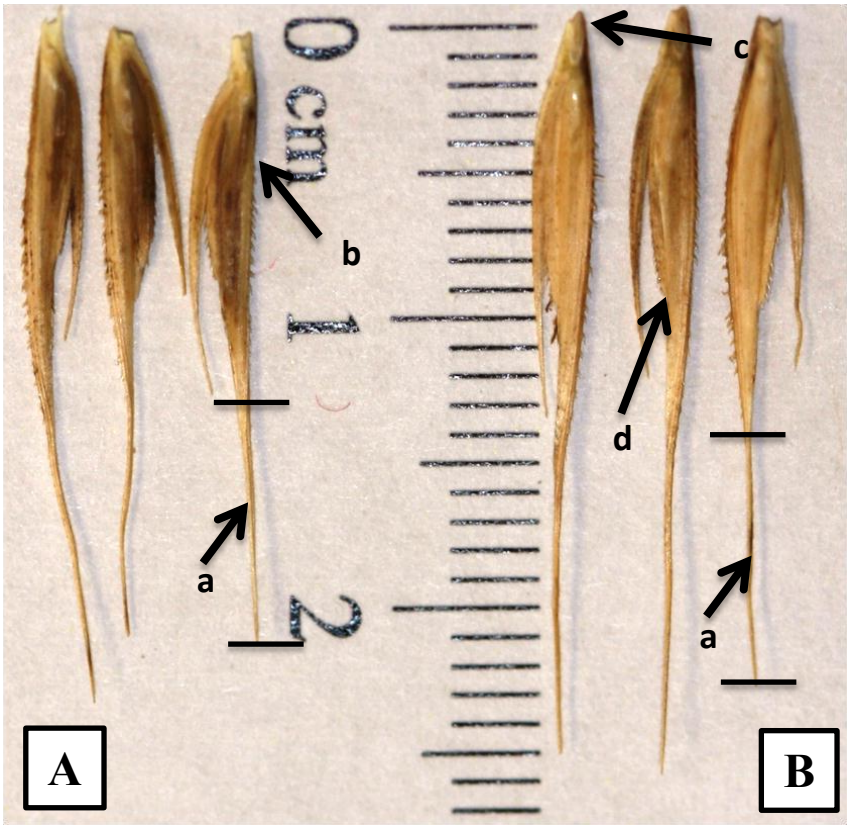

Figure 4.7 Spikelet of tetraploid (A) hexaploid (B) prairie cordgrass: a, awn; b, upper glume; c, callus; d, lemma. Awn length measured with the distance between two black bars.

REFERENCES

Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. Curr Opin Plant Biol 8:135–141

Appels R, Morris R, Gill BS, May CE (1998) Chromosome Biology. 1st Ed., Kluwer Academic Press, Canberra

Auger DL, Gray AD, Ream TS, Kato A, Coe EH, Birchler JA (2005) Nonadditive gene expression in diploid and triploid hybrids of maize. Genetics 169:389–397

Baack EJ (2004) Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). Am J Bot 91:1783–1788

Barkwoth ME, Anderton LK, Capels KM, Long S, Piep MB (2007) Manual of grasses for North America. Utah State Univ. Press, Logan, U.T

Baumel A, Ainouche ML, Levasseur JE (2001) Molecular investigations in populations of *Spartina anglica* C. E. Hubbard (Poaceae) invading coastal Brittany (France). Mol Ecol 10:1689–1701

Bennett MD (1987) Variations in genome form in plants and its ecological implications. New Phytol 106:177-200

Biomass Research and Development Board (2006) National biofuels action plan. Available at http://www1.eere.energy.gov/biomass/pdfs/nbap.pdf (verified 27 July 2010)

Bao-Quan Hu AL, Xue WY, Chen L, Wang WX, Song WZ, Chen CB, Wang CG (2011) DNA methylation in genomes of several annual herbaceous and woody perennial plants of varying ploidy as detected by MSAP. Plant Mol Biol Rep 29:784-793

Boe A, Lee DK (2007) Genetic variation for biomass production in prairie cordgrass and switchgrass. Crop Sci 47:929–934

