

REVIEW

## Domestication to Crop Improvement: Genetic Resources for *Sorghum* and *Saccharum* (Andropogoneae)

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• **Background** Both sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum*) are members of the Andropogoneae tribe in the Poaceae and are each other's closest relatives amongst cultivated plants. Both are relatively recent domesticates and comparatively little of the genetic potential of these taxa and their wild relatives has been captured by breeding programmes to date. This review assesses the genetic gains made by plant breeders since domestication and the progress in the characterization of genetic resources and their utilization in crop improvement for these two related species.

• **Genetic Resources** The genome of sorghum has recently been sequenced providing a great boost to our knowledge of the evolution of grass genomes and the wealth of diversity within *S. bicolor* taxa. Molecular analysis of the *Sorghum* genus has identified close relatives of *S. bicolor* with novel traits, endosperm structure and composition that may be used to expand the cultivated gene pool. Mutant populations (including TILLING populations) provide a useful addition to genetic resources for this species. Sugarcane is a complex polyploid with a large and variable number of copies of each gene. The wild relatives of sugarcane represent a reservoir of genetic diversity for use in sugarcane improvement. Techniques for quantitative molecular analysis of gene or allele copy number in this genetically complex crop have been developed. SNP discovery and mapping in sugarcane has been advanced by the development of high-throughput techniques for ecoTILLING in sugarcane. Genetic linkage maps of the sugarcane genome are being improved for use in breeding selection. The improvement of both sorghum and sugarcane will be accelerated by the incorporation of more diverse germplasm into the domesticated gene pools using molecular tools and the improved knowledge of these genomes.

**Key words:** Genomics, sorghum, *Sorghum bicolor*, sugarcane, *Saccharum officinarum*, crop improvement, domestication.

### INTRODUCTION

Crop plants were first cultivated around 10 000 years ago. However, crop domestication and development began much more recently (Doggett, 1970). Innumerable varieties, races and cultivars of agricultural plants have been developed to support human and animal demand for food, fibre and building materials. The Poaceae are an important global source of dietary protein, carbohydrates and other nutrients. Sorghum [*Sorghum bicolor* (L.) Moench] is the fourth most important cereal crop behind wheat, rice and maize, and is grown throughout the arid and semi-arid tropics (Smith and Frederiksen, 2000). Sugarcane (*Saccharum officinarum* L.) is the leading sugar-producing crop globally and is grown throughout tropical and subtropical parts of the world (Cordeiro *et al.*, 2006b). Sorghum and sugarcane are each other's closest relatives among cultivated crops. Their evolutionary divergence is estimated as occurring as recently as 5 million years ago, with maize having separated 15–20 million years ago (Paterson *et al.*, 2004). Intergeneric hybrids between the two groups have been reported, reinforcing their close relationship (Bowers *et al.*, 2003). Both are more recent domesticates than the other major grass crops

and despite ongoing breeding programmes using diverse germplasm, comparatively little of the genetic potential of these taxa and their wild crop relatives has been captured by breeding programmes to date.

### SORGHUM AND ITS WILD RELATIVES

#### *Sorghum domestication*

Archaeological evidence suggests that hunter-gatherers consumed sorghum as early as 8000 BC (Smith and Frederiksen, 2000). The domestication of sorghum has its origins in Ethiopia and surrounding countries, commencing around 4000–3000 BC. Numerous varieties of sorghum were created through the practice of disruptive selection, whereby selection for more than one level of a particular character within a population occurs (Doggett, 1970). This results from a balance of farmer selection for cultivated traits and natural selection for wild characteristics, generating both improved sorghum types, wild types and intermediate types (Doggett, 1970). These improved sorghum types were spread via the movement of people and trade routes into other regions of Africa, India (approx. 1500–1000 BC), the Middle East (approx. 900–700 BC) and eventually into the Far East (approx. AD 400). By the time sorghum was

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transported to America during the late 1800s to early 1900s, the diversity of new sorghum types, varieties and races created through the movement of people, disruptive selection, geographic isolation and recombination of these types in different environments would have been large (Wright, 1931; Doggett, 1970).

