Wide Hybridization and Utilization of Wild Relatives of Sorghum

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Contents

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Abstract

The process of wide hybridization refers to hybrids created through interspecific or intergeneric crosses of related species to extract useful and novel traits that protect or enhance the yield or quality of the domesticated crop. *Sorghum* contains approximately 25 recognized species that show significant variation in plant morphology, genetic and genomic diversity with an eightfold range in DNA content, and geographic distribution. Traits that increase the value of sorghum production have been reported in many of these species including resistance to sorghum midge, shootfly, and spotted stem borer. However, introgression of any traits has only been possible with species in the section *Eusorghum* due to pre- and post-fertilization barriers that isolate the other species. Now the creation of wide hybrids has been expanded beyond section *Eusorghum*. The *Inhibition of Alien Pollen* (*Iap*) gene that makes it possible to overcome pre-fertilization barriers by reducing adverse pollen–pistil interactions has been used to produce additional interspecific hybrids with species from sections *Chaetosorghum*, *Parasorghum*, and *Stiposorghum*. Post-fertilization barriers can be eliminated through embryo rescue techniques and the use of 2*n* gametes. Using 2*n* gametes as a vehicle to transfer genes by creating bridges that overcome ploidy and genomic differences between species is now being explored. With the chemical hybridizing agent trifluoromethanesulfonamide (TFMSA) the number of parental combinations and the number of florets that are emasculated are no longer limiting factors when developing strategies for creating wide hybrids. Accessing via wide hybridization novel traits that were previously unavailable is now possible.

Keywords

Cytogenetics · Cytological analysis · Cytometry · Embryo rescue · Eusorghum · Pollen–pistil interaction

1 Introduction

Successful breeding relies on genetic variability from which traits of agronomic importance are selected. Breeders access this variability from domestic lines, landraces, weedy accessions, and if variability is absent, from other species. Wild relatives have been exposed to biotic and abiotic stresses for a very long time and have acquired a full range of genetic traits that have ensured their survival. By comparison domesticated crops are fairly new, are usually derived from genetically restricted isolates and lack the range of traits found in its wild relatives (Harlan 1976). Use of these wild relatives therefore has the effect of increasing genetic diversity in the domestic crop. Species that have been isolated either by genetic incompatibility or geographic isolation can add diversity that was not previously

available (Dwivedi et al. 2008; Dempewolf et al. 2017). However, many useful traits documented in wild species have yet to be introgressed into their crop relatives due to barriers that inhibit the transfer.

Wide hybridization refers to hybrids created through interspecific or intergeneric hybridization of distantly related species in an attempt to extract useful and novel traits that protect or enhance the yield or quality of the domesticated crop. The benefits of wide hybridization have been recognized for at least a century (Vavilov 1938). While Vavilov recognized intraspecific hybridization as the principal means of crop improvement, he also recognized that interspecific and intergeneric hybrids could potentially contribute important traits that enhance resistance to biotic and abiotic stresses. As a wheat breeder, he was especially interested in its wild and weedy relatives, *Aegilops, Secale, Haynodia* and *Agropyrum* (Vavilov 1949/1950). In the past 40 years, the introgression of alleles from wild relatives has accelerated. These alleles from wild relatives condition disease and pest resistance, adaptation to a wider range of growing conditions, and improved quality and yield (Harlan 1976). Among the major domesticated crops, wheat, rice, potato, and tomato breeders have established successful programs focused on exploiting beneficial traits from related species (Hajjar and Hodgkin 2007; Dwivedi et al. 2008; Dempewolf et al. 2017).

Over 80% of the traits introgressed from related species into cultivated crops are for disease and pest resistance. This may reflect the limited pool of effective resistance genes within the crop while very high levels may be available in its wild relatives (Hajjar and Hodgkin 2007). One of the first documented examples of the benefit of an introgressed trait was to address the Irish potato blight famine of 1846-1851 in Europe. The famine was a direct result of susceptibility of the potato (*Solanum tuberosum* L.) to *Phytophthora infestans* (Mont.) de Bary (Salaman 1985). Resistance was initially introgressed from a wild Mexican species *Solanum demissum* Lindl. (Singh et al. 1993). Modern sugarcane cultivars are interspecific hybrids of *Saccharum officinarum* L. and *S. spontaneum* L. (Berding and Roach 1987). *S. spontaneum* is the source of disease resistance and vigor while *S. officinarum* provides high-quality sugar traits. Prescott-Allen and Prescott-Allen (1986) and Dwivedi et al. (2008) have listed many examples of wide hybridization and introgression in wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and other crops wherein resistance to pests and environmental stresses have been improved and agronomic potential and quality have been enhanced. Resistance to bacterial blight (*Xanthimonas oryzae pv. oryzae*) in rice was transferred from *Oryza longistaminata* A. Chev. & Roehr. (Brar and Khush 1997) and brown plant hopper (*Nilaparvata lugens* (Stål) resistance was derived from *O. officinalis* Wall. Ex Watt (Jena and Khush 1990). Resistance to corn leaf blight (*Cochliobolus heterostrophus* Drechsler) in maize was introgressed from *Tripsacum dactyloides* (L.) L. (Goodman et al. 1987) and hessian fly (*Mayetiola destructor* Say) resistance present in goatgrass, *Triticum tauschii* (Coss.) Schmalh. was transferred to wheat *T. aestivum* (Cox et al. 1994). Goatgrass was also a source of drought tolerance for wheat (Gororo et al. 2002). Sources of cytoplasmic male sterility have been transferred to rice from *O. rufipogon* Griff. (Hoan et al. 1997).

The introgression of desirable traits can be difficult due to pre- and post-fertilization barriers that isolate the species. These barriers may exist in any part of the reproductive cycle including pollen–pistil incompatibilities, lack of fertilization, endosperm failure, embryo abortion, seedling lethality, hybrid sterility, and linkage drag (Stebbins 1958; Price et al. 2005a). Methods used to overcome barriers include ploidy manipulation, crossability traits, somatic hybridizations, and genetic engineering.

Formal taxonomic descriptions have been useful for providing a framework for classification of plants into related groups, but taxonomy is sometimes less useful in terms of classification of species for their potential utility for crop improvement. To address this issue, Harlan and de Wet (1971) described a simple pragmatic system using taxonomic classification for defining relationships of wild relatives and related species for their potential use to breeders. Three informal gene pool classifications (primary, secondary, and tertiary) are based on ease of hybridization and the potential for introgression with the domesticated species. The primary gene pool (GP-1) includes cultivated, wild, and weedy types of the biological species which are easily hybridized, produce fertile progeny, and have good allelic recombination. The secondary gene pool (GP-2) consists of species that will hybridize with the crop where gene transfer is possible but barriers must be overcome. Sterility issues, poor chromosome pairing, or weak hybrids are difficulties that are commonly encountered in the GP-2 pool. The tertiary gene pool (GP-3) includes the outer extremes of the related genera and or species (Harlan and de Wet 1971). Hybrids of these species with the domesticated type may be recovered but they are usually sterile or do not survive to maturity. Further processes such as embryo culture, chromosome doubling, or the use of a bridge species are usually necessary to move beyond the hybrid generation.

Sorghum (*Sorghum bicolor* L. Moench) has a broad genetic base that has been made more accessible through several systematic introgression approaches. One approach has been to convert tropical photoperiod-sensitive sorghums to photoperiod insensitive types. In 1963, a continuing program was initiated to provide breeders in the temperate zone environments greater access to this genetic base (Stephens et al. 1967). Recently, a method to effectively introgress this allelic diversity into elite breeding material has been described (Jordan et al. 2011). For these approaches, sorghum breeders have relied almost exclusively on the primary gene pool (GP-1) for allelic diversity (Duncan et al. 1991; Rosenow and Dahlberg 2000). There has been interest in accessing the secondary gene pool (GP-2) (*S. halepense* (L.) Pers., *S. propinquum* (Kunth) Hitch., and *Sorghum* × *almum* Parodi) but success in this case has been modest (Price et al. 2006). Finally, to date, no traits have been introgressed from the tertiary gene pool (GP-3). Within that context, this chapter presents the taxonomic status, traits of utility present, and factors that influence the success of interspecific and intergeneric hybridization in *Sorghum*.

2 *Sorghum* Genus

2.1 Species and Distribution

Sorghum L. Moench contains approximately 25 recognized species that show significant variation in plant morphology, genetic diversity, and geographic distribution. The genus is separated into five taxonomic subsections based upon node, panicle, and spikelet morphology. *Eusorghum* (containing the domesticated, progenitor, and weedy GP-1 and GP-2 species), *Chaetosorghum*, *Heterosorghum*, *Parasorghum*, and *Stiposorghum* that contain the undomesticated GP-3 species (Garber 1950; Lazarides et al. 1991).

The *Eusorghum* include the cultivated species and their closest wild relatives: *Sorghum bicolor* subsp. *bicolor*, *S. almum* Parodi, *S. bicolor* subsp. *verticilliflorum* (Steud.) de Wet ex Wiersema and J. Dahlb (a progenitor of cultivated sorghum), *S. bicolor* subsp. *drummondii* (Steud.) de Wet ex Davidse, the widespread weedy species *S. halepense* (L.) Pers. and *S. propinquum* (Kunth) Hitchc. The *Eusorghum* originate from Africa and Asia and are $2n = 20$ or 40 chromosomes (Table 1) (de Wet and Harlan 1971; Doggett 1988; Duvall and Doebley 1990; Price et al. 2005b).