Boe A, Owens V, Gonzalez-Hermandez J, Stein J, Lee DK, Koo BC (2009) Morphology and biomass production of prairie cordgrass on marginal lands. GCB Bioenergy 1:240–250

Bonilla-Warford CM, Zedler JB (2002) Potential for using native plant species in stormwater wetlands. Environ Manage 29:385–394

Bose RB, Choudhury JK (1962) A comparative study of the cytotaxonomy, palynology, physiology of diploid plants from *Ocimum kilimandscharicum* Guerke and their yield of raw material and volatile contents. Caryologia 15:435-453

Bretagnolle F, Lumaret R (1995) Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. Euphytica 84:197–207

Brown RC (2003) Biorenewable Resources Engineering New Products from Agriculture. Iowa State Press, Ames, Iowa

Byrne MC, Nelson CJ, Randall DD (1981) Ploidy effects on anatomy and gas exchange of tall fescue leaves. Plant Physiol 68:891-893

Cain S (1944) Foundations of plant geography. Harper and Row, New York

Carvalho A, Polanco C, Lima-Brito J, Guedes-Pinto H (2010) Differential rRNA gene expression in hexaploid wheat realted to NOR methylation. Plant Mol Biol Rep 28:403-412

Cavalier-Smith T (1978) Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J Cell Sci 34:247–278

Church GL (1940) Cytotaxonomic studies in the Gramineae Spartina, Andgropogon and Panicum. Am J Bot 27:263-271

Cires E, Cuesta C, Angeles Revilla M, Fernández Prieto JA **(**2010) Intraspecific genome size variation and morphological differentiation of *Ranunculus parnassifolius* (Ranunculaceae), an Alpine–Pyrenean–Cantabrian polyploid group. Biol J Linn Soc 101:251–271

Dolezel J, Bartos J (2005) Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot (London) 95:99–110

Dolezel J, Bartoš, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51:127-128

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF (2008) Evolutionary genetics of genome merger and doubling in plants. Ann Rev Genet 42:443-461

Felber F (1991) Establishment of a tetraploid cytotype in a diploid population: effect of relative fitness of the cytotypes. J Evol Biol 4:195–207

Fortune PM, Schierenbeck K, Ainouche A, Jacquemin J, Wendel JF, Ainouche ML (2007) Evolutionary dynamics of Waxy and the origin of hexaploid *Spartina* species. Mol Phylogenet Evol 43:1040-1055

Fortune PM, Schierenbeck K, Ayres D, Bortolus A, Clatrice O, Ainouche ML (2008) The enigmatic invasive *Spartina densiflora*: a history of hybridizations in a polyploidy context. Mol Ecol 17:4304-4316

Freeman JL, Rayburn AL (2004) Metamorphosis in *Xenopus laevis* is not associated with large scale nuclear DNA content variation. J Exp Biol 207:4473–4477

Garbutt K, Bazzaz FA (1983) Leaf Demography, Flower Production and Biomass of Diploid and Tetraploid Populations of Phlox drummondii Hook. On a Soil Moisture Gradient. New Phytologist 93:129-141

Giles N H Jr. (1942) Autopolyploidy and geographical distribution in Cuhbertia graminea Small. American Journal of Botany 29:637-645

Goldblatt P (1980) Polyploidy in angiosperms. In W.H. Lewis [ed.], Polyploidy-biological relevance, Plenum, New York, USA. pp 219-239

Gonzalez-Hernandez JL, Sarath G, Stein JM, Owens V, Gedye K, Boe A (2009) A multiple species approach to biomass production form native herbaceous perennial feedstocks. In Vitro Cell Dev Biol Plant 45:267-281

Gopalakrishnan G, Negri MC, Wang M, Wu M, Snyder SW, Lafreniere L (2009) Biofuels, land, and water: A systems approach to sustainability. Environ Sci Technol 43:6094–6100

Grant V (1981) Plant speciation. Columbia University Press, New York, New York, USA

Gregory TR (2001) Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol Rev Camb Philos Soc 76:65–101

Groves H, Groves J (1980) *Spartina townsendii* nobis. Reports of the Botanical Society and Exchange Club of the British Isles. pp 1

Hancock JF, Bringhurst RS **(**1981) Evolution of California populations of diploid and octoploid Fragaria (Rosaceae): a comparison. Am J Bot 68:1–5

