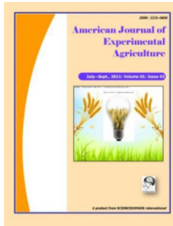

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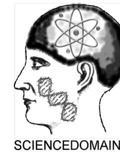

Molecular Characterization of Non-flowering Perennial *Sorghum* spp. Hybrids

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ABSTRACT

Aims: The goal of this study was to characterize recently identified, non-flowering, putative tetraploid *Sorghum* spp. hybrids utilizing bulked segregant analysis with SSRs and compare them to *S. bicolor*, *S. halepense*, and triploid putative *Sorghum* spp. hybrids. Confirmed species hybrids between *S. bicolor* and *S. halepense* would provide resources for investigating risks of invasiveness and transgene escape alongside potential for identifying novel perennial *Sorghum* feedstocks of value.
Study design: Bulked segregant analysis of *Sorghum* species and *S.* species hybrids.
Place and Duration of Study: Department of Soil & Crop Sciences; Texas A&M University; College Station, TX, USA; 2009 - 2011
Methodology: A bulked segregant analysis approach was conducted using SSRs mined from the *S. bicolor* genome sequence. Bulked samples of *S. bicolor*, typical flowering *S. halepense*, non-flowering tetraploid putative *Sorghum spp.* hybrids, and triploid putative *Sorghum spp.* hybrids were surveyed to identify both unique markers specific to each bulk and markers indicative of *S. bicolor* genetic material introgressed into the *Sorghum spp.* hybrids.
Results: Thirty-nine and 23 markers were found to be unique to the *S. bicolor* and typical flowering *S. halepense* bulks, respectively. These unique markers could be utilized in breeding programs to identify interspecific hybrids. A subset of 23 unique SSRs were found in the non-flowering tetraploid putative *Sorghum spp.* hybrid, and they may be useful in the characterization of the non-flowering phenotype.
Conclusion: Markers identified in this study provide: 1) species-specific tools for confirmation of interspecific *Sorghum* spp. hybrids and quantification of gene flow between *Sorghum* spp., 2) candidate gene and genomic region resources for dissection of the non-

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flowering phenotype, and 3) *Sorghum* spp. hybrid-specific markers suitable towards development of perennial biofuel and forage sorghum feedstocks.

Keywords: Sorghum bicolor; Sorghum halepense; Sorghum almum; bulked segregant analysis; SSR; biomass; forage; biofuel.

DEFINITIONS

MAS: marker assisted selection; PCR: polymerase chain reaction; SSR: simple sequence repeat; BSA: bulked segregant analysis.

1. INTRODUCTION

Natural interspecific hybridization is an important process in evolution (Stebbins, 1959; Barton, 2001) and a common event across plant species (Mallet, 2005). Many field crops have been developed from weedy progenitors (Simmonds, 1976; Linder, 1998), and crop-to-weed gene flow poses a significant problem when the crop and weed are sexually compatible and have synchronous flowering times (Doggett, 1988; Arriola and Ellstrand, 1997; Ellstrand et al., 1999). Wild alleles influencing traits such as seed dormancy and germination impact the persistence of crop-wild hybrids and opportunities for introgression of crop genes into wild populations (Linder and Schmitt, 1995). Beyond initial reports of gene flow and hybridization between crop and wild populations, little is known about hybridization frequencies, crop allele effects on weediness, and crop trait persistence in wild populations (Arriola and Ellstrand, 1997; Spencer and Snow, 2001). With increasing global commercialization of transgenic crops improved for herbicide resistance, insect resistance, and other high-value traits, additional gene flow studies are essential towards assessing potential risks associated with transgene escape into wild populations (Tiedje et al., 1989; Ellstrand and Hoffman, 1990). Understanding the frequency and fitness of crop-wild hybrids can accordingly provide baseline information regarding the persistence of such hybrids and the potential for transgene introgression (Ellstrand et al., 1999). As crop-wild hybrids can often be perennial and possess increased vegetative:reproductive sink ratios, however, they are not completely lacking in value and offer intriguing potential towards developing novel biofuel feedstocks with reduced invasiveness, improved agronomic characteristics, and perennial life cycles.