The monotypic sections *Chaetosorghum* and *Heterosorghum* contain *S. macrospermum* E. D. Garber and *S. laxiflorum* F. M. Bailey with the former endemic to a small area of the Northern Territory and the latter native to northern Australia and Papua New Guinea. Both species have $2n = 40$ chromosomes (Table 1) (Garber 1950; Lazarides et al. 1991; Price et al. 2005b). The *Parasorghum* section consists of seven species: *S. grande* Lazarides, *S. leiocladum* (Hack.) C. E. Hubb., *S. matarankense* E. D. Garber and Snyder, *S. nitidum* (Vahl) Pers., *S. purpureosericeum* (Hochst. ex. A. Rich.) Asch. and Schweinf., *S. timorense* (Kunth) Buse, and *S. versicolor* Andersson. These species vary in ploidy from $2n = 10$ or 20, and are native to northern monsoonal Australia, Africa, and Asia (Table 1) (Garber 1950; Lazarides et al. 1991; Phillips 1995; Price et al. 2005b).

Section *Stiposorghum* (Table 1) contains ten species that range in ploidy from $2n = 10$, 20, 30 or 40, with all endemic to northern Australia: *Sorghum amplum* Lazarides, *S. angustum* S. T. Blake, *S. brachypodum* Lazarides, *S. bulbosum Lazarides*, *S. ecarinatum* Lazarides, *S. exstans* Lazarides, *S. interjectum* Lazarides, *S. intrans* F. Muell. Ex Benth., *S. plumosum* (R. Br.) P. Beauv., and *S. stipoideum* (Ewart and Jean White) C. A. Gardner and C. E. Hubb (Garber 1950; Lazarides et al. 1991; Price et al. 2005b).

The geographic distribution of *Sorghum* species, which span a wide range of environments and climatic conditions, is shown in Fig. 1. These distributions show the natural geographic origin of species, and do not include the cultivation areas or the non-native distributions of the weedy species. Most of the tertiary gene pool species are native or endemic to Australia (Lazarides et al. 1991). The natural environments and climatic conditions where *Sorghum* species inhabit have imposed abiotic and biotic stresses that have resulted in a range of traits that could potentially be used to improve the production of cultivated sorghum. Wild sorghums are

Table 1 Sorghum taxonomy, gene pool, and DNA content (Lazarides et al. 1991; Price et al. 2005b; Dillon et al. 2007a)

				DNA (pg)		2*n* chromosome #	
Species	Section	Life form	Gene pool	2C DNA	$X = 5$ genome	Lazarides et al. (1991)	Price et al. (2005 b)
S. bicolor	Eusorghum	Annual	GP-1	1.67	0.42	NA	20
S. almum	Eusorghum	Perennial	GP-2	NA	NA	NA	NA
S. halepense	Eusorghum	Perennial	GP-2	3.28	0.41	NA	40
S. propinquum	Eusorghum	Perennial	GP-2	1.52	0.38	NA	20
S. macrospermum	Chaetosorghum	Annual	GP-3	2.07	0.26	40	40
S. laxiflorum	Heterosorghum	Annual	GP-3	2.49	0.31	40	40
S. grande	Parasorghum	Perennial	GP-3	NA	NA	30,40	NA
S. leiocladum	Parasorghum	Perennial	GP-3	4.6	2.30	20	10
S. matarankense	Parasorghum	Annual	GP-3	2.51	1.26	10	10
S. nitidum	Parasorghum	Perennial	GP-3	8.79	2.20	10,20	20
S. purpureosericeum	Parasorghum	Annual	GP-3	4.18	2.09	10	10
S. timorense	Parasorghum	Annual	GP-3	1.27	0.64	10,20	10
S. versicolor	Parasorghum	Annual	GP-3	3.25	1.62	NA	10
S. versicolor	Parasorghum	Annual	GP-3	6.67	1.67	NA	20
S. amplum	Stiposorghum	Annual	GP-3	7.69	1.28	10	30
S. angustum	Stiposorghum	Annual	GP-3	3.70	1.85	10	10
S. brachypodum	Stiposorghum	Annual	GP-3	3.36	1.68	10	10
S. bulbosum	Stiposorghum	Annual	GP-3	2.30	1.15	10	10
S. ecarinatum	Stiposorghum	Annual	GP-3	2.10	1.05	10	10
S. exstans	Stiposorghum	Annual	GP-3	2.75	1.38	10	10
S. interjectum	Stiposorghum	Perennial	GP-3	7.29	1.22	30	30
S. intrans	Stiposorghum	Annual	GP-3	2.28	1.14	10	10
S. plumosum	Stiposorghum	Perennial	GP-3	7.65	1.28	10,20,30	30
S. plumosum	Stiposorghum	Perennial	GP-3	10.30	1.29	10,20,30	40
S. stipoideum	Stiposorghum	Annual	GP-3	2.19	1.10	10	10

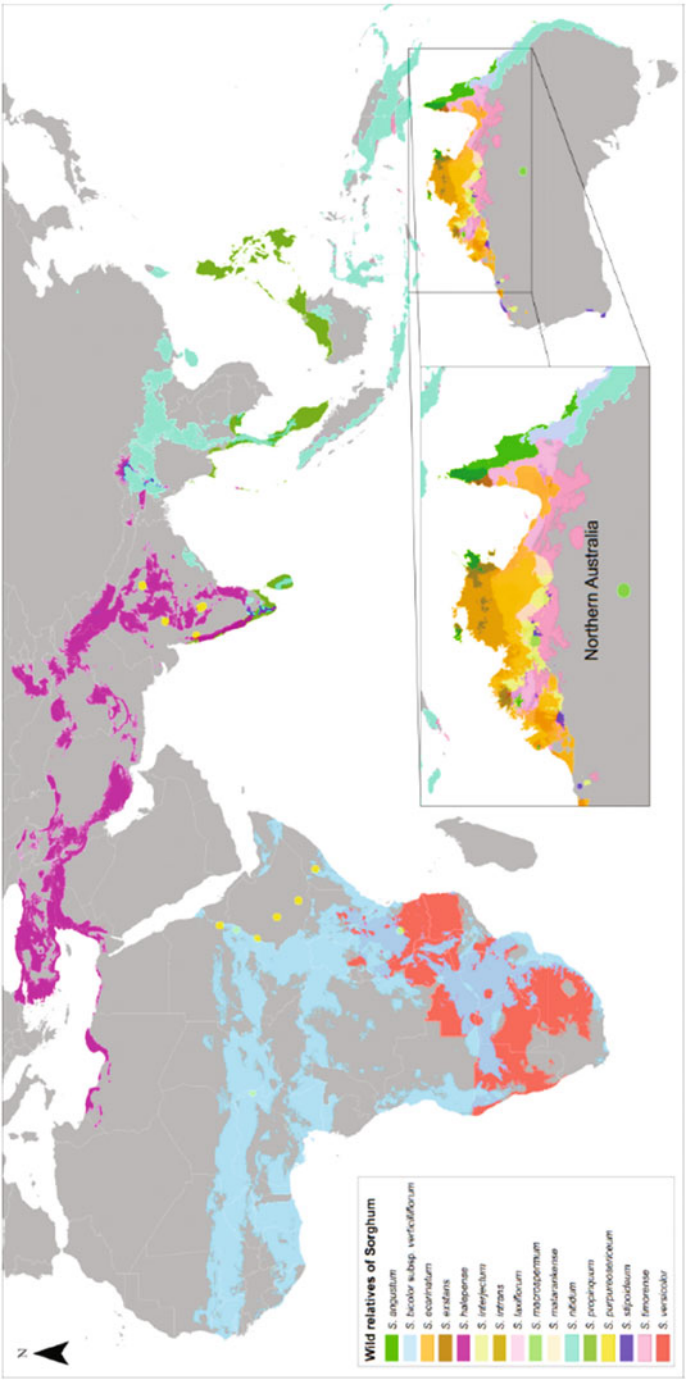

Fig. 1 Global distribution of wild sorghum species, showing Australia as the origin for most of tertiary genepool species. Figure shows natural distribution and does not include cultivated non-native distributions of the weedy species (Shapter et al. 2018)

established across diverse microenvironments with variable soil and moisture conditions, including very hot, dry, nutrient-limited environments, and have a strong ability to adapt and survive. Many of the wild *Sorghum* species have developed resistance to the suite of pests and diseases that affect global sorghum grain production. Many Australian wild species contain resistance to the major pest/diseases of Africa and America, which are not yet present within Australia that are yet to be exploited by plant breeders (Bapat and Mote 1982; Franzmann and Hardy 1996; Kamala et al. 2002; Komolong et al. 2002; Sharma and Franzmann 2001).

A number of studies have been undertaken to determine the genetic relationships among *Sorghum* species using either cytology (see next section) or molecular techniques. The majority of molecular studies have identified two major clades in *Sorghum*, one containing the *Eu/Chaeto/Heterosorghum* and the second consisting of the *Para/Stiposorghum* species (Duvall and Doebley 1990; Sun et al. 1994; Spangler et al. 1999; Dillon et al. 2004, 2007a; Ng'uni et al. 2010; Liu et al. 2014; Hawkins et al. 2015). Most of these studies included a limited number of non-*Sorghum* taxa for comparison and as such had insufficient resolving power to evaluate the infrageneric relationships and monophyly of the genus.

