Poa arachnifera×Poa secundaの種間交雑を用いた雄性発生による半数体作出

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Generation of Androgenic Haploids from Interspecific Hybridization of *Poa arachnifera*×*Poa secunda*

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Synopsis

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The generation of haploids in most grass species is relatively infrequent, but has continually been considered a potential tool for the development of fixed inbred lines, a method for cytoplasmic transfer, or polyploid genome manipulations. This study was originally undertaken to identify the frequency of successful hybridization following crosses between *Poa arachnifera*×*P. secunda*. However, during the research, four androgenic haploids representing the polyploid *P. secunda* parent were obtained with varying chromosome numbers. Each of the haploids exhibited the fibrous root system characteristic of the *P. secunda* parent. The generation of androgenic haploids offers an opportunity to observe *P. secunda* germplasm in a *P. arachnifera* cytoplasm as well as an opportunity to utilize such materials in genetic studies of polyploid *P. secunda*.

Key words : Androgenesis, Bluegrass, Haploid, Interspecific hybridization, *Poa*.

Introduction

Haploid inducement or double haploid breeding methods are widely used in many cereal crops but are not well characterized in many forage species. In addition, for many forage grass species, little information is available regarding meiotic or reproductive anomalies which can induce major genome manipulations. *Poa* represents one of the world's most highly regarded cool-season grasses for both turf and forage use. In addition, it is a variable genus with a tremendous number of diversity among species[1]. Within this genus, the high level of genetic diversity and its predominately apomictic form of reproduction make it a taxonomically difficult one for study.

Among the many diverse and wide spread Poa species, *P. arachnifera* Torr. (Texas bluegrass) is a potentially useful but little studied germplasm resource. *Poa arachnifera* is a vigorous, rhizomatous, sod-forming perennial grass native to the Southern Plains Region of the USA[10]. *Poa arachnifera* is dioecious, with male and female plants and as a consequence, cross pollinations are accomplished with relative ease. To date, all of our collections of native *P. arachnifera* have been completely sexual in their form of reproduction.

Poa secunda J. S. Presl. (Big bluegrass) is a perennial, native bluegrass and is an important component of the sagebrush grassland vegetation of the Western United States[10]. Its range extends from Southeastern Alaska through the Western and Great Plains Regions of the United States. In contrast to *P. arachnifera*, it possesses a fibrous root system, which can occasionally develop short rhizomes, and it is a facultative apomictic species exhibiting aposporic embryo sac development[2,3,5].

This study was originally undertaken to identify the frequency of successful hybridization following crosses between *P. arachnifera*×*P. secunda*. However, during the research, androgenic haploids were identified to occur at a low frequency. This research reports on the generation of androgenic haploids in hybridization with *P. arachnifera*.

Materials and Methods

1. Plant Materials

A mature female *P. arachnifera* individual (2n=54), originally collected near El Reno, Canadian County, Oklahoma, USA, was transplanted from a field nursery at the USDA-ARS, Grazinglands Research Laboratory (GRL), El Reno, OK in February 2000 and re-established in pots. Seeds of *P. secunda* cv. Sherman (2n=63) (PI578850) were obtained from the Western Regional Plant Introduction Station, Pullman, WA and were sown to the field nursery at the GRL. In February 2000, mature *P. secunda* individuals were transplanted and re-established in pots. All transplants were grown in a lighted, temperature controlled (20℃) growth chamber. Lighting was supplied by overhead units (Sunlight Supply Co., Boulder, CO) possessing one OSRAM metal halide M/MS

lamp and one OSRAM mercury vapor S106 LU600 super lamps at a distance of 1.5 meters from the plant canopy. The lamps were timed to provide a 12 hour light and 12 hour darkness regime. Humidity was not controlled but was kept at a reasonably low level. Following their transfer to the growth rooms, flowering initiated for both accessions in March, 2000. Pollinations were achieved by placing the accessions adjacent to each other and agitating the *P. secunda* individuals between 7 : 30-8 : 00 AM. As a consequence, pollen was dusted onto the *P. arachnifera* females from the *P. secunda* pollen source.