Sorghum species have been utilized worldwide for the production of grain, forage, sugar, and more recently biofuels (Rooney et al., 2007). *Sorghum bicolor* (L.) Moench (2n=2x=20) is the 5th most important grain crop in the world (Doggett, 1988). It is utilized primarily for human consumption throughout Asia, Africa, and Central America and for animal feed in the US, Australia, and South America (ICRISAT, 2011). The subspecies *S. bicolor* ssp. *drummondii* (Nees ex. Steud.) de Wet & Harlan, sudangrass, and hybrids between *S. bicolor* and *S. bicolor* spp. *drummondii* have been used extensively as a source of high biomass forage and hay feedstocks in the US (Armah-Agyeman et al., 2002). *Sorghum* spp. have more recently been evaluated as bioenergy feedstocks, including a grain starch substitute for corn-derived bioethanol, a stem sugar substitute for sugarcane-derived bioethanol, and a dedicated lignocellulosic energy crop (Rooney et al., 2007; Wu et al., 2008; Miller and Ottman, 2010; Sattler et al., 2010). All reports to date concerning *Sorghum* bioenergy

feedstocks have utilized *S. bicolor*, which has an annual growth cycle in temperate climates and exhibits only weak perenniality in tropical and subtropical climates. A perennial biomass feedstock by comparison would offer ecological and environmental benefits such as increased soil organic carbon, reduced soil erosion, reduced fertilizer and pesticide inputs, and a higher net energy return (Costanza et al., 1997; Lewandowski et al., 2003; Kort et al., 1998; McLaughlin and Walsh, 1998; Khanna et al., 2010). Because sorghum is widely adapted and utilized across 588 million acres of range and pasture land and 61.5 million acres of hay land in the US (USDA/RMA, 2011), it could also serve as a dual-use forage:biofuel feedstock in the near-term while biofuel refineries are under construction and markets develop.

Most sorghum species are highly self-pollinated but capable of varied frequencies of outcrossing. As a result, natural interspecific hybridization between culitivated and weedy sorghum spp. is possible. *Sorghum propinquum* (Kunth) Hitchcock (2n=2x=20) is an undomesticated, perennial, rhizomatous relative of *S. bicolor* (de Wet, 1978); however, its limited natural distribution and lack of germplasm in the USDA-ARS National Plant Germplasm System (NPGS) have greatly limited its potential for hybridization to *S. bicolor*. The natural distribution of *S. halepense* (L.) Pers. (2n=4x=40), by comparison, spans throughout Africa, Southern Europe, and Asia (Price et al., 2005; de Wet, 1978). *S. halepense* has also become naturalized and is considered a noxious invasive weed throughout the US (USDA/GRIN, 1992). *S. halepense* possesses one subgenome that is similar to *S. bicolor*'s genome. The common wild sorghum (*S. bicolor* ssp. *arundinaceum* [Desv.] Stapf) is believed to be the progenitor of modern *S. bicolor* (Hadley, 1953; de Wet, 1978; Celarier, 1958). *S. halepense* is theorized to have originated from hybridization between *S. propinquum* and *S. bicolor* ssp. *arundinaceum* followed by chromosome doubling (de Wet, 1978). *S. halepense* has approximately double the DNA content of *S. bicolor* and *S. propinquum,* with all three species having similar haploid chromosome complement sizes (Price et al., 2005). The difference in DNA content between these species is largely due to levels of ploidy, with *S. halepense* being tetraploid (4x) and both *S. bicolor* and *S. propinquum* being diploid (2x). Paterson *et al.* (1995) found that 117 out of 125 RFLP alleles identified in *S. halepense* were accounted for by species-representative germplasm collections of *S. bicolor* and *S. propinquum* collectively. This finding further supports the theory that *S. halepense* is a polyploid containing one subgenome in common with *S. bicolor*.