The most recent study of genetic relationships among *Sorghum* species used sequence data from eight low copy number nuclear loci and confirmed the two distinct clades within *Sorghum* as the *Eu/Chaeto/Heterosorghum* and the second consisting of the *Para/Stiposorghum* species, with the genus again identified as polyphyletic in origin (Hawkins et al. 2015). This study was able to show the hybridization and polyploidization events that produced the *Eusorghum* species recognized today. The resolving power of the sequences used was also able to show the clear genome-specific association of the orthologous polyploid alleles of *S. macrospermum* and *S. laxiflorum*, the two members of *Chaeto/Heterosorghum* (Hawkins et al. 2015). The second clade was strongly resolved of *Para/Stiposorghum* species; however, the infrageneric relationships among the species were difficult to delineate but followed similar clustering to previous studies (Dillon et al. 2007a; Hawkins et al. 2015). Additional analysis by Hawkins et al. (2015) including a wide range of Andropogoneae taxa explored the infrageneric relationships between *Sorghum* and closely related genera and confirmed the two distinct clades and the polyphyletic nature of *Sorghum*. The first clade contained the *Eu/Chaeto/Heterosorghum*, confirming the close relationships between these species. The second strongly resolved clade encompassed the *Para/Stiposorghum* and included a basal sister sub-clade of *Miscanthus* and *Saccharum*. The inclusion of these species into this *Sorghum* clade provides support to the proposal of Spangler (2003) for the reclassification of the *Para/Stiposorghum* species into the distinct genus *Sarga*.

Saccharum, Miscanthus, and *Erianthus* are within the *Saccharum* complex, an interspecific breeding group within Andropogoneae tribe, with *Sorghum* considered to be one of the closest relatives of this complex (Dillon et al. 2007b; Hodnett et al. 2010; Kim et al. 2014). Within Andropogoneae, the divergence of the Saccharinae-Sorghinae occurred c. 5.4 million years ago (MYA), with the *Miscanthus-Saccharum* polyploidization event c. 3.8 MYA, and the divergence of

Miscanthus-Saccharum c. 3.1 MYA (Kim et al. 2014). Genome analysis shows that whole-genome duplication is shared by *Miscanthus* and *Saccharum*, but after their divergence from *Sorghum*, and that $x = 10$ is ancestral in Saccharinae-Sorghinae species (Kim et al. 2014). The close genetic relationships between *Sorghum*, *Saccharum*, and *Miscanthus* indicate that hybridization between the Saccharinae-Sorghinae species with a common ancestor has significant potential for the improvement of sorghum. Within *Sorghum*, the tertiary genepool species in *Chaeto/Heterosorghum* offer the best potential for introgression of traits into cultivated sorghum and are discussed in the later hybridization section of this paper.

2.2 Cytology and Cytogenetics

The genus *Sorghum* is divided into two groups based on genome size (Table 1). Sections *Eusorghum, Chaetosorghum*, and *Heterosorghum* have smaller chromosomes and less DNA, the $x = 5$ genome ranging from 0.26 to 0.42 pg, a 1.6-fold difference, while *Parasorghum* and *Stiposorghum* have larger chromosomes with an $x = 5$ DNA content of 0.64–2.3 pg (Price et al. 2005b). Owing to their similarities to *Eusorghum*, Wu (1993) has proposed *S. macrospermum* Garber and *S. laxiflorum* F. M. Bailey of sections *Chaetosorghum* and *Heterosorghum* be included in section *Eusorghum*. Most reported chromosome counts in *Sorghum* are in agreement but multiple ploidy levels have been reported for *S. amplum* ($2n = 10, 30$), *S. leiocladum* ($2n = 10, 20$), *S. nitidum* ($2n = 10, 20$), *S. plumosum* ($2n = 10, 20, 30, 40$), and *S. timorense* ($2n = 10, 20$) (Table 1). DNA content ranges from 1.27 to 10.30 pg, an 8.1-fold variation in the *Sorghum* genus (Price et al. 2005b).

Most cytology has focused on the *Eusorghums* as interspecific hybrids readily occur among the *Eusorghum* species. In this group, the domesticated species *S. bicolor* is classified as a diploid ($2n = 2x = 20$) and is meiotically regular with 10 bivalents at metaphase I with rare multivalent formations. There has been discussion as to whether *S. bicolor* is a diploid or tetraploid (Garber 1950; Hadley 1953; Endrizzi and Morgan 1955; Doggett 1988; Tang and Liang 1988; Gomez et al. 1998; Zwick et al. 2000). Brown (1943), Kidd (1952), and Endrizzi and Morgan (1955) observed meiotic bivalents in haploid sorghums while others have reported quadrivalents in diploids (Bennett and Merwine 1966). A tandemly repeated DNA centromeric sequence (CEN38) bound differentially to the centromeres of *S. bicolor* chromosomes with a strong signal from 10 of the chromosomes and little or no signal from the other 10 (Gomez et al. 1998; Zwick et al. 2000). Gomez et al. (1998) have proposed the differential binding of CEN38 to sorghum chromosomes is evidence of two subgenomes supporting a polyploid origin of sorghum. Tang and Liang (1988) have assigned *S. bicolor* the genomic formula AAB_1B_1.

Sorghum propinquum is interfertile with *S. bicolor,* but is considered a distinct species due to spatial isolation; *S. bicolor* is from Africa and *S. propinquum* is found in southern India, south-eastern Asia, and the southeast Asian islands (de Wet 1978). *Sorghum halepense* ($2n = 4x = 40$) also known as Johnsongrass has been considered

an autotetraploid (Casady and Anderson 1952; Duara and Stebbins 1952), an auto-octoploid (Bennett and Merwine 1966) and an auto-allo-octoploid (Hadley 1953; Tang and Liang 1988) assigning a genomic formula of $AAAAB_1B_1B_2B_2$, the subgenomes A and B_1 having homology with *S. bicolor*. These two species can hybridize producing triploid and tetraploid progeny. *Sorghum almum* ($2n = 4x = 40$), also known as Columbusgrass, is considered a naturally occurring hybrid of *S. bicolor* and *S. halepense* but is difficult to separate from *S. halepense* (Parodi 1943; Endrizzi 1957). One study has compared the genomic relationship of the intersectional species *S. bicolor*, a *Eusorghum*, and *S. macrospermum* of section *Chaetosorghum*. Kuhlman et al. (2008) noted homology exists between the two species in the A and B1 genome, with higher homology in the A genome, proposed the *S. macrospermum* genomic formula as AAB_1B_1YYZZ, the Y and Z genomes having no known relation with other sorghum species. Genomic formulae of other sorghum species are lacking. With the rapid developments of genomic methods our increased understanding of *Sorghum* genomic relatedness will provide data useful for establishing effective introgression strategies.

3 Desirable Traits in Other *Sorghum* Species

Access to the secondary and tertiary gene pools in sorghum has been limited due primarily to pollen–pistil interactions (Hodnett et al. 2005). Other sorghum species within *Eusorghum* have been assessed for a few traits, principally traits of perenniality (Cox et al. 2002; Jessup et al. 2017a, b; Washburn et al. 2013). As in most other crops, sources of pest resistance are a priority and resistance has been reported in other *Sorghum* sections. A number of species (Table 2) have been tested for resistance to the insect pests sorghum midge [*Contarinia sorgicola* (Coquillett)], shootfly (*Atherigona soccata* Rondani), and stem borer [*Chilo partellus* (Swinehoe) (Lepidoptera: Pyralidae)].

3.1 Resistance to Sorghum Midge

Sorghum midge is one of the most damaging insects in sorghum production worldwide (Young and Teetes 1977). In the 1970s, resistance to midge was bred into commercial cultivars with resistance due to ovipositional preference or antixenosis (Franzmann 1993). However, the effectiveness of ovipositional preference is limited under no choice conditions such as in large acreages of a crop (Henzell et al. 1994). In 1985, a sorghum accession with antibiosis resistance, DJ 6514, was developed at ICRISAT and has been incorporated into breeding programs (Sharma 1985). DJ 6514 was not effective in all locations; it and its derivatives were susceptible to midge in Kenya (Sharma et al. 1999). Resistance to pests break down over time and therefore, the search for new sources of resistance is ongoing in this case to sorghum midge (Sharma and Franzmann 2001). In Australia the sorghum midge is restricted to *S. bicolor*; midge do not infest the native sorghum species. As such, they have

Table 2 Pest resistance for sorghum midge (*Stenidiplosis sorghicola*), shootfly (*Atherigona soccata*), and spotted stem borer (*Chilo partellus*) in *Sorghum*

	Sorghum midge[a]	Shootfly[b]		Stem borer[c]	
	Antixenosis	Antixenosis	Antibiosis		
Species	Eggs/spikelet	% plants w/eggs	% adults emerged	% dead hearts	Recovered larvae
S. almum	NA	NA	NA	NA	NA
S. bicolor	NA	71.3	50.8	43	8
S. halepense	1.3	95.1	68.9	98	13
S. propinquum	NA	NA	NA	NA	NA
S. macrospermum	0.02	76.6	NA	NA	NA
S. laxiflorum	0.0	61.3	6.2	15	0
S. grande	NA	NA	NA	NA	NA
S. leiocladum	0.02	NA	NA	NA	NA
S. matarankense	0.0	0.0	0.0	5	0
S. nitidum	0.0	57.6	0.0	0	0
S. purpureosericeum	NA	1.8	0.0	0	0
S. timorense	0.0	100	45.8	0	0
S. versicolor	NA	2.1	0.0	0	0
S. amplum	0.0	NA	NA	NA	NA
S. angustum	0.0	9.0	0.0	0	0
S. brachypodum	0.0	NA	NA	NA	NA
S. bulbosum	0.004	NA	NA	NA	NA
S. ecarinatum	NA	8.5	0.0	0	0
S. exstans	0.0	0.0	0.0	0	0
S. interjectum	0.0	2.3	0.0	0	0
S. intrans	0.0	7.1	0.0	0	0
S. plumosum	0.02	NA	NA	NA	NA
S. stipoideum	0.02	0.0	0.0	0	0

[a]Franzmann and Hardy (1996) and Sharma and Franzmann (2001)
[b]Kamala et al. (2009)
[c]Kamala et al. (2012)

potential to contribute additional resistance (Harris 1979). Fifteen sorghum species indigenous to Australia were tested in no choice conditions (Franzmann and Hardy 1996; Sharma and Franzmann 2001) (Table 2). Midge females did not lay eggs on accessions of 10 species. *Sorghum macrospermum* had a moderate number of eggs that were oviposited on a single panicle (Franzmann and Hardy 1996). Oviposition was very low on all other species. While these species appear to have midge tolerance, there are no reports of their introgression into *S. bicolor*.