Initial domestication of sorghum would have focused primarily on converting wild types with small, shattering (dehiscent) seed to improved types with larger, non-shattering seed. Disruptive selection resulted in sorghum types with vastly different characteristics in height, inflorescence type, and of course, end use (food, fodder, fibre, building materials, etc). Over time, sorghum has been described and redescribed by numerous taxonomists (Fig. 1), and is now described under the family Poaceae, tribe Andropogoneae, subtribe Sorghinae and genus *Sorghum* Moench (Clayton and Renvoize, 1986).

The *Sorghum* genus as currently proscribed consists of 25 species (USDA ARS, 2007), although this varies in different scientific publications confirming the dynamic nature of the classification of cultivated sorghum and its wild relatives. The genus is separated into five taxonomic subgenera or sections: *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum* and *Stiposorghum* (Garber, 1950). Section *Eu-Sorghum* contains all domesticated/cultivated sorghum races and varieties as *Sorghum bicolor* subsp. *bicolor*, as well as the wild and weed species *S. halepense* (L.) Pers. (Johnsons grass), *S. propinquum* (Kunth) Hitchc, *S. × alnum* Parodi, *S. × drummondii* (Steud.) Millsp. & Chase, and *S. arundinaceum* (Desv.) Stapf. (the known progenitor of *S. bicolor*) (Harlan and de Wet, 1971; Doggett, 1988). All *S. bicolor* subsp. *bicolor* have  $2n = 2x = 20$  chromosomes, and are described as annual, with thick culms up to 5 m in height, often branched with many tillers. They have been classified into five basic races: *bicolor*, *guinea*, *caudatum*, *kafir* and *durra*, with ten intermediate races of these also recognized (Harlan and de Wet, 1972). These 15 races of cultivated sorghum are recognizable on spikelet/panicle morphology alone, and can be linked back to their specific environments and the nomadic peoples that first cultivated them (Smith and Frederiksen, 2000).

A comprehensive analysis of genetic diversity in sorghum landraces and core collections based on race, latitude of origin, photoperiod, seed quality, agronomic traits and DNA markers has demonstrated sorghum has considerable polymorphism that has been poorly exploited in terms of crop improvement (Wu *et al.*, 2004; Abu Assar *et al.*, 2005; Deu *et al.*, 2006; Kayode *et al.*, 2006). At the DNA level, two high-density maps have been completed, one intraspecific and another from an interspecific cross (between *S. bicolor* and *S. propinquum*). These maps showed a high colinearity from which the divergence between *Sorghum* species and the diversity within cultivated *S. bicolor* has been indicated (Feltus *et al.*, 2006).

#### *Changing characteristics/traits of domesticated sorghum and effects on yield*

Early domestication of sorghum was associated with changing the small-seeded, shattering open panicles

towards larger, non-shattering seeds and more compact panicles. This involved several factors: significantly increasing the number of branches within the inflorescence; decreasing the internode length of the rachis; and an increase in seed size so it protruded out of the glumes (House, 1985). These changes contributed to an increase in yield over the original sorghum landrace varieties.

Stable, high-yielding sorghum varieties have been recently developed through breeding/improvement programmes utilizing sorghum landrace varieties from Africa, India and China. This has involved selecting traits such as photoperiod insensitivity, reduced height (to reduce lodging), drought tolerance, and pest and disease resistance (Reddy *et al.*, 2006).

Plant height and photoperiod insensitivity were the focus of conversion programmes that developed sorghum lines with desirable plant height and maturity that were usable in breeding programmes in both tropical, short-day environments and in long-day, temperate and subtropical environments. As sorghum originated in north-eastern Africa, the many landraces and early varieties were photoperiod sensitive, with a critical photoperiod of 12 h: once the day length is shorter than 12 h, the sorghum plant changes from vegetative to reproductive growth (Reddy *et al.*, 2006). Growing these photoperiod-sensitive landraces/lines as a summer crop in temperate zones of America and Australia where the day length is longer than 13 h was difficult, especially as many growth-related characteristics are poorly expressed under these long-day conditions (Reddy *et al.*, 2006). This made breeding improved varieties in semi-arid temperate and subtropical climates difficult. Cultivars and landraces were identified in India that had higher critical photoperiods, with no delay in flowering observed when grown in day lengths up to 17 h. These photoperiod-insensitive sorghum cultivars have since been widely adopted in breeding programmes throughout the world (Rai *et al.*, 1999; Reddy *et al.*, 2006).

