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SHORT COMMUNICATION

High proportion of diploid hybrids produced by interspecific diploid × tetraploid *Sorghum* hybridization

Stan Cox · Pheonah Nabukalu · Andrew H. Paterson · Wenqian Kong · Susan Auckland · Lisa Rainville · Sheila Cox · Shuwen Wang

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Abstract A perennial version of grain sorghum [*S. bicolor* (L.) Moench] would create opportunities for greatly reducing tillage and preventing soil degradation. Efforts to select for perenniality and grain production among progeny of hybrids between *S. bicolor* (2n = 20) and the weedy tetraploid perennial *S. halepense* (L.) Pers. (2n = 40) are complicated in that F_1 hybrids produced by diploid × tetraploid sorghum crosses are usually tetraploid. In 2013, a set of random pollinations between 19 diploid cytoplasmic male-sterile inbred lines and 43 tetraploid perennial plants produced 165 F_1 hybrid plants, more than 75% of which had highly atypical plant, panicle, and seed phenotypes. Phenotypic segregation in F_2 populations derived from atypical hybrids was also anomalous. Examination of mitotic metaphase cells in F_1 or F_2 root tips revealed that 129 of the 165 hybrids were diploid. Parentage of the diploid progenies was confirmed using simple-sequence repeat analysis. The mechanism by which diploid hybrids arise from diploid × tetraploid crosses is unknown, but it may involve either production of monohaploid (n = 10) pollen by the tetraploid parent or chromosome elimination during early cell divisions following formation of the triploid zygote. The ability to produce diploid germplasm segregating for *S. bicolor* and *S. halepense* alleles could have great utility, both for the development of perennial sorghum and for the improvement of conventional grain sorghum.

Keywords Genetic resource · Germplasm enhancement · Sorghum breeding · Ploidy · Perennial · Interspecific hybridization · Chromosome

Introduction

Development of perennial grain sorghum (Paterson et al. 2013; Nabukalu and Cox 2016) would create opportunities for greatly reducing tillage and other aspects of annual grain sorghum cultivation that damage soil structure and lead to erosion. Efforts to develop perennial sorghum germplasm, underway since 2002, involve producing hybrids between *S. bicolor* and the weedy perennial grass *S. halepense* (L.) Pers., selecting for rhizome development and winter survival in segregating populations, backcrossing to *S. bicolor*, and repeating the cycle (Nabukalu and Cox 2016).

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S. Cox (✉) · P. Nabukalu · S. Cox · S. Wang
The Land Institute, 2440 E. Water Well Rd., Salina, KS 67401, USA
e-mail: cox@landinstitute.org

A. H. Paterson · W. Kong · S. Auckland · L. Rainville
Plant Genome Mapping Laboratory, University of Georgia, 111 Riverbend Road, Rm 228, Athens, GA 30605, USA

S. bicolor is diploid with 2n = 20, while *S. halepense* is tetraploid with 2n = 40. Hybridization can be accomplished by using an induced tetraploid line of *S. bicolor* as either the male or the female parent (Piper and Kulakow 1994) or by fertilizing male-sterile diploid *S. bicolor* plants with pollen from perennial tetraploid plants and obtaining 40-chromosome hybrids through the infrequent but consistent production of 20-chromosome gametes by the male-sterile parent (Hadley 1958; Nabukalu and Cox 2016). Either process results in tetraploid populations that may be used in selection and germplasm development. In some cases, triploid hybrids occur, but they produce no seed when self-pollinated or pollinated by *S. bicolor*.

Introgressing useful germplasm from *S. halepense* via tetraploid hybrids presents several disadvantages: it limits the scope of the parental gene pool; the approach to homozygosity with self-pollination is slower than in diploid sorghum; partial sterility is common; and genetic analysis is complicated and difficult (Haldane 1930; Gupta 2007). Perennial sorghum germplasm development will be greatly simplified if alleles for salient traits such as cold tolerance and rhizome development can be introgressed from *S. halepense* directly into diploid grain sorghum.

In published research on controlled hybridization between diploid *S. bicolor* and *S. halepense*, chromosome numbers of hybrids have varied. Hadley (1958) and Sengupta and Weibel (1971) obtained both triploid and tetraploid hybrids. When pollinating thousands of nuclear and cytoplasmic male-sterile panicles with tetraploid plants during 2002–2012 as part of our perennial sorghum germplasm research (Nabukalu and Cox 2016), we obtained hundreds of germinable tetraploid F_1 seeds and only one germinable triploid seed (although we have often observed shriveled, inviable seeds that we assumed were triploid).

Dweikat (2005) reported surprising results from crossing eight nuclear male-sterile (*ms3ms3*) sorghum plants with *S. halepense*. Pollinating an estimated 36,000 florets, he obtained 380 shriveled, inviable seeds (presumably triploids) and two normally developed seeds. One of the plants from a normal seed was found to be diploid. Phenotypic and molecular-marker analysis confirmed the plant's parentage.