Overlapping distributions provide significant opportunities for natural hybridization to occur between *S. bicolor* and *S. halepense*. *S. almum* Parodi (2n=2x=40), commonly referred to as 'Columbusgrass', has in fact been theorized to be a naturally occurring hybrid between *S. bicolor* and *S. halepense* Parodi, 1943). This species hybrid was discovered in Argentina and generally has been difficult to morphologically separate it from *S. halepense* (Parodi, 1943; Endrizzi, 1957). Arriola and Ellstrand (1996) determined that natural hybridization occurred when *S. halepense* plants were located up to 100m away from *S. bicolor.* They also determined that hybridization generally increased to approximately 12% as the distance between *S. bicolor* fields and the *S. halepense* stands decreased. This maximum frequency of hybridization was also shown to be near the average frequency, 11%, of hybridization in controlled pollinations under greenhouse conditions. The resulting hybrids also had similar germination rates and seedling vigor to that of the *S. halepense* parents (Arriola and Ellstrand, 1997). Introgression of *S. bicolor* genetic material into *S. halepense* has further been documented from a survey of 77 *S. bicolor* specific alleles on multiple populations of *S. halepense* from the eastern and central United States (Morrell et al., 2005). This survey revealed that *S. bicolor* specific allele frequencies reached 0.134 in *S. halepense*

populations with long term exposure, suggesting that introgression is not only occurring but that hybrid progeny are persistent for long periods of time. This naturally occurring hybridization presents a serious concern for both development of novel crop-wild hybrids and escape of engineered genes such as herbicide resistance from *S. bicolor* into “weedy” *Sorghum* spp. hybrid populations.

Despite these concerns, Columbusgrass hybrids offer a potential germplasm base from which improved perennial sorghum biomass feedstocks can be developed. Controlled hybridization between *S. bicolor* and *S. halepense* has been successful in previous studies with varying results (Bennett and Merwine, 1966; Merwine and Bennett, 1966, Sengupta and Weibel, 1971; Dweikat, 2005). Hybrid fertility varies across ploidy levels, with diploid (2n=2x=20), triploid (2n=3x=30) and tetraploid (2n=4x=40) F_1 hybrids having 90%, 1.1%, and 66% average seed set, respectively (Endrizzi, 1957; Dweikat, 2005). Such hybrids range phenotypically from intermediate between the parent species to indistinguishable from *S. halepense* using characters such as seed production, number of panicles per plant, number of tillers per plant, and above- and belowground biomass production. Evidence that rhizome production and perenniality vary in Columbusgrass populations (Yim and Bayer, 1997) indicates that their potential for invasiveness can be moderated. The development of improved forage Columbusgrass cultivars, such as 'Krish' (Krishnaswamy et al., 1956), 'Crooble' (Davis and Edye, 1959), 'Silk' (CSIRO, 1978a), and 'Sucro' (CSIRO, 1978b), with reduced rhizome production and varied crop life spans reinforce this finding. Seed weed concerns in Columbusgrass could also likely be addressed through breeding and selection efforts focused on non-shattering, delayed flowering, photoperiodism, and genetic sterility mechanisms.

Markers unique to *S. halepense* and *S. bicolor* would be beneficial in identifying Columbusgrass hybrids and determining the amount of gene flow between species. Towards this goal, the recent annotation of the complete *S. bicolor* genome (Paterson et al., 2009) provides an abundant resource from which sequence based DNA markers can be developed. Bulked segregant analysis (BSA) is a technique of quickly identifying markers linked to genomic regions or traits of interest through utilization of segregating populations or collections and phenotypically pooled groups (Michelmore et al., 1991). BSA has been successfully used in numerous species for diverse traits such as disease resistance, freezing tolerance, and apomixis (Rémus-Borel et al., 2010; Fondevilla et al., 2008; Singh et al., 2006; Boora et al., 1999; Dwivedi et al., 2007). Beyond genetic mapping of traits, BSA also offers potential towards identifying species-specific genomic regions in interspecific hybrids. The objective of this research was to characterize recently identified, non-flowering, putative *Sorghum* spp. hybrids utilizing BSA and to compare the hybrids to *S. bicolor*, *S. halepense*, and putative triploid *Sorghum* spp. hybrids.

2. MATERIALS AND METHODS

2.1 Plant Materials

Ten parental lines of diploid *S. bicolor* with diverse genetic backgrounds were obtained from the USDA-ARS NPGS (Table 1). Nine genotypes of flowering, tetraploid *S. halepense* were collected: seven genotypes were collected within TX; one genotype was collected in NC; and one unidentified genotype was attained from the USDA NPGS (Table 1). Three tetraploid putative *Sorghum* spp. hybrids that did not flower either under field and greenhouse conditions between 2009 and 2011, or under short-day (8 hr light: 16 hr dark) growth

chamber conditions at College Station, TX were included. Four flowering, triploid putative *Sorghum* spp. hybrids were also included (Table 1).