Plant height and grain yield are highly correlated in some populations of sorghum, with maximum productivity achieved at heights of around 1.75–1.80 m and flowering at 68–70 d (Miller, 1982; Rao and Rana, 1982). However, plants of these heights easily lodge, and are not easily cultivated under modern farming practices. A selection of high-yielding, tall sorghum landraces/lines were crossed to shorter, photoperiod-insensitive sorghum lines to develop improved high-yielding cultivars with a shorter stature (Miller, 1980; Rosenow and Dahlberg, 2000).

Sorghum is grown predominantly in low-rainfall, arid to semi-arid environments. The occurrence of drought stress is a major constraint to sorghum production globally. Two forms of drought stress have been identified in sorghum: ‘pre-anthesis’ where plants are stressed during panicle differentiation prior to flowering; and ‘post-anthesis’ when moisture stress occurs during the grain fill stage (Rosenow and Clark, 1995). The identification of varieties and lines with naturally high levels of pre-anthesis drought tolerance and the selection of these for higher yields has developed sorghum varieties with stable, high yields (Ellis *et al.*, 1997). Post-anthesis drought stress can result in significant yield loss due to small grain size, premature plant death and susceptibility to diseases.

Pliny described sorghum <sup>1</sup>	60–70 AD	
Named <i>Milium</i> sp <sup>1</sup>	1500s	
Named <i>Sorghum</i> and <i>Sorgo</i> <sup>1</sup>	1600s	
Michelli named <i>Sorghum</i> genus <sup>1</sup>	1729	
Michelli's work, Duna as cultivated sorghum <sup>1</sup>	1737	
Linnaeus named the gems <i>Holcus</i> , cultivated species <i>H. dora</i> , <i>H. docha</i> , <i>H. cafer</i> <sup>1</sup>	1751	
	1753	<sup>2</sup> <i>Holous sorghum</i> L. and <i>H. saccoratist</i>
	1794	<sup>2</sup> <i>Holous bicolor</i>
Moench renamed genus <i>Sorghum</i> , <i>S. bicolor</i> <sup>1</sup>	1805	<sup>2</sup> Moench included <i>H. bicolor</i> and <i>H. saccar</i>
	1848	<sup>2</sup> <i>S. halepense</i> L. (Persoon) described
<i>S. halepense</i> recognized <sup>1</sup>	1866	
Alefold names cultivated sorghum <sup>1</sup> <i>Andropogon sorghum</i>	1888	
Koemicke names cultivated sorghum <sup>1</sup> <i>Andropogon sorghum</i>	1911	<sup>2</sup> Ewart used <i>Sarga</i> for <i>S. stipoideum</i>
Chiovenda returned to <i>Sorghum</i> genus <sup>1</sup>	1912	
Stapf returned to <i>Sorghum</i> genus <sup>1</sup>	1917	
Snowden ID 31 cultivated sp and 17 wild sp <sup>1</sup>	1936	<sup>2</sup> Snowden described <i>S. bicolor</i> races
Hitchcock and Chase, Sorghos for sweet sorghums <sup>3</sup> <i>S. bicolor</i> sub sp. <i>sacroratist</i>	1950	<sup>4</sup> Garber names 5 sections; <i>Eu-Sorghum</i> , <i>Heterosorghum</i> , <i>Chaetosorghum</i> , <i>Stiposorghum</i> and <i>Para-Sorghum</i>
	1961	<sup>2</sup> Clayton confirmed Moench description of <i>Sorghum</i>
Harlan and de Wet; five races of <i>S. bicolor</i> , <i>bicolor</i> , <i>guinea</i> , <i>candatum</i> , <i>kafir</i> and <i>Duna</i> <sup>1</sup>	1971	
	1976	<sup>3</sup> Harlan described four wild races in <i>S. bicolor</i> : <i>arundinaceum</i> , <i>virgatum</i> <i>aethiopicum</i> , <i>vertiflorum</i>
De Wet, 28 of Snowden's cultivated taxa into <i>S. bicolor</i> and 13 wild taxa into <i>S. bicolor</i> subsp. <i>arundinaceum</i> <sup>1</sup>	1978	<sup>2</sup> De Wet, all cultivated sp into <i>S. bicolor</i> , split into races by particle morphology and described <i>S. propinquum</i> and <i>S. halepense</i>
	1988	<sup>3</sup> Doggett described four weedy races under <i>S. bicolor</i> sub sp. <i>verriciflorum</i>
	1991	<sup>5</sup> Lazarides confirms Garber's 5-section split
	2003	<sup>2</sup> Spangler splits <i>Sorghum</i> into three genera: <i>Sorghum</i> , <i>Vacoparis</i> and <i>Sarga</i> . <i>Para-Sorghum</i> and <i>Stiposorghum</i> species combined as <i>Sarga</i> .
Molecular evidence to maintain <i>Sorghum</i> genus as described by Lazarides in 1991 <sup>3</sup>	2005	
Further molecular evidence supporting Lazarides <i>Sorghum</i> description <sup>2</sup>	2007	