2. Seed Germination

Seed heads were harvested approximately 45 days following pollination and were air dried for an additional two weeks. Due to the cob-webby nature of the *P. arachnifera* seed heads, the infloresences were broken up by rubbing the seed heads between two hands. The seed heads were sown in pots containing a light potting soil mix, then lightly covered with the same potting soil mixture. The pots were then watered, covered to prevent evaporation and placed in a germination chamber set at 20℃. As seedlings emerged, they were transplanted to separate pots. As plants became established, they were transferred to the field nursery at the GRL.

3. Cytogenetic and RAPD Evaluations

Chromosome counts of mitotic metaphase cells were obtained from the rapidly dividing root-tips of hybrid individuals following a method for rice with the exception that the pretreatment time was limited to 30 minutes[9]. RAPD analyses were performed on selected individuals by isolating DNA from fresh leaf material. DNA was extracted from fresh leaf material utilizing the Master Pure Plant Leaf DNA Purification Kit (Epicentre Technologies, Madison, WI) and a traditional PCR-RAPD protocol was utilized[11]. Approximately 5 ng of plant DNA was used per reaction. AmpliTaq DNA polymerase and reaction buffer were purchased from Perkin Elmer (Branchburg, NJ). Primers utilized in the study were previously shown to generate products that display segregation and polymorphism among *P. arachnifera*. PCR amplification was carried out in a MWG-BioTech Primus 96 well thermocycler (High Point, NC). The PCR amplification reaction was programmed for 1 cycle of 94℃ for 1.5 min followed by 25 cycles of 94℃ for 1 min ; 37℃ for 2 min and 72℃ for 2 min and then 1 final cycle of 37℃ for 2 min and 72℃ for 5 min. Following amplification, RAPD products were held at 4℃ until they were separated on a 15 g/L agarose gel and run in 1×TBE (Tris-borate-EDTA) buffer at 100 V for 3.5 hours. Visualization was achieved under UV light following staining in EtBr for 1 hour.

Results and Discussion

Germination of the *P. arachnifera* seed heads resulted in 75 F_1 individuals being obtained. Of these, four (5.3%) exhibited a seedling and mature plant phenotype distinctively different from their siblings. The phenotype of these four was much more representative of the *P. secunda* parent than of a presumed intermediate form of *P. arachnifera*×*P. secunda* hybrid (PaPs). Chromosome counts of root-tip metaphase cells showed that these four individuals, (designated as H1, H2, H3 and H4), exhibited 16, 18, 20 and 16 chromosomes, respectively (Fig. 1 a). A random sample of ten PaPs hybrids exhibited chromosome numbers ranging from 54 to approximately 77 (Fig. 1 b). The H1-H4 individuals exhibited fewer tillers, possessed a more upright or erect leaf profile and smaller or compact seed heads (Figs. 2 a and 2 b). The H1-H4 individuals also expressed a lighter green color when compared the blue-gray color of *P. arachnifera* or their *P. arachnifera*×*P. secunda* counterparts. In addition, a comparison of the root systems of the H1-H4 with the PaPs hybrids showed that all the H1-H4 plants possessed a fibrous root system, similar to the *P. secunda* parent, whereas all the PaPs hybrids exhibited a rhizomatous root system, which is characteristic of the *P. arachnifera* parent (Figs. 3 a and 3 b).

PCR analysis utilizing 50 RAPD primer pairs and multiplex primer reactions on DNA extracted from the H1-H4 individuals, as well as the PaPs hybrids, revealed that the H1-H4 individuals possessed none of the *P. arachnifera* genome and that they possessed only DNA from the paternal *P. secunda* genome (Fig.

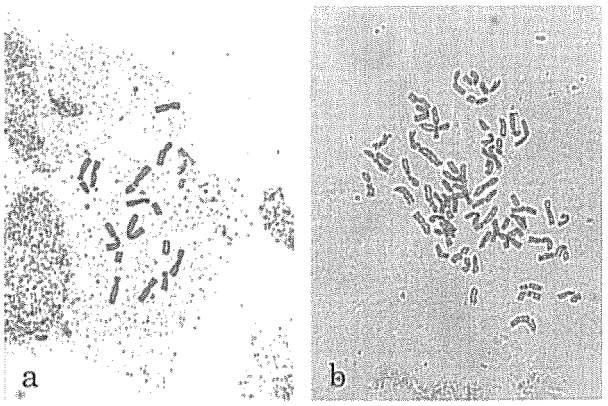

Fig. 1. Root tip metaphase cell of progeny resulting from a *P. arachnifera*×*P. secunda* hybridization. (a) Chromosome count of a root tip metaphase cell of a partial paternal polyhaploid possessing 18 chromosomes. (b) Chromosome count of a root tip metaphase cell of a true *P. arachnifera*×*P. secunda* hybrid possessing 70 chromosomes.