Hijmans RJ, Gavrilenko T, Stephenson S, Bamberg J, Salas A, Spooner DM (2007) Geographical and environmental range expansion through polyploidy in wild potatoes **(***Solanum section Petota*). Global Ecol Biogeogr 16:485–495

Hitchocock AS (1950) Manual of the grasses of the United States. 2nd ed. Rev. A. Chase. USDS Misc. Publ. 200. US.S. Gov. Print. Office, Washington, DC

Hoya A, Shibaike H, Morita T, Ito M (2007) Germination characteristics of native Japanese dandelion autopolyploids and their putative diploid parent species. J Plant Res 120:139–147

Hsiao C, Jacobs SWL, Chatterton NJ, Asay KH (1999) A molecular phylogeny of the grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). Austral Syst Bot 11:667–688

Huff Kao R (2008) Origins and widespread distribution of co-existing polyploids in *Arnica cordifolia* (Asteraceae). Ann Botany 101:145-152

Husband BC, Schemske DW **(**1998) Cytotype distribution at a diploid-tetraploid contact zone in *Chamerion* (*Epilobium*) *angustifolium* (Onagraceae). Am J Bot 85:1688–1694

Husband BC, Schemske DW (2000) Ecological mechanisms of reproductive isolation and coexistence of diploid and tetraploid *Chamerion angustifolium*. J Ecol 88:689-701

Jersàkovà J, Castro S, Sonk N, Milchreit K, Schödelbauerovà I, Tolashc T, Dötterl S (2010) Absence of pollinator-mediated premating barriers in mixed-ploidy populations of *Gymnadenia conopsea* s.l. (Orchidaceae). Evol Ecol 24:1199-1218

Jordan N, Warner KD (2010) Enhancing the multifunctionality of US agriculture. Bioscience 60:60–66

Kim S, Rayburn AL, Lee DK (2010) Genome size and Chromosome Analyses in Prairie Cordgrass. Crop Sci 50:2277-2282

Kim S, Rayburn AL, Voigt T, Parrish A, Lee DK (2011a) Salinity effects on Germination and Plant Growth of Prairie Cordgrass and Switchgrass. Bioenergy Res doi: 10.1007/s12155-011-9145-3

Kim S, Rayburn AL, Lee DK (In press) Neopolyploidy in Spartina pectinata: 1. Morphological Analysis of Tetraploid and Hexaploid Plants in a Mixed Natural Population. Accepted to Plant Systematics and Evolution

Kim S, Rayburn AL, Parrish A, Lee DK (In press) Cytogeographic distribution and genome size variation in prairie cordgrass (*Spartina pectinata* Bosc ex Link). Accepted to Plant Mol Biol Rep

Kim YS, Hahn EJ, Murthy HN, Paek KY (2004) Effect of polyploidy induction on biomass and ginsenoside accumulations in adventitious roots of ginseng. J Plant Biol 47:356–360

Kong F, Mai S, Jiang J, Wang J, Fang X, Wang Y (2011) Proteomic changes in Newly synthesized *Brassica napus* allotetraploids and their early generations. Plant Mol Bio Rep 29:927-935

Korban SS, Wannarat W, Rayburn CM, Tatum TC, Rayburn AL (2009) Genome size and nucleotypic variation in *Malus* germplasm. Genome 52:148-155

Krahulcová A, Krahulec F (2000) Offspring diversity in *Hieracium* subgen. *Pilosella*(Asteraceae): new cytotypes from hybridization experiments and from open pollination. Fragm Flor Geobot 45(1–2):239–255

Kron P, Suda J, Husband BC (2007) Applications of flow cytometry to evolutionary and population biology. Annu Rev Ecol Evol Syst 38:847–876

Leitch IJ, Bennett MD (1997) Polyploidy in angiosperms. Trends Plant Sci 2:470-476

Levin DA (1983) Polyploidy and novelty in flowering plants. Am Nat 122:1–25

Levin DA (2002) The Role of Chromosomal Change in Plant Evolution. Oxford University Press

Lewis WH (1976) Temporal adaptation correlated with ploidy in *Claytonia virginica*. Syst Bot 1:340–347