Table 1: Plant accessions used in bulked segregant analysis and their origins

ID	Origin	Bulk
09NC01	Collected: 36.33521° N, 80.79339° W	*S. halepense* (Flowering)
09TX01	Collected: 33.39121° N, 96.91875° W	*S. halepense* (Flowering)
09TX03	Collected: 30.54664° N, 96.43741° W	*S. halepense* (Flowering)
09TX04	Collected: 30.54606° N, 96.43741° W	*S. halepense* (Flowering)
09TX06	Collected: 30.63848° N, 96.45306° W	*S. halepense* (Flowering)
09TX07	Collected: 30.21450° N, 97.13771° W	*S. halepense* (Flowering)
09TX08	Collected: 30.03131° N, 97.30483° W	*S. halepense* (Flowering)
09TX09	Collected: 30.11890° N, 97.33909° W	*S. halepense* (Flowering)
09TX20	PI 271615 (Country of origin : India)	*S. halepense* (Flowering)
09TX13	Collected: 29.90665° N, 96.91128° W	*Sorghum spp.* (4x: Non-flowering)
09TX14	Collected: 29.90665° N, 96.91128° W	*Sorghum spp.* (4x: Non-flowering)
09TX15	Collected: 29.90665° N, 96.91128° W	*Sorghum spp.* (4x: Non-flowering)
09TX02	Collected: 30.54608° N, 96.43812° W	*Sorghum spp.* (3x: Flowering)
10TX01	Collected: 30.54276° N, 96.43402° W	*Sorghum spp.* (3x: Flowering)
10TX02	Collected: 30.54170° N, 96.43327° W	*Sorghum spp.* (3x: Flowering)
10TX03	Collected: 30.54758° N, 96.44058° W	*Sorghum spp.* (3x: Flowering)
09TX21	PI 598091	*S. bicolor*
09TX22	PI 598093	*S. bicolor*
09TX23	PI 598094	*S. bicolor*
09TX24	PI 598097	*S. bicolor*
09TX25	PI 598105	*S. bicolor*
09TX26	PI 598106	*S. bicolor*
09TX27	PI 598109	*S. bicolor*
09TX28	PI 598113	*S. bicolor*
09TX29	PI 598116	*S. bicolor*
09TX30	PI 598118	*S. bicolor*

2.2 Flow Cytometry

The ploidy level of all plant materials, other than the *S. bicolor* accessions, was assayed using flow cytometry. The chromosome number of a tetraploid *S. halepense* genotype (2n=4x=40) was verified by counting the chromosomes in its root tips and it was used as an internal standard. Leaf material from both the standard individual accessions was aseptically collected, kept on ice, and macerated using a standard razor blade in 0.25 mL of Galbraith's buffer in a Petri dish. An additional 1.0 mL of Galbraith's buffer was added and the material was then strained through a filter into a 2.0 mL microtube. Fifty µL of propidium iodide was added to each microtube and allowed to incubate for 15 minutes in a covered ice chest. The sample solutions were then analyzed for DNA content using a Partec CyFlow Counter (Partec GmbH, Münster, Germany), with a minimum of 3,000 particles assayed for each sample. Three replications were completed for each accession.

2.3 SSR Development

The Simple Sequence Repeat (SSR) markers to be used in the BSA were developed from the sequenced *S. bicolor* genome located online at http://www.phytozome.net. SSRs were identified and primer sequences designed using the SSRLocator software package located online at *http://www.ufpel.edu.br/faem/fitotecnia/fitomelhoramento/faleconosco.html*. The marker sequences ranged in size from 100-400 base pairs. Regions previously associated with "weedy" attributes such as rhizome production and length, seed shattering, basal tillering, and regrowth as described by Paterson et al. (1995) were saturated more so, by density, with markers than the rest of the genome. Markers outside of the "weedy" regions were located approximately every 2.5 million base pairs and within the regions the markers occur as frequently as every 200,000 base pairs.