FIG. 1. Time-line displaying the changes in *Sorghum* nomenclature over time. <sup>1</sup>House *et al.* (1995); <sup>2</sup>Spangler (2003); <sup>3</sup>Smith and Frederiksen (2000); <sup>4</sup>Garber (1950); <sup>5</sup>Lazarides *et al.* (1991); <sup>6</sup>Hodnett *et al.* (2005), Price *et al.* (2005a); <sup>7</sup>Dillon *et al.* (2007).

Post-flowering drought tolerance is referred to as stay-green, with plants maintaining green leaf area and photosynthetic capability under severe moisture stress, which results in higher grain yields compared with senescent varieties (Borrell and Douglas, 1997; Borrell *et al.*, 1999). The physiological components of stay-green (green leaf area at flowering; time of onset of senescence; rate of senescence) are

independently inherited and easily combined through breeding, resulting in new sorghum varieties exhibiting high levels of stay-green with stable high yields and good levels of insect resistance (Borrell *et al.*, 2000).

Sorghum production is affected by many pests and diseases globally. Some of the major pests include midge (*Stenodiplosis sorghicola* Coquillett), green bug (*Schizaphis*

*graminum* Rondani), various aphids, shootfly (*Atherigona soccata* Rondani) and stem borer (*Chilo partellus* Swinhoe) (Sharma, 1993). Major diseases include downy mildew, anthracnose, sorghum rust, leaf blight, ergot and head and kernel smut (House, 1985). Success in breeding for insect resistance in sorghum varieties has been varied. Resistance to some pests is quantitatively inherited and therefore difficult to transfer into high-yielding cultivars (Tao *et al.*, 2003). The exception to this is midge resistance, where high levels of midge immunity have been incorporated from Indian, American and Australian breeding lines into elite, high-yielding sorghum varieties in Australia, with greater than 80% of the planted area utilizing these resistant varieties (Jordan *et al.*, 1998; Tao *et al.*, 2003).

Development of disease-resistant sorghum varieties has relied on identifying sorghum varieties/landraces with natural genetic resistance to the particular disease. To date, commercial sorghum varieties have been developed with resistance to grain moulds and anthracnose (Reddy *et al.*, 2006).

The development of photoperiod-insensitive, dwarfed sorghum varieties with some levels of pest/disease resistance has improved the yields of cultivated sorghum varieties. However, the development of a hybrid cropping system is responsible for increases in yields of more than 300% since the 1950s (Rooney and Smith, 2000). Hybrid cultivars make use of male sterility to enhance the combining abilities of the parental lines, resulting in heterosis and significant increases in phenotypic traits such as yield, plant height and days to flowering (Reddy *et al.*, 2006).

Although the domestication and resulting super-domestication of sorghum has relied on principally *S. bicolor* subsp. *bicolor* varieties/landraces/lines for significant gains in agricultural production, the undomesticated *Sorghum* species offer an untapped wealth of novel traits for both biotic and abiotic stress resistance and yield.

#### *Undomesticated Sorghum species as genetic resources for sorghum improvement*

All cultivated sorghum varieties and landraces are *S. bicolor* subsp. *bicolor* of the *Eu-Sorghum* subgeneric section of the *Sorghum* genus. The other four sections, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum* and *Stiposorghum* contain 19, wild species native to Africa, Asia and Australia (Garber, 1950; Lazarides *et al.*, 1991). These species are briefly outlined below, and contain new sources of genetic diversity for agronomic traits affecting yield, survivability and novel traits that may create new markets for sorghum products.