Long SP, Incoll LD, Woolhouse HW (1975) C_4 photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*. Nature 257:622–624

Lumaret R (1988) Adaptive strategies and ploidy levels. Acta Oecol Oecol Plant 9:83–93

Madakadze IC, Coulman BE, McElroy AR, Stewart KA, Smith DL (1998) Evaluation of selected warm-season grasses for biomass production in areas with a short growing season. Bioresour. Technol 65:1–12

Marchant CJ (1967) Evolution in *Spartina* (Gramineae). I. The history and morphology of the genus in Britain. J Linn Soc Bot 60:1–24

Marchant CJ (1968a) Evolution in *Spartina* (Gramineae): II. Chromosomes, basic relationships and the problem of *S. x townsendii* agg. J Linn Soc Bot 60:381–410

Marchant CJ (1968b) Evolution in Spartina (Gramineae): III. Species chromosome numbers and their taxonomic significance. J Linn Soc Bot 60:411–417

Marhold K, Kudoh H, Pak JH, Watanabe K, Španiel S, Lihovà J (2010) Cytotype diversity and genome size variation in eastern Asian polyploid *Cardamine (Brassicaceae)* species. Ann Bot 105:249–264

Michaelson MJ, Price HJ, Ellison JR, Johnston JS (1991) Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. Am J Bot 78:183-188

Mishra MK (1997) Stomatal characteristics at different ploidy levels in Coffea L. Ann Bot (Loud.) 80:689-692

Mobberley DG (1956) Taxonomy and distribution of the genus Spartina. Iowa St Coll J Sci 30:471-574

Moncada KM, Ehlke NJ, Muehlbauer, GJ, Sheaffer CC, Wyse DL, DeHaan LR (2007) Genetic variation in three native plant species across the state of Minnesota. Crop Sci 47:2379–2389

Montemayor MB, Price JS, Rochefort L, Boudreau S (2008) Temporal variations and spatial patterns in saline and waterlogged peat fields. 1. Survival and growth of salt marsh graminioids. Environ Exp Bot 62:333–342

Mosquin T, Small E **(**1971) An example of parallel evolution in *Epilobium* (Onagraceae). Evolution 25:678–682

Mraz P, Singliarova B, Urfus T, Krahulec F (2008) Cytogeography of *Pilosella officinarum (Compositae):* altitudinal and longitudinal differences in ploidy level distribution in the Czech republic and Slovakia and the general pattern in Europe. Ann Bot 101:59-71

Müntzing A. (1936) The evolutionary significance of autopolyploidy. Hereditas 21:263-378

Nelson CJ, Asay KH, Sleper DA (1977) Mechanisms of canopy development of tall fescue genotypes. Crop Sci 17:449-452

Nelson JM, Lane B, Freeling M (2002) Expression of a mutant maize gene in the ventral leaf epidermis is sufficient to signal switch of the dorsoventral axis. Development 129:4581–4589

Nilsson E (1950) Some experiments with tetraploid tomatoes. Hereditas 36:181-204

OECD/IEA (2009) World Energy Outlook. http://www.iea.org/speech/2009/Tanaka/WEO2009_Press_Conference.pdf

Otto SP, Whitton J (2000) Polyploid incidence and evolution. Annu Rev Genet 34:401-437

Otto, SP (2007) The evolutionary consequences of polyploidy. Cell 131:452-462

Parisod C, Alix K, Just J, Petit M, Sarilar V, Mhiri C, Ainouche M, Chalhoub B,Grandbastien M-A (2010) Impact of transposable elements on the organization and function of allopolyploid genomes. New Phytol 186:37–45

Perlack RD, Wright LL, Turhollow A, Graham RL, Stokes B, Eraback DC (2005) Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. Oak Ridge National Laboratory, Oak Ridge

Petit C, Thompson JD, Bretagnolle F (1996) phenotypic plasticity in relation to ploidy level and corn production in the perennial grass *Arrhenatherum elatius*. Can J Bot 74:1964-1973

Petit C, Bretagnolle F, Felber F (1999) Evolutionary consequence of diploid-polyploid hybrid zones in wild species. Trends Ecol Evol 14:306-311