2.4 DNA Isolation

Genomic DNA was isolated following a modified Aljanabi and Martinez (1997) protocol. Four hundred µL of homogenizing buffer (0.4 M NaCl, 10 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0) and 100 mg of fresh leaf tissue were added to 1.7 mL microtubes. Forty µL of 20% SDS and 8 µL of 20 mg mL-1 proteinase K were added and vortexed for 5 sec. Following incubation in a water bath at 65° C for 1 h, 300 µL of NaCl saturated H_2O was added and vortexed for 30 sec. Samples were spun at 12,000 rpm for 10 min, supernatant was transferred to new tubes, samples were spun at 12,000 rpm for 20 min, and supernatant was transferred to new tubes without disturbing any remaining pellet. Following the addition of 800 µL of cold isopropanol and 20 gentle inversions, samples were incubated at -20° C for 1 h. Samples were spun at 10,000 rpm for 5 min, supernatant was removed, 500 µL of cold 70% ethanol was added, samples were spun at 10,000 rpm for 5 min, and supernatant was removed. Microtubes were inverted until dry, and DNA was resuspended in 100 µL of sterile ddH_2O.

2.5 Bulked Segregant Analysis

The DNA concentrations of all samples were quantified using a spectrophotometer (Eppendorf, Hamburg, Germany). The sample bulks were as follows: *S. bicolor* accesions, flowering *S. halepense* genotypes, non-flowering putative *Sorghum* spp. hybrids, and flowering putative triploid *Sorghum* spp. hybrids. Each genotype was represented equally within its respective bulk and the final DNA concentration of each bulk was 50 ng μL^{-1}. Once the bulks were made, 329 SSR primers were surveyed across all bulks. Each PCR reaction consisted of 11.8 µL of H_2O, 2 µL of 25 mM $MgCl_2$, 2 µL of 10X reaction buffer, 1 µL of 50 ng μL^{-1} bulk template DNA, 1 µL of 25mM deoxynucleoside triphosphates (dNTPs), 0.2 µL of 5 U μL^{-1} *Taq* DNA polymerase, 1 µL of the 40 mM forward primer, and 1 µL of the 40 mM reverse primer. The PCR conditions were as follows: initial denaturation of 95℃ for 3 min; 40 cycles of 95℃ for 25 s, 55℃ for 25 s, and 70℃ for 45 s; and a final extension of 72℃ for 10 min. Amplification products were separated by electrophoresis using a MEGA-GEL (C.B.S. Scientific, Del Mar, CA) high-throughput vertical unit and nondenaturing gels with final concentrations of 6% acrylamide, 0.5X TBE (tris-borate-EDTA) Buffer, 0.07% ammonium persulfate, and 0.08% TEMED (Tetramethylethylenediamine) as described by Wang et al. (2003). Gels were stained with 50 µL of 10 mg mL^{-1} ethidium bromide. Gels were then scored for the presence or absence of allele bands according to the procedure set forth in Rodriguez et al. (2001). Cluster and ordination analysis were performed using NTSYS-pc version 2.0 (Rohlf, 1997). Similarity coefficients were calculated using Jaccard's

coefficient, SJ=a/(a+u), where a is the number of bands in which the two operational taxonomic units (OTUs) agree and u the number of bands present in one OTU but absent in the other (Jaccard, 1908) with the SIMQUAL function. Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) algorithm within the SAHN function.

3. RESULTS AND DISCUSSION

Results from flow cytometry analyses were in agreement with phenotypic assessments of all accessions. The ten *S. bicolor* accessions were diploid, the nine flowering *S. halepense* and three non-flowering *Sorghum* spp. hybrids were tetraploid, and the four flowering *Sorghum* spp. hybrids were triploid. These findings provided supporting evidence that the flowering putative triploid *Sorghum* spp. hybrids are Columbusgrass.

A total of 155 unique SSRs were surveyed. Four SSRs were discarded due to PCR amplification failure and were considered primer design errors. Three additional SSRs were discarded because of smeared PCR amplification products and were likely high-copy loci. The remaining 148 SSRs produced a total of 330 scorable alleles, and both unique and overlapping associations of these SSR alleles were obtained across the four bulks (Figure 1).