4). These data, together with the chromosome counts, suggest that individuals H1-H4 possess only a subset of the *P. secunda* genome and represent true androgenic haploids. RAPD PCR evaluations on several PaPs hybrids authenticated their hybrid genome constitutions by exhibiting bands specific to both parents.

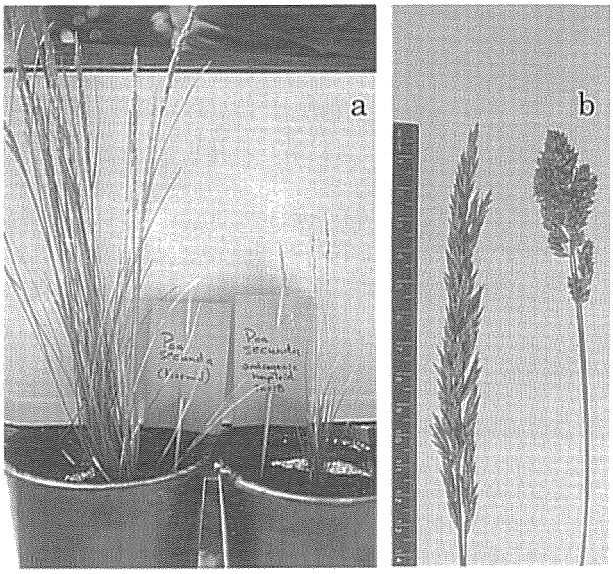

Fig. 2. (a) Photograph of androgenic haploid individual H 2 (2n=16, right) and a normal *P. secunda* parent (left) at 1 year maturity. (b) Photograph of the infloresence of a true *P. arachnifera*×*P. secunda* F_1 hybrid (left) and that of *P. secunda* androgenic haploid H3 (2n=20, right).

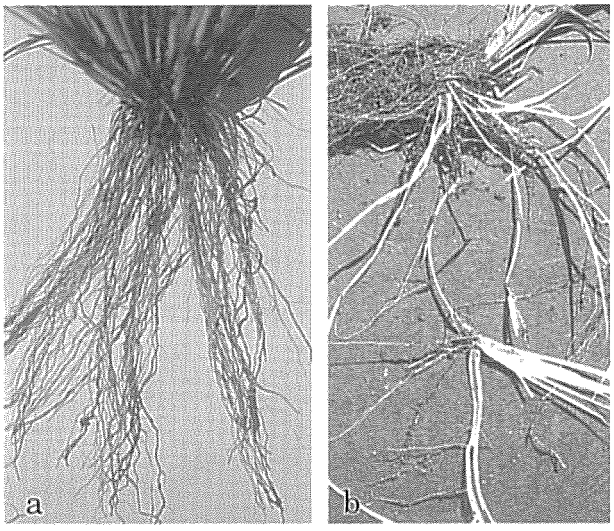

Fig. 3. Photograph of root systems specific to each product arising from the *P. arachnifera*×*P. secunda* hybridization. (a) The fibrous root system characterizing *P. secunda* androgenic haploids. (b) An example of the rhizomatous root system which characterizes the true *P. arachnifera* ×*P. secunda* hybrids.