3.2 Resistance to Shootfly

Several of the same wild sorghum species also possess effective resistance or immunity to shootfly (Table 2) through ovipositional non-preference and antibiosis (Kamala et al. 2009). Plants were screened 3 weeks after inoculating the young seedlings at the coleoptile or one-leaf stages during the rainy seasons of 1990, 1991, 1998, and 1999 at ICRISAT (Kamala et al. 2009). Under no choice conditions shootfly females did not lay eggs on *S. matarankense, S. exstans,* or *S. stipoideum*. Overall, very little damage occurred to the species in the section *Stiposorghum* with the number of plants with eggs, eggs per plant, and dead hearts being very low. Within section *Parasorghum,* accessions of *S. purpureosericeum* and *S. versicolor* had little damage while other accessions were more susceptible to shootfly. Several traits present in these species may contribute to ovipositional antixenosis through absence of attractants, the presence of repellent compounds and physical barriers such as hairiness of the leaves and pubescence of the leaf blade (Kamala et al. 2009). Antibiosis was present in all *Stiposorghums* and in accessions of *S. purpureosericeum*. When inoculated with shootfly eggs, accessions of *S. matarankense*, *S. purpureosericeum*, *S. exstans,* and *S. stipoideum* had no dead hearts, and no adult emergence. *Stiposorghum* species overall had no or very low incidents of dead hearts, the highest proportion being 5.4% in *S. ecarinatum*. While larvae did feed on these plants they did not complete their life cycle (Kamala et al. 2009). No adults emerged from dead hearts of accessions of *S. nitidum, S. purpureosericeum*, or *S. versicolor* although the proportion of plants with dead hearts was 51.8, 12.7, and 19.4% respectively.

3.3 Resistance to Spotted Stem Borer

The spotted stem borer *Chilo partellus* is one of the most damaging pests in Africa and Asia (Kamala et al. 2012). While moderate levels of stem borer resistance have been bred into sorghum cultivars, more effective sources of resistance are needed. Antixenosis and antibiosis were assessed in 17 sorghum species (Kamala et al. 2012). Under no choice conditions, females of the spotted stem borer were capable of laying eggs on all species ranging from 0.1 to 4.3 egg masses/plant and 2.7–64.8 eggs/egg mass. *Chaetosorghum* and *Eusorghums* incurred extensive damage and were susceptible to and had a great deal of damage from the stem borer while *Stipo-*,

Para-, and *Heterosorghums* had low or no levels of damage. No damage occurred from leaf feeding larvae on any of the *Stiposorghums* (*S. angustum*, *S. ecarinatum, S. extans, S. interjectum*, *S. intrans, S. stipoidium*) nor in an accession of *S. purpureosericeum*. While there was slight damage on accessions of *S. australiense, S. matarankense, S. timorense*, and *S. versicolor*, no dead hearts developed and no larvae were recovered. In the *Heterosorghum, Parasorghum*, and *Stiposorghums*, all larvae died before becoming adults. Resistance to the stem borer may be due to an antibiosis effect or to the inability of larvae to feed due to anatomical features of the plant (Kamala et al. 2012).

3.4 Variations in Starch Physicochemistry

Cereal starch development and its physico-chemistry are distinct for each species. Rice and oats have compound starch granules where multiple small granules develop within a single amyloplast while in wheat a single large granule forms within and smaller granules form independently of the amyloplasts (Shapter et al. 2008). Within the amyloplast of *S. bicolor*, a single large starch granule forms. The size of these starch grains is the primary indicator of how it will be used in foods or other industrial applications (Ji et al. 2004). Variation in the number of pores and channels on the surface of starch granules as well as protein bodies and the protein matrix can affect digestibility (Fannon et al. 2004; Benmoussa et al. 2006). In *S. bicolor*, there are two regions to the endosperm, a vitreous outer layer and a central floury endosperm. The floury endosperm is more loosely packed with the presence of protein bodies but no matrix (Duodu et al. 2002), while the vitreous endosperm has closely packed starch granules surrounded by a protein matrix embedded with protein bodies (Serna-Saldivar and Rooney 1995). Higher proportions of vitreous endosperm increase the hardness of the grain and are more resistant to diseases and pests but reduce digestibility (Tesso et al. 2006). A recent study on the nature of the starch of several native species of Australia that included wild sorghum relatives was conducted (Shapter et al. 2008). Variation exists in the vitreous endosperm of four of the 13 wild sorghum species examined while all the species varied from *S. bicolor* in the nature of its floury endosperm. The distribution of the matrix and protein bodies was also variable as was the occurrence of pores and channels. *Sorghum leiocladum* produces a rice-like starch granule which might be used to improve digestibility of the grain (Shapter et al. 2008). *Sorghum amplum, S. nitidum*, and *S. extans* had properties that made them potentially more digestible than *S. bicolor*. Their starch granules were more spherical, had pores and channels and a lower proportion of protein bodies in the matrix. *Sorghum laxiflorum* was uniform throughout the endosperm with no distinct layers and few protein bodies. *Sorghum matarankense* and *S. timorense* have a uniform starch distribution throughout the grain but also a larger volume of protein bodies (Shapter et al. 2008). These unique combinations of starch, matrix, protein bodies, pores, and channels may provide additional genetic options for the breeder depending on the end-product requirements.

4 Factors Influencing Wide Hybridization in Sorghum

4.1 Pollen–Pistil Interactions

Pollen–pistil incompatibility in wide crosses is a common occurrence, so an understanding of the process is useful when developing strategies to eliminate or promote fertilization. Successful hybridizations occur when the male and female gametes, housed in a pollen grain and pistil, unite forming a seed with good embryo and endosperm development. The pistil not only houses the female gamete which is embedded in the ovary, but also determines what kind of male gametes will be welcomed (Bedinger et al. 2017). It possesses the ability to allow or stop pollen tube growth. Pollen–pistil incompatibility provides a species a means of species continuity, allowing only pollen of the same species access as the male parent in seed production. It is not surprising then that recovering interspecific and intergeneric hybridizations can be difficult. These interactions can be extremely complex with numerous peptides involved (Qu et al. 2015). Over the last two decades, our understanding of the interaction between a pollen grain and a pistil has dramatically increased. For more details on this important topic please refer to Sanchez et al. (2004), Dresselhaus and Franklin-Tong (2013), Qu et al. (2015), Dresselhaus et al. (2016), and Higashiyama and Yang (2017). The process of pollination to fertilization can be divided into several general steps (Hiscock and Allen 2008; Lausser and Dresselhaus 2010; Dresselhaus et al. 2011). Pollen must be captured (adhesion) by pistils usually on a stigma branch. Pollen grains must hydrate followed by germination of a pollen tube that penetrates the stigma branch on which it is bound. Pollen tubes then grow through the stigma and style and into the ovary which houses the egg and central cell. At the base of the ovary, the pollen tube will enter the micropyle and grow into one of the synergids of the egg apparatus and discharge its sperm. The sperm then enter the egg and central cell from the intercellular space between them fusing with the female nuclei from which an embryo and endosperm develop.

4.2 Pre-fertilization Factors

4.2.1 Pollen Adhesion, Hydration, and Germination

Pollen adhesion does not appear to be a strong barrier between species within a genus nor within a family. However, the more distant the relationship, the weaker the adhesive forces may be. Reciprocal interspecific pollinations within the *Brassicaceae* family among *Brassica oleracea* L., *B. napus* L., *Cheiranthus cheiri* L., *Hirschfeldia incana* (L.) Lagr.-Foss, *Raphanus raphanistrum* L., and *Sinapis arvensis* L. had similar levels of adhesion but with reciprocal crosses of *B. oleracea* and *Arabidopsis thaliana* (L.) Heynh., adhesive forces were significantly reduced (Luu et al. 1998). Pollinations of *A. thaliana* also showed an increased reduction in pollen adhesive forces with increasing distance in relationship of dicot relatives and virtually no adhesion with monocot pollen (Zinkl et al. 1999).