In 2014, when we grew out 154 F_1 plants resulting from pollinations between male-sterile grain sorghum inbred lines and perennial tetraploid plants, a large proportion of them were phenotypically very different from diploid × tetraploid F_1 plants we had observed in our nurseries over the previous decade. In 2016, after reading for the first time the report by Dweikat (2005), we formed a hypothesis that the phenotypically novel F_1 plants we had observed in 2014 were diploids produced by diploid × tetraploid pollination, and we began examining chromosome numbers of their F_2 progeny.

Materials and methods

In May, 2013, 43 tetraploid sorghum plants were selected from among the self-pollinated progeny of *S. bicolor* × *S. halepense*, (*S. bicolor* × *S. halepense*) × *S. bicolor*, or (*S. bicolor* × *S. halepense*) × *S. bicolor*^{2} hybrids and were used to pollinate inbred lines of grain sorghum carrying A1-type male-sterile cytoplasm (Suppl. Table 1). The perennial parents were tetraploid sorghum plants selected in the course of developing perennial sorghum germplasm (Suppl. Table 1; Nabukalu and Cox 2016). Nineteen different inbred lines produced F_1 seed. A total of 154 F_1 plants from 55 different parental combinations (Suppl. Table 1) were grown to maturity and harvested. In 2016, 11 remnant F_1 seeds from crosses made in 2013 were removed from storage and germinated, and the seedlings were transplanted in the field. These F_1 seeds had been produced during the same time period as the 154 hybrids discussed above, using the same pool of parents.

In 2016–2017, root tips were obtained from F_2 progeny of each of the 154 F_1 plants grown in 2014, the 11 F_1 plants grown in 2016, and plants derived by self-pollination of the male parents of 2013 crosses. Root tips were fixed and used for determining mitotic metaphase chromosome numbers. The technique was based on procedures reported by Singh (2016) and Sharma and Sharma (2014), with modifications. Slide preparations were examined under a compound light microscope (Axiostar plus; Zeiss, Oberkochem, Germany) with a magnification of 40×. Chromosomes were observed, counted and photographed using AxioCamERc5 s camera and Zen 2 (version 10.0) digital imaging software (Zeiss, Oberkochen, Germany).

Simple-sequence repeat (SSR) genotyping was used to verify the recorded parentages of a sample of the diploid hybrids. Young leaf tissue was obtained from 13 to 26 seedlings belonging to each of nine F_2 populations and from all 11 new F_1 plants that were being grown out that year. Leaf tissue was also sampled from 12 seedlings of each male parent and one seedling of each female parent involved in producing the sampled F_1 and F_2 plants. DNA extraction from leaf samples and SSR genotyping were performed as described by Kong et al. (2013).

Results and discussion

Phenotypically, the 165 F_1 plants grown out in 2014 and 2016 fell into two distinct groups. A minority exhibited traits typically seen in tetraploid hybrids between domesticated sorghum and tetraploid perennial plants. Compared with *S. bicolor*, these hybrids displayed more profuse tillering and upper-node branching, thinner culms, narrower leaves, longer panicle branches, lower seed-set, and smaller seeds. As is typical in such hybrids, seed shattering was delayed or absent. The second group, a majority of the plants, had phenotypes intermediate between the first group of hybrids and *S. bicolor*. They produced fewer tillers and branches than is typical of tetraploid hybrids. They had thicker culms and wider leaves, shorter panicle branches (panicles would have been classified as semi-compact to semi-lax), higher seed-set, and larger seed than had been seen in tetraploid hybrids in previous years. Some displayed early, complete seed shattering.

Somatic chromosome numbers were determined for one random F_2 plant from each of 127 populations that we suspected of being diploid on the basis of their F_1 parents' phenotypes and F_2 segregation, finding that 121 were indeed diploid and six were tetraploid. Fifteen additional plants that we hypothesized to be tetraploid on the basis of phenotype were examined cytologically as controls, and all were tetraploid. Figures 1 and 2 show images of cells at mitotic metaphase from diploid and tetraploid F_2 plants. Chromosome numbers of F_2 plants from an additional 15 populations assumed to be tetraploid on the basis of phenotypic segregation were not confirmed cytologically.

Of the 11 F_1 plants grown in 2016 from remnant seed, we found that eight were diploid and three tetraploid. Adding those counts to those from F_2 plants, we concluded that at least 129 of the 165 F_1 hybrids produced in 2013 had been diploid and the remainder had been tetraploid (Suppl. Table 1).