The production of the four androgenic haploids (H 1-H4) would appear to be similar to the indeterminate gametophyte system of maize, which generates paternal haploids at low frequency[6,8]. However, polyembryony or twinning was not observed in *P. arachnifera, P. secunda* or any of the seeds following hybridization. Within the genus *Poa*, this behavior is not without precedent. In an earlier study involving a comparison of twins arising from a series of apomictic *P. pratensis* individuals (2n=63-90), one plant with a "subhaploid" set of 2n=18 was obtained[7]. Progeny from this individual included an array of stable and viable individuals possessing chromosome numbers ranging from 2n=14 thru 2n=19. The majority of the progeny possessed 2n=16 for their genome constitution. In the H1 thru H4 individuals, their low vigor, weak perennial nature and varied chromosome numbers suggest that these individuals do not possess a balanced genome contribution from their polyploid *P. secunda parent.*

Since only a single *P. arachnifera* female was utilized in the crosses, it is not clear whether the occurrence of androgenic haploids can be attributed to a unique maternal *P. arachnifera* individual. However, additional crosses in 2001 with utilizing a second *P. arachnifera* female have provided at least two additional paternal haploid individuals. As a consequence, the generation of androgenic haploids in *P. arachnifera*×*P. secunda* hybridizations would appear to be a constant, but infrequent event. At the time of this publication, the haploids are over 2 years

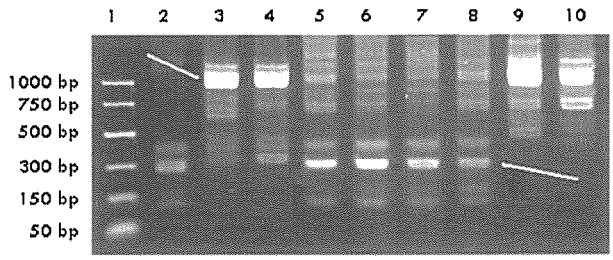

Fig. 4. PCR amplification products of a series of *P. secunda* androgenic haploids, *P. arachnifera*×*P. secunda* hybrids and their parents utilizing UBC decamer primer UBC15.
Lane 1 : Molecular weight markers ; Lane 2, *P. arachnifera* maternal parent ; Lanes 3 and 4, *P. secunda* androgenic haploids H1 and H3 ; Lanes 5 to 8, *P. arachnifera*×*P. secunda* F_1 hybrids ; Lanes 9 and 10, *P. secunda* cv. Sherman. Arrows indicate *P. secunda* genome amplification in lanes 3 and 4 and *P. arachnifera* genome amplification in lanes 5 to 8. Though not shown in this figure, the partial paternal polyhaploids did exhibit variation between each other depending on the decamer primer used.

old and are classified as weak perennials. None appear adapted to the hot and dry environment which characterizes Central Oklahoma and as a consequence are maintained in pots in the growth chamber rooms. Each of the haploids was pollen sterile and backcrossing with *P. arachnifera* and *P. secunda* pollen fail to induce seed formation. Consequently, the haploids appear to be both pollen and seed sterile. Their tendency to tiller has not been prolific and the diagnosis for their long term maintenance is questionable. Colchicine treatments to induce chromosome doubling in the rhizomatous root tip meristems has thus far been unsuccessful. The generation of androgenic haploids in this interspecific hybridization remains novel and of limited breeding value. However, these individuals will prove useful for genome mapping and genetic analysis with regards to polyploid genome of *P. secunda*.

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要　　旨

Bryan KINDIGER (2004) : *Poa arachnifera*×*Poa secunda* の種間交雑を用いた雄性発生による半数体作出. 日草誌 **49**, 577-580. (USDA-ARS, Grazinglands Research Laboratory, 7207 West Cheyenne St., El Reno, OK 73036)

多くのイネ科牧草種において半数体の作出は比較的少ない。しかし，半数体植物の作出は，従来から，遺伝的に固定された自殖系の育成，細胞質置換のための一つの方法，あるいは倍数体のゲノム操作のための潜在的な手法として認識されてきた。本研究は，当初，*Poa arachnifera*×*P. secunda* の交雑による雑種頻度を明らかにするために行われた研究であった。しかしながら，この過程において，雄性発生により，花粉親とした倍数体の *P. secunda* と同様の形質を示し，染色体数変異がある4個体の半数体が得られた。これら半数体のすべては *P. secunda* と同様の繊維状の根系を持つことが本研究によって明らかにされた。この雄性発生による半数体作出は，今後，*P. arachnifera* 細胞質内における *P. secunda* 遺伝子の発現を知ることを可能にするとともに，倍数体の *P. secunda* の遺伝的研究を行うための有効な材料を提供する。

キーワード：種間交雑，半数体，ブルーグラス，*Poa*，雄性発生.