Interspecific and intergeneric pollen adhesion also differs when pollinating *S. bicolor*. Fourteen sorghum species were used as pollinators with ATx623 as the sorghum seed parent. On average there were 70 pollen grains per stigma (Hodnett et al. 2005; Price et al. 2006). Pollen germination values ranged as low as 52.2%. The remaining un-germinated pollen grains remained attached, an indication of strong adhesive forces. In contrast, pollen germination of the more distantly related species was reduced (Bartek et al. 2012). Using accessions of *Zea*, *Pennisetum*, and *Miscanthus* as pollinators, 144 sorghum pistils were pollinated, with an average of 1.5 pollen grains per pistil remaining on the stigmas after panicles were fixed in a 3:1 solution of ethanol:acetic acid and then excised. Contrary to Zinkl et al. (1999), who found 1 M acetic acid removed all pollen grains from *Arabidopsis* stigmas, pollen grains from species within the *Sorghum* genus remained attached indicating strong adhesive forces are present. However, more distantly related species exhibited low or no adhesion (Bartek et al. 2012).

Pollen hydration in grasses is loosely controlled but also highly susceptible to ambient humidity (Heslop-Harrison et al. 1984a). When pollinating maize, *Sorghum bicolor* and *Pennisetum americanum* pollen had similar levels of germination as did maize pollen at given levels of humidity. Very few pollen grains hydrated at low humidity (5–10%) but at 70% and 90–95% humidity hydration and germination readily occurred (Heslop-Harrison et al. 1984b).

Of particular interest is the increase in pollen grains adhering to the stigma, hydrating and germinating when using a sorghum line recessive for the *Inhibition of Alien Pollen* (*Iap*) gene. Bartek et al. (2012) compared pollen grains remaining after fixation on stigmas of Tx3361(*iap*) and ATx623(*Iap)* when pollinated with accessions of the distant relatives *Zea*, *Miscanthus*, and *Sorghastrum*. Comparable amounts of pollen were used for each pollination but the difference in the number of pollen grains that remained on the stigma was striking. Pollen adhesion for ATx623 (*Iap*) was similar to results of Luu et al. (1998) and Zinkl et al. (1999) when making very wide crosses. Very few pollen grains remained demonstrating weak adhesion. In contrast, more pollen grains remained on the stigmas of Tx3361(*iap*) for each pollination averaging three to 300 times more pollen. While 85% of the more than 22,000 pollen grains adhering to the pistils of Tx3361(*iap*) germinated, the 3000+ that did not germinate remained attached to the stigma by adhesive forces only.

The *Iap* allele in ATx623 inhibited adhesion with distant relatives but *iap,* in Tx3361, removed inhibition as demonstrated by pollen of *Zea, Miscanthus*, and *Pennisetum* species. It may be the result of a recognition of mechanisms that trigger an inhibitory response independent of the adhesion process and when removed, adhesion can proceed. Whatever the cause, the use of *iap* provides a method to increase pollen adhesion events in extremely wide crosses opening the door for many more species combinations. Because they have a similar genetic background the differences for pollen adhesion mentioned above are very likely influenced by *iap*. Tx3361 is a BC_1F_3 from a cross between BTx623(*Iap)* and NR481(*iap*) (Laurie and Bennett 1989).

4.2.2 Penetration of the Stigma and Pollen Tube Growth

While very loose controls are present for pollen adhesion, hydration, and germination, penetration of the stigma is tightly controlled and could be considered the "first gatekeeper" (Dresselhaus et al. 2011). After stigma penetration two other barriers immediately arise; the pollen tube must find the transmitting tissue and then, once pollen resources are exhausted, receive nutrients from the pistil. *Poa nemoralis* L., *Lolium multiflorum* Lam., and *Oryza sativa* pollen germinated on maize and *Tripsacum dactyloides* stigmas, but pollen tube growth was arrested prior to entering the transmitting tract (Lausser and Dresselhaus 2010).

Pollen tubes readily grow with little resistance in interspecific pollinations among the *Eusorghums*. However, attempted hybridizations between *S. bicolor* and species from other sorghum sections failed. Only a few reports address these reproductive barriers but they show inhibition of pollen tube growth is a primary barrier to hybridization. In pollinations of *S. bicolor* with *S. versicolor* only a few pollen tubes grew into the ovary and most did not grow beyond the stigma. While no hybrids were recovered there were significant differences among the sorghum lines for pollen tube inhibition indicating pollen tube growth was influenced by genotype (Sun et al. 1991). Shivanna and Seetharama (1997) made reciprocal pollinations of *S. bicolor* and *S. purpureosericeum,* but the pollen tubes were inhibited from entering the stigma. In a broader study by Hodnett et al. (2005), 14 species were used as pollen parents with sorghum line ATx623 as the female parent. Most of the alien sorghum species exhibited very strong pollen tube inhibition in the stigma. Seventy-one percent of the pollen grains germinated but only 28% entered the stigma branch and 6% grew to the stigma axis. While all species had some pollen tubes reach the stigma axis, pollen tubes of only six of the species (*S. angustum*, *S. ecarinatum*, *S. macrospermum*, *S. matarankense*, *S. plumosum*, and *S. purpureosericeum*) grew into the style. In three of these six species, *S. ecarinatum, S. macrospermum,* and *S. matarankense*, a small number of pollen tubes had entered the ovary. Embryos from additional pollinations of these three species were found in 0.9% of *S. ecarinatum* pistils, 0.08% of *S. macrospermum* pistils, and 0.2% of *S. matarankense* pistils. Just one seedling, a *S. bicolor* × *S. macrospermum* hybrid, was recovered (Price et al. 2005a).

4.2.3 Genes That Control Some Aspect of the Pollen–Pistil Interaction

Only a few genes have been identified in grasses that control some aspects of the pollen–pistil interaction. Four crossability genes, Kr_1, Kr_2, Kr_3, and Kr_4 identified in hexaploid wheat are used extensively in wide hybridizations (Lein, as reported in Riley and Chapman 1967; Krolow 1970; Luo et al. 1992). It was determined that the dominant form of these alleles inhibits crossability of alien species with wheat. Using substitution lines, *Kr1* actively inhibited pollen tubes from penetrating the stigma and growing in the stigma, style and ovary wall while the recessive allele did not (Riley and Chapman 1967). No contribution to crossability either positive or negative could be attributed to the recessive allele (Riley and Chapman 1967; Lange and Wojciechowska 1976; Jalani and Moss 1980; Koba 1997). An additional gene in wheat *(Triticum aestivum)*, *Pairing homeologous* (*Ph*), found on the long arm of

chromosome 5B inhibits homeologous chromosomes from pairing, but when 5B is replaced with an alien homeologue, homeologous pairing occurs (Chapman and Riley 1970). Chromosome translocations of alien chromosome segments have been instrumental in the introgression of important traits into wheat cultivars (Zhang et al. 2017). A *Ph*-like locus in sorghum has not yet been reported.

Inhibition of alien pollen (Iap) in sorghum has a similar function as *Kr* genes in wheat. The *Iap* (dominant) allele increases pollen–pistil incompatibilities that prevent hybridization among divergent species of the *Sorghum* genus (Price et al. 2006). Laurie and Bennett (1989) demonstrated the inhibition of maize pollen tube growth on the sorghum stigma is genetically controlled. In an initial study of three sorghum genotypes maize pollen tubes never grew more than 100–300 μm even though the maize pollen grain has enough endogenous reserves to grow about 20 mm (Heslop-Harrison et al. 1984b), an indication that maize pollen tube growth in sorghum was inhibited. An additional 10 diverse accessions were selected and one single accession (Nr481) did not inhibit maize pollen tube growth (Laurie and Bennett 1989). Pollen of five genotypes of maize germinated and grew through Nr481 stigmas and at least to the base of the style. Out of 469 ovaries pollinated with the maize line Seneca 60, five showed entry of pollen tubes into the embryo sac with endosperm development in three of them. Evidence of a hybrid endosperm was reported in one of the three ovaries where approximately 30 chromosomes were observed during mitosis. Additional crosses were left on the panicles to develop but no embryos were recovered. It was determined that maize pollen tube growth on sorghum stigmas was inhibited by a single dominant allele (Laurie and Bennett 1989). *Iap,Iap* × *iap,iap* sorghum hybrids inhibited maize pollen tubes but the $BC_1$of Nr481 segregated 1:1 inhibiting:noninhibiting demonstrating the trait was controlled by a single allelic variation at a single locus.

Among Sorghum species, a *Chaetosorghum* (*S. macrospermum, 2n* = 4*x* = *40*), a *Parasorghum* (*S. nitidum,* 2*n* = 2*x* = 20), and a *Stiposorghum* (*S. angustum,* 2*n* = 2*x* = 10) were used to pollinate male sterile sorghum line ATx623(*Iap*) and a male sterile derivative of Nr481 homozygous for *iap*. ATx623 was not receptive to *S. nitidum* or *S. angustum* and only slightly receptive to *S. macrospermum* pollen where pollen tubes entered the ovaries of two of 15 pistils (Price et al. 2006). In contrast, seven of eight pistils pollinated with *S. angustum* pollen, nine of 11 pistils pollinated with *S. nitidum* pollen and all four pistils pollinated with *S. macrospermum* pollen had pollen tubes in the ovary of the Nr481 derivative. The *iap* genotype removed some inhibition but was not as successful as *S. bicolor* × *S. bicolor* where more pollen tubes reached the ovary than pollen tubes from the three sorghum relatives. Pollen tubes from the intraspecific pollination grew straighter and were smoother in appearance than species pollen tubes which tended to meander. However, inhibition was reduced enough that some pollen tubes of at least one accession of *Z. mays*, *Z. mays* subsp. *Mexicana, Pennisetum,* and *Sorghastrum* entered ovaries of the Nr481 CMS derivative (Bartek et al. 2012). It was also clear that successful pollen tube growth is genotype-dependent.