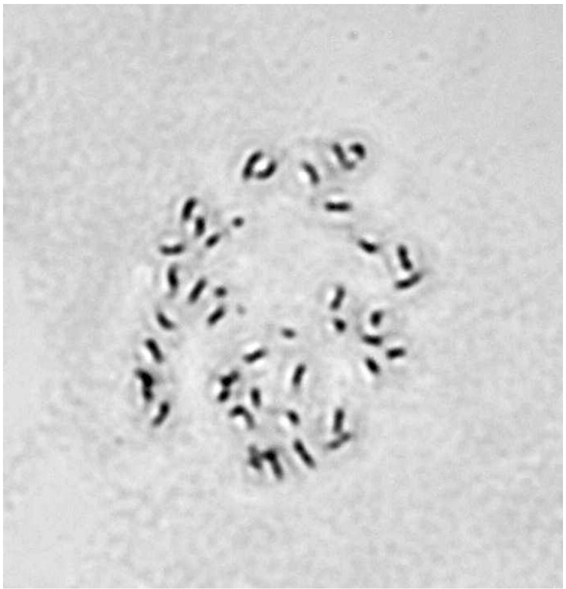

Fig. 1 Nucleus at metaphase in a tetraploid F_2 plant from the cross KS105A ($2n = 20$) × H6-70-8 ($2n = 40$)

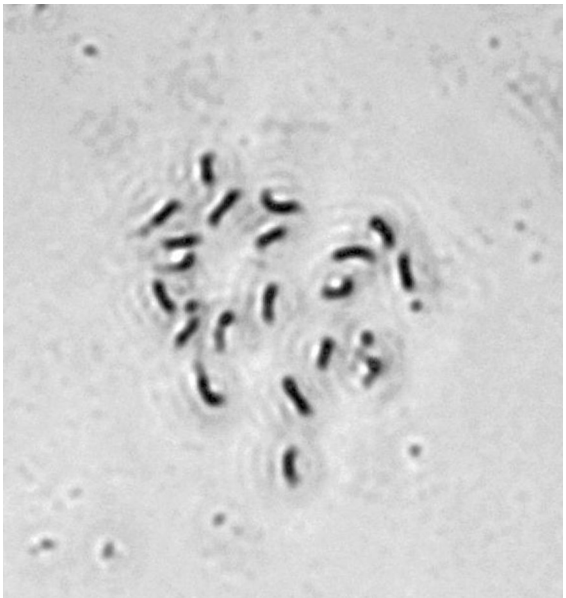

Fig. 2 Nucleus at metaphase in a diploid F_2 plant from the cross KS105A × H6-70-8

From each of nine of the diploid hybrids confirmed by counts of 20 chromosomes at mitotic metaphase, 13–26 F_2 progeny were screened with 20 SSRs previously known to be polymorphic between BTx623 and Gypsum 9E, one sampling each sorghum chromosome arm (Suppl. Table 2). All eight diploid F_1 plants were similarly screened (Supp. Table 3). Among the SSRs, 15 to 18 were informative between the *S. bicolor* and (*S. bicolor*^{n} × *S. halepense*) parents of the nine F_2 progeny arrays. SSR genotypes of F_1 plants and F_2 families were consistent with their recorded parentage. Among the nine F_2 families, the portion of loci with *S. halepense* alleles ranged from 13.9 to 33.2%, with a mean of 25%. No individual progeny completely lacked *S. halepense* alleles, although several had only one. At one locus, *Xiabt-p121*, no *S. halepense* allele was found in any plant; other loci showed *S. halepense* alleles in 1.1–56.3% of individuals, demonstrating that most *S. halepense* chromosome arms can be recovered in such lines. Among F_1 plants, the share of loci with a *S. halepense* allele derived from the male parent ranged from 8.3 to 75.0%, with an overall presence of 25.9%.

The SSR analysis showed that we can recover in diploid x tetraploid hybrids most chromosome arms from the tetraploid parent. Across all loci, the *S. halepense* allele was detected in 26% of F_1 plants and 25% of F_2 plants. Those values are consistent with the 25% expected when the tetraploid male parent used to pollinate a diploid plant carries a single copy of the *S. halepense* allele, and then, as postulated by Dweikat (2005), half of the male parent's chromosomes are eliminated either during pollen formation or after fertilization. Because the male parents we used were derived from repeated backcrossing to *S. bicolor*, it would not be surprising if many *S. halepense* alleles were present as single copies in these parents. More extensive analysis will be required to resolve questions surrounding the transmission of alleles from tetraploid parents to diploid progeny.

The parentage of the diploid F_1 plant produced by Dweikat (2005) contrasts with the parentage of the diploid hybrids reported herein. His hybrid arose from a cross between a nuclear male-sterile (*ms3ms3*) *S. bicolor* plant and a *S. halepense* plant, whereas ours arose from crosses between cytoplasmic male-sterile plants and species-backcross tetraploid plants. Dweikat (2005) hypothesized that his diploid hybrid resulted from either (1) production of a monohaploid ($n = 10$) gamete by the *S. halepense* parent or (2) elimination of chromosomes in early cell division following fertilization. With either mechanism, a central question is whether specific chromosomes were selectively retained or eliminated.

Further studies will be required to determine which (if either) of Dweikat's (2005) proposed mechanisms is the cause of diploid production by such crosses and to explain the unprecedented numbers of diploid hybrids we produced in 2013. More broadly, it would be useful to identify genetic or environmental factors that might be manipulated to increase the likelihood that diploid × tetraploid sorghum crosses will produce diploid hybrids. The diploid *S. bicolor* × *S. halepense* germplasm thus produced could have high utility for the development of perennial sorghum and sorghum improvement in general.

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