Pires JC, Zhao J, Schranz ME, Leon EJ, Quijada PA, Lukens LN, Osborn TC (2004) Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). Biol J Linn Soc 82:675–688

Potter L, Bingham MJ, Baker MG, Long SP (1995) The potential of two perennial C_4 grasses and a perennial C_4 sedge as ligno-cellulosic fuel crops in N.W. Europe: Crop establishment and yields in E. England. Ann Bot (London) 76:513–520

Raybould AF, Gray AJ, Lawrence MJ, Marshall DF (1991) The evolution of *Spartina anglica* C. E.Hubbard (Gramineae): origin and genetic variability. Bot J Linn Soc 43:111–126

Rayburn AL, McCloskey R, Tatum TC, Bollero GA, Jeschke MR, Tranel PJ (2005) Genome Size Analysis of Weedy Amaranthus Species. Crop Sci 45:2557-2562

Rayburn AL, Crawford J, Rayburn CM, Juvik JA (2009) Genome size in three Miscanthus species. Plant Mol Biol Rep 27:184–188

Ramsey J, Schemske DW (1998) Pathways, mechanisms and rates of polyploid formation in flowering plants. Annu Rev Ecol Syst 29:477-501

Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Annu Rev Ecol Syst 33:589-639

Ramsey J (2011) Polyploidy and ecological adaptation in wild yarrow. Proc Natl Acad Sci USA 108:7096-7101

Ranney TG (2006) Polyploidy: From evolution to new plant development. Combined Proc Intl Plant Prop Soc 56:137–142

Reeder JR (1977) Chromosome-numbers in western grasses. Am J Bot 64:102-110

Renny-Byfield S, Ainouche M, Leitch IJ, Lim KY, Le Comber SE, Leitch AR (2010) Flow cytometry and GISH reveal mixed ploidy populations and Spartina nonaploids with genomes of S. alteriflora and S. maritima origin. Ann Bot (London) doi: 10.1093/aob/mcq008

Rousseau-Gueutin M, Lerceteau-Kohler E, Barrot L, Sargent DJ, Monfort A, Simpson D, Arus P, Guerin G, Denoyes-Rothan B (2008) Comparative genetic mapping between octaploid and diploid Fragaria species reveals a high level of colinearity between their genomes and the essentially disomic behavior of the cultivate octaploid strawberry. Genetics 179:2045–2060

Sapra VT, Hughes JL, Sharma GC (1975) Frequency, size and distribution of stomata in triticale leaves. Crop Sciences 15:356-358

Seneca ED (1974) Germination and seedling response of Atlantic and Gulf Coast populations of *Spartina altinaflora*. Am J Bot 58:48-55

Setter TL, Schrader LE, Bingham ET1978. Carbon dioxide exchange rates, transpiration and leaf characteristics in genetically equivalent ploidy levels in alfalfa. Crop Sci 18:327-332

Shapiro HM (1995) Practical flow cytometry. 3rd ed. Wiley-Lis, New York

Shitsukawa N, Kinjo H, Takumi S, Murai K (2009) Heterochronic development of the floret meristem determines grain number per spikelet in diploid, tetraploid and hexaploid wheats. Ann Bot 104:1–9

Shilman R, Brand Y, Brand A, Hedvat, I, Hovav R (2011) Identification and molecular characterization of homeologous Δ9-stearoyl acyl carrier protein desturase 3 genes from the allotetraploid peanut (Arachis hypogaea). Plant Mol Bio Rep 29:232-241

Simmons BA, Loque D, Blanch HW (2008) Next-generation biomass feedstocks for biofuel production. Genome Biol 9:242.1–242.5

Skinner RH, Zobel RW, Van der Grinten M, Skaradek W (2009) Evaluation of native warm-seson grass cultivars for riparian zones. J S W Con 64:413-422

Soliman MH (1980) Ploidy and strain differences in seed germination of *Glycine wightii* at different PH levels. Theor Appl Genet 56:175-182

Soltis DE, Soltis PS, Schemske DW, Hancock JF, Thompson JN, Husband BC, Judd WS (2007) Autopolyploidy in angiospers: have we grossly underestimated the number of species? Taxon 56:13-30