4.2.4 CHA, Genetic Male Sterile, and CMS Lines

One of the limitations when screening sorghum is the small number of genotypes that possess genetic or cytoplasmic male sterility (Laurie and Bennett 1989). Small quantities of seed of any cross can be produced with mechanical sterility induction methods such as hand removal of anthers or emasculation with plastic bags (Schertz and Clark 1967) but it is limited by the time and skill needed to perform the task. To overcome these limitations, the use of a chemical hybridizing agent (CHA) that induces male sterility would be useful. In a greenhouse study, trifluoromethanesulfonamide (TFMSA) effectively induced temporary male sterility in two sorghum lines BTx623 and ARG-1 (Hodnett and Rooney 2018). As little as 2 mg TFMSA applied to the leaves induced sterility in BTx623 and as much as 40 mg were applied to ARG-1 without any observed phytotoxic effects on the plant or on the progeny. The larger quantities were effective even when applied 30 days prior to flag leaf emergence. TFMSA affects the free amino acid ratios in anthers and pollen and in particular proline (Loussaert 2004). While proline is the most abundant free amino acid in pollen, including sorghum pollen, accounting for more than half of the free amino acid pool (Bathurst 1954; Kern and Atkins 1972; Krogaard and Andersen 1983; Leport and Larher 1988), proline levels are low in male sterile sorghums (Kern and Atkins 1972; Brooking 1976). Proline has been identified as a key amino acid required for pollen development (Funk et al. 2012; Mattioli et al. 2012; Biancucci et al. 2015). Loussaert (2004) induced temporary male sterility in maize therefore it is not unreasonable to expect TFMSA to be effective on all sorghum species as well as other grasses. With effective CHAs any wide hybridizations of interest can be explored.

4.3 Post-fertilization Barriers

Post-fertilization barriers include ploidy differences, cytoplasmic incompatibilities, hybrid breakdown, or a lack of genetic recombination (Price et al. 2005a; Dwivedi et al. 2008). When working with polyploids a reduction of ploidy can be accomplished by backcrossing with the crop species as the recurrent parent. This may reduce any extra chromosomes through the next generations to the desired ploidy level (de Wet et al. 1976). For example, the triploid F_1 hybrid of sorghum ($2n = 20$) × Johnsongrass ($2n = 40$) was pollinated with diploid sorghum recovering 20 and 21 chromosome progeny (Hadley and Mahan 1956).

Parental ploidy differences usually cause endosperm failure thus they must be addressed simultaneously. The most common interploidy post-fertilization barrier of wide crosses is degeneration of the endosperm which leads to embryo death (Brink and Cooper 1947a, b). Lin (1984) demonstrated the importance of a 2:1 maternal: paternal genomic ratio for developing endosperm in maize also demonstrating the endosperm develops independently of the embryo. Since the endosperm and the embryo develop independently, if the endosperm fails to develop the embryo can be rescued, a process that is commonly used. However, since it is always more productive to produce viable seed, fully developing endosperm is preferred. Because

there are exceptions to a 2:1 ratio for normal endosperm development, the concept of Endosperm Balance Number (EBN) in *Solanum* (Johnston and Hanneman 1980) and the Polar-Nuclei Activation (PNA) in *Avena* species (Nishiyama and Yabuno 1983) were independently developed but are considered to be the same biological concept (Katsiotis et al. 1995). Instead of the 2:1 genomic ratio of the endosperm the EBN or PNA number predicts endosperm development irrespective of ploidy. Now that hybrids outside of the *Eusorghums* are possible, applying this concept in sorghum for both interspecific and intergeneric crosses may be useful. In sorghum the 2:1 maternal:paternal genome ratio of the endosperm produces healthy endosperm. However, *S. bicolor* ($2n = 20$ chromosomes) × *S. macrospermum* ($2n = 40$ chromosomes) seed produces well developed endosperm and a viable triploid embryo (Price et al. 2006). Other interspecific hybrids may be predicted by EBN when a 2:1 maternal:paternal ratio does not function. Genomic imbalances can be overcome in several ways. An F_1 hybrid that is sterile due to pairing failure will not produce viable gametes, but may have fertility restored by doubling its chromosomes. With fertility restored it can be used in a backcross program to introgress traits of interest. Alternatively, selfing over several generations, which also provides opportunities for additional recombination, may reduce ploidy (Dwivedi et al. 2008).

For species that do not produce fertile hybrids or that breakdown in succeeding generations the use of a bridge species prior to increasing ploidy may be possible. Simpson (1991) introgressed a high level of resistance to early (*Cercopsora arachidicola* Hori) and late [*Cercospridium personatum* (Berk. And Curt.) Deighton] leaf spot in groundnut *Arachis hypogaea* L., a tetraploid composed of genomes A and B. Simpson (1991) used three diploid species by first creating a hybrid of *A. cardenasii* Krapov. & W. C. Greg. × *A. chacoensis* Krapov. & W. C. Greg., diploids composed of the A genome and which carry separate resistance mechanisms for leafspot, and then making the tri-species hybrid *A. batizocoi* Krapov. & W. C. Greg. × (*A. cardenasii* × *A. chacoensis*) to include the B genome of *A. batizocoi*. It was necessary that both parents possessed the A and B genomes for successful introgression of these traits. Bridge species in *Sorghum* may be a method for trait transfer. Although hybrids of *S. bicolor* × *S. angustum* and *S. nitidum* were created, they did not develop beyond the juvenile growth phase as a result of genomic differences (Price et al. 2006). Including species with common genomes may reduce or eliminate these conditions thus eliminating hybrid breakdown. As genomic relationships within *Sorghum* are better understood effective strategies to overcome post-fertilization barriers such as hybrid breakdown will provide additional tools for this work.

Increasing the ploidy of the lower ploidy parent to match that of the upper ploidy parent prior to crossing is another method. This can be accomplished in two ways. The chromosomes of the parent can be doubled using a spindle poison preventing chromosomes to migrate during anaphase or when present $2n$ gametes can be used. $2n$ gametes, gametes with the somatic chromosome number, are widespread among plants and are thought to play a major role in polyploid formation in nature (Harlan and de Wet 1975; Kreiner et al. 2017). Harlan and de Wet (1975) compiled a list of

hundreds of species from 85 genera in which 2*n* gametes are produced including wheat, maize, rice, sorghum, and *Saccharum*. They principally form from irregularities in meiosis that disrupt segregation either during meiosis I or meiosis II and are respectively termed First Division Restitution (FDR) and Second Division Restitution (SDR) (Mok and Peloquin 1975). If the disruption occurs during FDR chromosomal segregation does not occur thus the somatic chromosome number is retained. If the irregularity occurs during SDR, the somatic chromosome number is restored (Bretagnolle and Thompson 1995). FDR will retain most of the allelic heterozygosity present in the parent while SDR will contain less. It has been determined 2*n* gamete production is genetically controlled in both the pollen and egg (Bretagnolle and Thompson 1995). Unreduced (2*n*) gametes are commonly used to overcome ploidy imbalances to avoid endosperm failure. Potato breeders have used 2*n* gametes extensively for moving favorable traits into the cultivated species *Solanum tuberosum* ($2n = 4x = 48$) from diploid to hexaploid relatives (den Nijs and Peloquin 1977). This system is an effective method not only for trait improvement but also for increasing allelic diversity and maximizing heterozygosity (Carputo et al. 1999). Using this strategy improved potato cultivars have expanded to environments previously unsuitable for them.

In sorghum, Endrizzi (1957), Hadley (1958), McClure (1962, 1965), and Sengupta and Weibel (1968) reported recovering a total of 166 tetraploids and 51 triploids from sorghum × Johnsongrass implying the presence of *2n* gametes but because they were limited studies, inferences could not be made. Although there has been little attention regarding 2*n* gametes for sorghum improvement, they offer a means to transfer traits from wild to domestic sorghum. In addition to eliminating genomic imbalances, polyploids are generally more tolerant of chromosomal manipulations including aneuploidy which provides a mechanism for alien chromosome translocations. As an illustration, *Saccharum officinarum* accepts a wide variety of interspecific and intergeneric hybrids because of its high ploidy level (Dwivedi et al. 2008). Hybrids have been created using all of the species within *Saccharum* as well as *Erianthus, Miscanthus,* and *Sorghum* (Sreenivasan et al. 1987).

4.4 Confirming Hybrids by Flow Cytometry and Cytological Analyses

In the process of creating wide hybrids multiple ploidies may be created from the same cross. Seedlings recovered from diploid sorghum × tetraploid Johnsongrass are triploid, tetraploid, or hexaploid (Hadley 1958). Hexaploids are the union of a 2*n* gamete from each parent (Harlan and de Wet 1975) or have undergone a somatic chromosome doubling event. Flow cytometric analysis provides a powerful method for rapidly identifying the ploidy of these seedlings. By using a standard of known DNA content ploidies can be estimated and then confirmed by cytological analysis of a small subsample. In this way the ploidy of large numbers of interspecific sorghum hybrids is quickly determined in our lab.

4.5 Embryo Rescue

The most desirable outcome of a cross is to produce viable seed. If endosperm failure does occur, a common practice is to rescue the embryo by excising it and placing it on an artificial medium designed to provide the nutrients needed for embryo development (Price et al. 2005a). Seedlings recovered from *S. bicolor* × *S. angustum, S. bicolor* × *S. nitidum,* and *S. bicolor* × *Saccharum* ssp. are examples of wide hybridizations that were successfully rescued (Price et al. 2006; Hodnett et al. 2010).