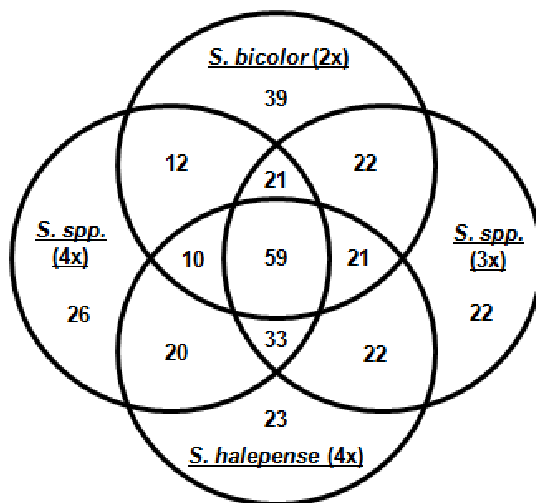

Figure 1: Venn diagram showing unique and overlapping SSR associations across *Sorghum* spp. and *Sorghum* spp. hybrid bulks.

Clustering analyses revealed similar yet distinct genetic relationships between the bulks as graphically represented in a dendrogram (Figure 2). Both the triploid and non-flowering, tetraploid *Sorghum* spp. hybrid bulks had closer relationships with the *S. halepense* bulk than did the *S. bicolor* bulk, providing evidence that accessions in both bulks are interspecific hybrids between *S. bicolor* and *S. halepense* as suspected. Assuming the triploid *Sorghum* spp. hybrid bulk consisted of true (n+n) interspecific hybrids between *S. bicolor* and *S. halepense*, approximately 66.67% similarity would be expected and reflect the two haploid genome complements from the *S. halepense* parent and one haploid genome complement from the *S. bicolor* parent. The highest degree of similarity was observed between the

flowering *S. halepense* and non-flowering, tetraploid putative *Sorghum* spp. hybrid bulks. This comparatively close relationship supports phenotypic similarities between these bulks. Interestingly, it also suggests that the non-flowering, tetraploid Sorghum spp. bulk accessions are likely backcross derivatives involving *S. halepense* following interspecific *S. halepense* x *S. bicolor* hybridization. Unreduced maternal gamete (2n+n) derived F_1 progeny from *S. bicolor* x *S. halepense* hybridization or unreduced paternal gamete (n+2n) derived F_1 progeny from *S. halepense* x *S. bicolor* hybridization, in contrast, would both be expected to have had an even more distant relationship to the *S. halepense* bulk than the triploid *Sorghum spp.* bulk. These results confirmed that the non-flowering, tetraploid putative *Sorghum* spp. hybrids were also Columbusgrass.

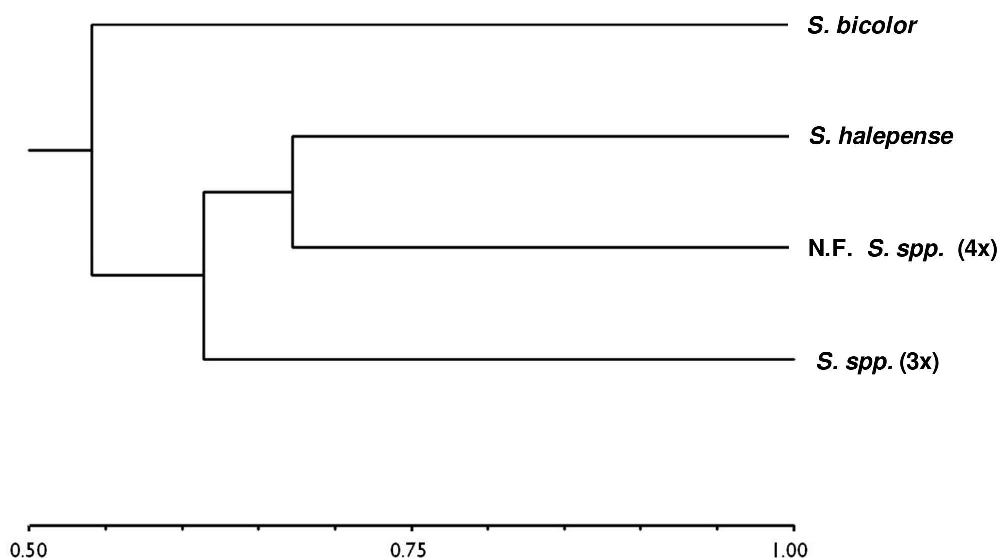

Figure 2: Dendrogram showing relatedness between bulks. *S. bicolor* is the diploid *S. bicolor* bulk, *S. halepense* is the typical *S. halepense* bulk, N.F. *Sorghum* spp. is the tetraploid non-flowering *Sorghum* spp. bulk, and *Sorghum* spp. (3x) is the triploid *Sorghum* spp. bulk.