Soltis DE, Albert VA, Leebens-Mack J. Bell CD, Paterson AH, Zheng C, Sankoff D, dePamphilis CW, Wall PK, Soltis PS (2009) Polyploidy and angiosperm diversification. Am J Bot 96:336-348

Somers GR, Grant D (1981) Influence of seed source upon phenology of flowering of *Spartina alterniflora* Loisel. and the likelihood of cross pollination. Am J Bot 68:6-9

Souer D, Krol A, Kloos D, Spelt C, Bliek M, Mol J, Koes R (1998) Genetic control of branching pattern and floral identity during Petunia inflorescence development. Development 125:733-742

Stebbins GL (1950) Variation and evolution in plants. New York, USA: Columbia University Press

Stebbins GL (1971) Chromosomal evolution in higher plants. Addison Wesley, Reading, Mass

Stubbendieck J, Hatch SL, Kjar KJ (1982) North American range plants. 2nd ed. Univ. Nebraska Press, Lincoln

Suda J, Krahulcovà A, Tràvnícek P, Rosenbaumovà R, Peckert T, Krahulec F (2007a) Genome size variation and species relationships in *Hieracium* sub-genus *Pilosella* (Asteraceae) as inferred by flow cytometry. Ann Bot 100:1323–1335

Suda J, Weiss-Schneeweiss H, Tribsch A, Schneeweiss GM, Trávníček P, Schönswetter P (2007b) Complex distribution patterns of di-, tetra- and hexaploid cytotypes in the European high mountain plant *Senecio carniolicus* Willd. (*Asteraceae*). Am J Bot 94(8):1391-1401

Thompson LD, Lumaret R (1992) The evolutionary dynamics of polyploid plants: origins, establishment and persistence. Trends Ecol Evol 7:302-307

Thomson JA, Alonso A, Miguel E (2002) Clarification of the taxonomic status and relationships of Pteridium caudatum (Dennstaedtiaceae) in Central and South America. Bot J Linn Soc 140:237–248

Tilman D, Socolow R, Foley JA, HillJ, Larson E, Lynd L, Pacala S, Reilly J, Searchinger T, Somerville C, willians R (2009) Beneficial biofuels-The food, energy, and environment tilemma. Science, 325:270-271

Tràvnícek P, Kuàtovà B, Curn V, Rauchovà J, Krajníkovà E, Jersàkovà J, Suda J (2011a) Remarkable coexistence of multiple cytotypes of the *Gymmadenia conopsea* aggregate (the fragrant orchid): evidence form flow cytometry. Ann Bot 107:77-87

Tràvníček P, Dočkalovà Z, Rosenbaumovà R, Kubàtovà B, Szeląg Z, Chrtek J (2011b) Bridging global and microregional scales: ploidy distribution in *Pilosella echioides* (Asteraceae) in central Europe. Ann Bot 107:443-454

United States Department of Agriculture, Natural Resources Conservation Service (2002) Prairie cordgrass plant guide. Available at http://plants.usda.gov/plantguide/pdf/pg_sppe.pdf (verified 27 July 2010)

United Nations (2007) Sustainable bioenergy: A framework for decision makers. Available at http://esa.un.org/un-energy/pdf/susdev.biofuels.fao.pdf (verified 27 July 2010)

United States Department of Agriculture, Natural Resources Conservation Service (2002) Prairie cordgrass plant guide. Available at http://plants.usda.gov/plantguide/pdf/pg_sppe.pdf (verified 27 July 2010)

United States Census Bureau, Statistical Abstract of the United States (2011) (130th Edition) Washington, DC. http://www.census.gov/statab (verified 25 Oct 2011)

United States Department of Agriculture (2011) Plant Feedstock Genomics for Bioenergy. http://genomicscience.energy.gov/research/DOEUSDA/usda_doe_handout.pdf (verified 25 Oct 2011)

Van Dijk P, Hartog M, Van Delden W (1992) Single cytotype areas in autopolyploid Plantago media L. Biol J Linn Soc 46:315-331

Warner DA, Edwards GE (1993) Effects polyploidy on photosynthesis. Photosynth Res 35:135-147

Weaver JE (1954) North American prairie. Lincoln, NE: Johnson Publishing Company. pp 348