5 Interspecific/Intergeneric Hybridization in Sorghum

5.1 Hybridization Within the *Eusorghums*

5.1.1 *Sorghum propinquum*

Sorghum propinquum is the closest relative of *S. bicolor*. They both are 20 chromosome species, have about the same amount of DNA and are fully interfertile. *Sorghum bicolor* × *S. propinquum* hybrids have been used to produce genetic maps that identify QTLs associated with useful traits related to senescence (Feltus et al. 2006). QTLs for rhizomatousness, tillering and regrowth were found in *S. propinquum* that may benefit forage and biomass genotypes (Paterson et al. 1995). Studies of rhizomatousness and overwintering resulted in the release of *S. bicolor* × *S. propinquum* hybrid PSH12TX09 for forage and biofuel feedstock development that survives temperatures as low as −12 °C (Washburn et al. 2013; Jessup et al. 2017b). Kong et al. (2014, 2015) found QTLs for rhizomatousness and vegetative branching. An understanding of branching may be used to produce lines with better apical dominance or for increased branching depending on the requirement of the crop (Kong et al. 2015).

5.1.2 *Sorghum halepense* (Johnson Grass)

The most commonly reported interspecific hybrid is sorghum × Johnsongrass. While they differ in ploidy they readily hybridize and have been used to develop forage lines Silk (CSIRO 1978a), Sucro (CSIRO 1978b), and Co27 (Surendran et al. 1988). Jessup et al. (2012) report the use of *S. halepense* for improvement of Columbusgrass (*S. almum*) and have registered a seed sterile Columbusgrass hybrid PSH09TX15 for developing perennial hay, forage, and biofuel cultivars (Jessup et al. 2017a). PSH09TX15 has good leaf production and survives temperatures as low as −12 °C and of particular interest, does not flower in Texas latitudes ensuring no gene flow to weedy relatives (Jessup et al. 2012).

5.1.3 Perennial Grain Sorghum

The Land Institute in Kansas has an ongoing program for breeding perennial grain sorghum using *S. halepense* as a source of perenniality (Piper and Kulakow 1994; Cox et al. 2002). Proposed benefits of perennial sorghum are reduced soil erosion and fertilizer inputs, conservation of soil organic matter and reduced tillage

operations (Cox et al. 2006). In 2016 the project determined that they can simultaneously select for perenniality and yield (Nabukalu and Cox 2016). Progress has been made in grain size, grain yield, and over wintering but excess branching continues to limit their progress (Cox et al. 2018b). However, with the development of additional QTLs for branching and perenniality selection against excessive branching may be possible (Washburn et al. 2013; Kong et al. 2014, 2015). An interesting development has been the production of diploid progeny from a diploid × tetraploid cross with introgression from *S. halepense* (Cox et al. 2018a). A diploid interspecific hybrid of *S. bicolor* × *S. halepense* had previously been reported by Dweikat (2005). The mechanism for diploid progeny is still to be resolved but producing diploid progeny from a diploid × tetraploid cross would increase the efficiency of trait transfer.

5.2 Interspecific Hybrids Beyond the *Eusorghums*

Until recently strong reproductive barriers have eliminated any interspecific hybridizations, except within the *Eusorghums* (Garber 1950; Schertz and Dalton 1980; Doggett 1988; Hodnett et al. 2005). However, a few accounts of attempted intersectional hybridizations have been reported. Sun et al. (1991) made reciprocal pollinations with three lines of sorghum and *S. versicolor*. While no hybrids were recovered, there was differential pollen tube growth among the genotypes. A few pollen tubes of *S. versicolor* reached the ovary with some near the micropyle of two genotypes but not of the other, while *S. bicolor* pollen tubes were limited to the stigma and style. Huelgas et al. (1996) were not successful in obtaining hybrids of *S. bicolor* and *S. macrospermum*, *S. timorense*, *S. matarankense,* or *S. stipoideum*. Embryo rescue techniques were used in an attempt to rescue any putative hybrids but none were recovered. Hodnett et al. (2005) excised hybrid embryos of *S. bicolor* and *S. ecarinatum*, *S. macrospermum*, or *S. matarankense* with the frequency being respectively 10/1119, 1/1237, and 13/533 embryos/pollinated florets. Only the *S. bicolor* × *S. macrospermum* hybrid survived (Price et al. 2005a). This hybrid was morphologically intermediate between the parents and was as expected triploid ($2n = 30$ chromosomes).

Viable seeds developed on 10% of *S. bicolor* (*iap*) florets when pollinated with *S. macrospermum* eliminating the need for embryo rescue (Price et al. 2006). When using the same seed parent, pollinations with *S. nitidum* and *S. angustum* of sections *Parasorghum* and *Stiposorghum* formed embryos on 18.8 and 10.2% of the florets, but embryo rescue was necessary. Hybrids were confirmed by chromosome analysis. Each hybrid had the expected chromosome number of $2n = 30$, 20, and 15 (Fig. 2). Hybrids of *S. macrospermum* were partially fertile while hybrids of *S. nitidum* and *S. angustum* never developed beyond the juvenile growth stage. As mentioned previously, *S. bicolor* and *S. macrospermum* have homology in genomes A and B_1 which promises to be useful for introgressing traits of interest. Introgression of up to 18.6% was found on a *S. bicolor* × *S. macrospermum* BC_2F_1 and in some families introgression was random indicating its potential as a source for genetic

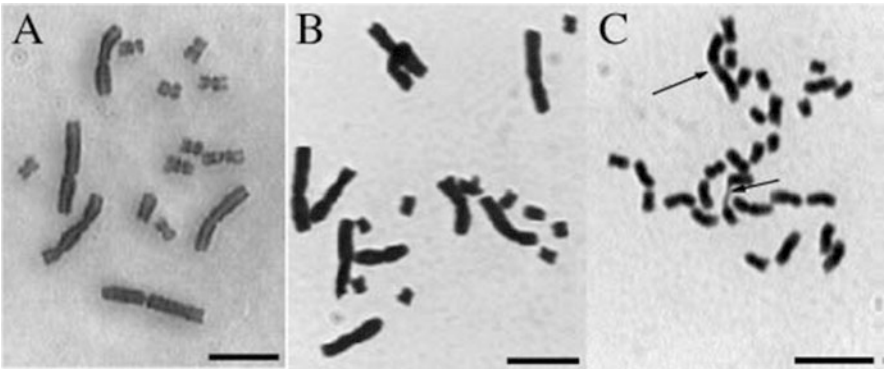

Fig. 2 Somatic chromosomes of hybrids between *S. bicolor* ($2n = 2x = 20$) and *S. angustum* ($2n = 2x = 10$), *S. bicolor* and *S. nitidum* ($2n = 2x = 20$), and *S. bicolor* and *S. macrospermum* ($2n = 4x = 40$). (**a**) Chromosomes of a hybrid between *S. bicolor* and *S. angustum* consisting of five large chromosomes from *S. angustum* and 10 small chromosomes from *S. bicolor*. (**b**) Chromosomes of a *S. bicolor* × *S. nitidum* with 10 large chromosomes from *S. nitidum* and 10 small chromosomes from *S. bicolor*. (**c**) Chromosomes of a *S. bicolor* × *S. macrospermum* hybrid with 20 chromosomes from *S. macrospermum* and 10 from *S. bicolor*. Upper arrow shows two chromosomes. Lower arrow shows a chromosome in which the centromere is not fully condensed and appears as a strand. Scale bars = 5 μm. (Source: Price et al. 2006)

improvement (Kuhlman et al. 2010). The recovery of new hybrid combinations and additional hybrids of existing wide crosses aids in maximizing genetic recombination and increases the probability that genomic regions can be introgressed from wild species into *S. bicolor*, as demonstrated by Kuhlman et al. (2008, 2010).

5.3 Intergeneric Hybridization

5.3.1 Saccharum

The *Saccharum* complex is considered a close relative of *Sorghum* having diverged from a common ancestor about 5.4 million years ago (Al-Janabi et al. 1994; Kim et al. 2014). Since this close relationship has been recognized, *Saccharum* × *Sorghum* crosses have been attempted with some success (Venkatraman and Thomas 1932; Bourne 1935; Moriya 1940; De Wet et al. 1976; Subramonian 1991). In an analysis of a BC_4 population with a tetraploid *S. bicolor* as the recurrent parent, tetraploid progeny were recovered with $2n = 40$ chromosomes that retained some of the characteristics of *Saccharum* (de Wet et al. 1976; Gupta et al. 1978). While univalents, bivalents, trivalents, and quadrivalents formed during diakinesis, 40, 41, and 42 chromosome seedlings were recovered (Gupta et al. 1976). Recently, *S. bicolor* has been used in an attempt to broaden the genetic base of sugarcane in India (Singh et al. 2002). Clones of *Saccharum officinarum* × *S. bicolor* were recommended for production of biomass in Japan (Terajima et al. 2007). Expression

profiling of sucrose metabolizing genes function similarly in sucrose accumulating sugarcanes, sweet sorghums, and in sugarcane × sorghum (Ramalashmi et al. 2014). Other traits of interest may be pursued without compromising sugar accumulation in the stems.