Pairwise genetic similarity coefficients paralleled clustering results closely (Table 2). Typical *S. halepense* and non-flowering, tetraploid *Sorghum spp.* bulks had genetic similarities of 0.5265 and 0.4983 with the *S. bicolor* bulk, respectively. These values are near the 0.5 value that would be expected if *S. halepense* is an allotetraploid with the *S. bicolor* genome representing half of its genetic material (Hadley, 1953; Clearier, 1958; de Wet, 1978). The triploid *Sorghum spp.* hybrid bulk had a pairwise similarity value of 0.6724 with the typical *S. halepense* bulk, which is near the expected value of 0.6667 expected if the hybrids were true (n+n) F_1 hybrids between *S. bicolor* and *S. halepense*. The typical *S. halepense* and non-flowering, tetraploid *Sorghum spp.* bulks had a similarity value of 0.6296, which is much lower than would be expected if they were both *S. halepense*. The lower than expected value strongly indicates that the non-flowering, tetraploid *Sorghum spp.* bulk accessions have introgressed genetic material from *S. bicolor*. The similarity coefficients for both triploid and non-flowering, tetraploid *Sorghum* spp. bulks compared to *S. halepense* also indicate the possibility that these accessions are backcross derivatives involving *S. halepense*. At least a portion of the departures from the expected values may also be explained by bulks

consisting of fewer genotypes as compared to previous BSA's, where in some cases over 40 genotypes were used to create a single bulked sample (Michelmore et al., 1991; Singh et al., 2006; Fondevilla et al., 2008; Remus-Borel et al., 2010). A larger quantity of heterogenic genotypes in future studies would increase the possibility of identifying a higher number of alleles unique to specific bulks and clearer delineation of syntenic vs. non-syntenic genomic regions between bulks.

Table 2: Pairwise genetic similarity coefficients for *Sorghum* spp. and Sorghum spp. hybrid bulks

	S. bicolor	***S. halepense***	**N.F. *Sorghum spp.* (4x)**	***Sorghum spp.* (3x)**
S. bicolor	1.0000			
S. halepense	0.5265	1.0000		
N.F. *Sorghum spp.* (4x)	0.4983	0.6296	1.0000	
Sorghum spp. (3x)	0.5979	0.6724	0.5987	1.0000

The SSR markers unique to the *S. bicolor* and *S. halepense* bulks in this study (Figure 1) have utility for utilization in future studies as marker-assisted hybrid verification tools in lieu of flow cytometry. The 23 markers that were unique to the non-flowering, tetraploid *Sorghum spp.* bulk can serve as a baseline for candidate genomic regions potentially involved in the non-flowering phenotype. The 12 markers found in both the *S. bicolor* and non-flowering, tetraploid *Sorghum spp.* bulks may similarly serve as an additional resource for selecting genomic regions potentially involved in the *S. halepense* non-flowering phenotype.

4. CONCLUSION

BSA of *S. bicolor* SSRs effectively identified species-specific alleles in *S. bicolor* and *S. halepense*, as well as confirmed that the putative *Sorghum spp.* hybrids in this study were derived from natural interspecific hybridization between *S. bicolor* and *S. halepense*. These methods confirmed the hybrids in this study are in fact Columbusgrass. Marker-assisted hybrid verification is therefore feasible with these tools and will facilitate future Columbusgrass breeding efforts. BSA also indicated that the triploid hybrids are F_1 hybrids, while the non-flowering tetraploid hybrids are backcross derivatives involving *S. halepense*. These findings will enhance Columbusgrass breeding strategies that incorporate the non-flowering trait identified in this study. Future dissection of the genetics underlying this trait offers potential for novel sources of floral suppression. Whether the mechanism suppressing flowering in these hybrids is controlled by novel alleles or altered expression of previously characterized genes in floral induction pathways, its characterization and exploitation offers great potential towards designing environmentally benign transgenic sorghums as well as perennial biofuel sorghums with greatly reduced invasiveness risks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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