Sorghum × *Saccharum* crosses have been attempted with limited success (Bourne 1935; Nair 1999) the primary barrier being pollen tube inhibition (Hodnett et al. 2010). When using a sorghum parent homozygous for *iap,* an average of 56 seed were produced per sorghum panicle with seed set as high as 53%. Because the seeds were viviparous and the germinated seedlings were unable to penetrate the seed coat, embryo rescue was necessary. Seedling recovery was 33% while 39% of the seed had no embryo and another 28% were not viable. Hybrids have also been created from *Miscanthus* spp. and *Erianthus* spp. (author unpublished data). The genetic and phenotypic variation among these hybrids was extensive providing opportunities for selection. The genetic variation that exists in sorghum and sugarcane provides opportunities to introgress valuable quantitative traits for either species or for producing *Sorghum-Saccharum* hybrids with enhanced water use efficiency and high sugar-accumulating capacity (Hodnett et al. 2010).

5.3.2 Maize

Attempts by Bernard and Jewell (1985) and Dhaliwal and King (1978) to hybridize maize × sorghum were not successful. Reger and James (1982) and Heslop-Harrison et al. (1984b) observed sorghum pollen tubes near the micropyle in maize ovules but no entry into the egg apparatus was seen. Ramesh and Reddy (1984) report two putative maize × sorghum hybrids that were male sterile. In two studies by James (1978, 1979) 32 hybrids were recovered from about 43,000 pollinations. Since endosperm breakdown occurred embryo rescue of the hybrids was necessary. Other putative hybrids were made but they were not recovered as the embryo was not viable. All of the recovered hybrids had 20 maize chromosomes, assumed from 2*n* gametes, and from two to ten sorghum chromosomes. Morphologically the hybrids exhibited unusual traits such as male and female sectors in the inflorescence. Two of the hybrids were recovered from a tetraploid maize parent but all other hybrids were from diploid parents. While sorghum chromosomes were eventually lost in backcrosses of the progeny and no introgression was documented, abnormal morphology was observed in some of the seedlings even after six generations of intercrosses among the backcross progeny. When making the reciprocal pollination, maize pollen tubes rarely grew beyond the stigma branches of sorghum (Dhaliwal and King 1978; Laurie and Bennett 1989). Maize pollen tubes would grow a short distance and stop due to interactions that inhibited pollen tube growth. However, with the use of the *iap* mutant, Laurie and Bennett (1989) observed possible endosperm development but no embryos or seed were recovered.

5.3.3 Other Species

Sixteen accessions of species belonging to the genera *Pennisetum* Rich., *Sorghastrum* Nash, *Miscanthus* Andersson, and *Zea* L. were used as pollen parents by pollinating sorghum line Tx3361 (*iap*). No attempts to recover hybrids were

made and a limited number of pistils were examined. Even so pollen tubes of seven of the 16 accessions, two accessions of *Zea mays*, two accessions of *Zea mays* subsp. *Mexicana* (Schrad,) Lltis, two accessions of *Pennisetum ciliare* (L.) Link and one accession of *Miscanthus floridulus* (Labill.) Warb. ex K. Schum.&Lauterb., grew into the ovary (Bartek et al. 2012). Pollen grains of distantly related grass species will germinate, pollen tubes will grow and may result in hybrids.

6 Manipulating Gene Flow in Sorghum

6.1 Pollen-Mediated Gene Flow from Sorghum Crop to the Wild/Weedy Congeners

Pollen-mediated gene flow produces a change in allele frequency in a population due to the movements of gametes or individuals. Gene flow within a species has a homogenizing effect against genetic drift (Slatkin 1987) but may result in novel evolutionary trajectories when interspecific hybrids are created.

Genome recombination may lead to the development of hybrids that are fertile and environmentally fit enough to reproduce and evolve as a new taxon (e.g., Johnsongrass). Heterosis or hybrid vigor and invasiveness of Columbusgrass is attributed to heterosis due to hybridization between Johnsongrass and sorghum (Ejeta and Grenier 2005). In most cases, hybridization and gene flow will not produce distinctive hybrid entities but rather may act as a conduit between species through which alleles and their associated traits introgress into the other species (Rieseberg and Wendel 1993). Such introgression is commonplace among the eusorghums where there is a long history of introgression between cultivated sorghum and Johnsongrass (Arriola and Ellstrand 1997; Morrell et al. 2005; Mutegi et al. 2010; Jessup et al. 2012). Gene flow is a concern when allelic combinations that confer a fitness advantage to cultivated crops, such as abiotic and biotic stress tolerances, are transferred into the wild or weedy species growing in the vicinity. Any fitness advantage conferred to the crop can be lost if these traits are introgressed into populations of weedy relatives (Ellstrand 2014). No matter how the gene of interest is incorporated into the crop (i.e., conventional breeding vs. genetically engineered), gene flow can encompass some direct and indirect consequences. Large-scale and continuous cultivation of crops increase the chance of gene escape to weedy congeners. In the case of resistance genes where selection pressure is high (such as herbicide resistance), a rapid shift in the frequency of resistance could occur in the weed populations. This would ultimately eliminate the benefit of the trait in the crop. A well-known example of crop-to-weed gene transfer is the hybridization between cultivated and weedy rice and the escalation of herbicide-resistant (ALS-inhibitor-resistant) weedy rice in less than 5 years after the release of an herbicide-resistant rice cultivar (Burgos et al. 2008). Further, if populations of wild species carrying the traits develop highly invasive forms, they can spread rapidly across different environments (Ohadi et al. 2017). If these invasive

genotypes become dominant, diversity in the wild gene pool may be reduced due to selective sweep and genetic swamping (gene contamination) (Ellstrand 2014).

While breeding programs seek methods to increase hybridization for accelerating the development of new cultivars, gene flow prevention requires methods that minimize crossability between the crop and wild or weedy relatives. Given that interspecific gene flow between sorghum and its weedy/wild congeners does occur, methods and techniques to reduce or eliminate gene flow should be considered. While physical isolation of sorghum from its weedy/wild congeners is not practical, effective weed management inside and at the edges of the field before planting, during growth, and after crop harvest, can decrease flowering overlap of crop and weed and reduce the seedbank size minimizing the probability of the establishment of hybrid progenies (Della Porta et al. 2008).

Gene flow containment methods attempt to decrease or eliminate the pollen/ovule availability during the flowering period. Development of cleistogamous, self-fertilizing cultivars is one containment strategy (Yoshida et al. 2007; Leflon et al. 2010). While cultivated sorghums are not cleistogamous, *S. laxiflorum* is. Introgression of the cleistogamy trait in *S. laxiflorum* might be a useful containment strategy. Another form of containment is to increase pollen–pistil incompatibility where pollen grain germination and pollen-tube growth is inhibited by the recipient sorghums/weeds (Rooney 2016). Pollen–pistil incompatibility traits might be found in sorghum lines, mutant populations (Ukai and Nakagawa 2012) or in other sorghum species. Cytoplasmic male sterility (CMS), widely used in sorghum breeding, is considered a viable tactic for gene flow containment by incorporating the gene of interest into the cytoplasmic genome reducing the chance of gene escape. However, maternal inheritance of the cytoplasmic genome is not absolute and a small rate (<0.4%) of cytoplasmic transmission can still occur (Avni and Edelman 1991). There is also evidence that cytoplasmic male sterility breaks under stress conditions (Weider et al. 2009). Finally, it is common for sorghum grain to be lost during harvest and transportation which produces seedlings that are receptive to pollen from nearby Johnsongrass populations thus providing an additional avenue of escape (Ohadi et al. 2017).

Given that most of the field-scale management techniques and to some extent containment techniques do not entirely prevent the gene flow, molecular transgenic techniques could be more effective for gene flow prevention. However, most of these techniques have been tested at small scales. In general, in these techniques the gene of interest to be inserted into the crop is accompanied by a deleterious malfunctioning, blocking construct (Kuvshinov et al. 2005) or genes that decrease the hybrids fitness (Gressel 2015). The deleterious construct should be chosen in such a way that it is neutral for the crop but detrimental for the hybrids. The tandem of transgene trait-deleterious trait can be inserted into a cytoplasmic genome, nuclear genome, or into the transposon elements (Kuvshinov et al. 2004; Gressel and Levy 2014; Gressel 2015). Genetic use restriction technology (GURT) (Hills et al. 2007) and the use of tissue-specific promoters (Roque et al. 2007) are some other plausible methods that can be used for sorghum improvement.

7 Conclusion

A study of wide hybridization must look beyond the success or failure of seed set. An understanding of pollen–pistil interaction and the reasons for success or failure of a hybridization must be assessed identifying pre- and post-fertilization barriers. Ploidy and genomic relationships and their use are necessary for successful introgression strategies. Sorghum breeders have had no tools at their disposal for wide hybridizations with species outside of the *Eusorghums*. Now some of the recently characterized genes and techniques should facilitate greater capacity to create additional interspecific and intergeneric hybrids to extract traits of value from those species for introgression into *S. bicolor*. For example, the discovery of the *Iap* locus has facilitated the study of genomic relationships beyond the *Eusorghums*. The presence of *iap* does not assure any given wide hybridization will succeed but increases the possibility. A second example is the development of chemical hybridizing agent trifluoromethanesulfonamide (TFMSA) (Hodnett and Rooney 2018). This CHA eliminates the need for hand emasculation or male sterility which opens hybridization potential to increased accession and/or numbers of florets. Finally, it is evident that 2*n* gametes are a major driver of polyploidy and exploiting them in *Sorghum* is just now beginning to be explored. Ploidy manipulation may prove to be key in creating bridges over which gene transfer will be possible. As we continue to define the genetic and genomic structure of each species, ploidy may be a significant player in the manipulation of the wild species as genes are introgressed into sorghum.

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