The monotypic sections *Chaetosorghum* and *Heterosorghum* contain the octaploid ( $2n = 40$ ) Australian species *S. macrospermum* E.D. Garber and *S. laxiflorum* F.M. Bailey, respectively. Section *Para-sorghum* contains the five Australian species *S. grande* Lazarides, *S. leiocladum* (Hack.) C.E. Hubb., *S. matarankense* E.D. Garber & Snyder, *S. nitidum* (Vahl) Pers., *S. timorensis* (Kunth) Buse, and the two African/Asian species *S. purpureo-sericeum* (Hochst. ex A. Rich.) Asch. & Schweinf. and *S. versicolor* Andersson. These species range

in ploidy from  $2n = 10$  to  $2n = 40$ , with *S. grande*, *S. nitidum* and *S. timorensis* showing varying ploidy within species. Ten Australian endemic species form section *Stiposorghum*: *Sorghum amplum* Lazarides, *S. angustum* S.T. Blake, *S. brachypodium* 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. stipoides* (Ewart & Jean White) C.A. Gardner & C.E. Hubb. (Garber, 1950; Lazarides *et al.*, 1991). Most of these species are diploid with  $2n = 10$  chromosomes, while *S. interjectum* has  $2n = 30, 40$  and *S. plumosum* has  $2n = 10, 20, 30$  (Garber, 1950; Lazarides *et al.*, 1991).

The adaptability of these undomesticated *Sorghum* species to colonize a wide range of soil and moisture conditions across a wide range of microenvironments is shown through their ability to survive very hot, dry, nutrient-limited environments. Due to their adaptability, many of the undomesticated *Sorghum* species have developed resistances to the many pests and diseases that affect sorghum grain production globally. Interestingly, many Australian undomesticated species contain resistances to the major pest/diseases of Africa and America, which are not yet present within Australia (Bapat and Mote, 1982; Karunakar *et al.*, 1994; Franzmann and Hardy, 1996; Sharma and Franzmann, 2001; Kamala *et al.*, 2002; Komolung *et al.*, 2002).

Recent controlled-environment glasshouse trials have shown that the undomesticated *Sorghum* species, though adapted to specific abiotic conditions in the wild, showed prolific growth under moderate temperature in a standard potting mix and watered regularly (Table 1). These data show useful variations to germination times and time to flowering. Representatives of the undomesticated *Heterosorghum*, *Para-Sorghum*, *Stiposorghum* and a *Eu-Sorghum* were grown concurrently to compare their development under controlled conditions (Fig. 2). Cultivated *S. bicolor* takes 3–10 d to germinate depending on soil temperatures, with the first 30–35 d post-germination undergoing lower leaf growth followed by a rapid elongation in non-dwarf varieties. Flowering in *S. bicolor* occurs 55–70 d post-germination and seeds reach physiological maturity 30–40 d post-anthesis. It then takes 20–25 d to reduce the moisture content to the 12% required for post-harvest storage (House *et al.*, 1995). There appears to be limited differences between undomesticated species and *S. bicolor* for these traits (Table 1).

#### *Undomesticated Sorghum species: grain attributes*

The morphology of seed size and shape within the *Sorghum* genus varies greatly. Figure 3 shows the morphology of the domesticated *S. bicolor* subsp. *bicolor* and undomesticated *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum* and *Stiposorghum* species. Variation in the grain morphology of representatives of the undomesticated *Heterosorghum*, *Para-Sorghum* and *Stiposorghum* species have also been evaluated at the microscopic level. Mature caryopses of 13 species were critically point dried, snap fractured and examined using a Leostereoscan 440 scanning electron



TABLE 1. Germination, growth rates and flowering times for undomesticated Sorghum species

Species	DTT*	Height (cm)				Days till flower	Number of panicles				
		30 d	60 d	90 d	130 d		10 dpa	20 dpa	30 dpa	40 dpa	Final max count
<i>S. amplum</i>	4.5	25	40	40	125 fl	136	md	16	29	–	–
<i>S. angustum</i>	5.5	18	40	62 fl	95 fl	86	9	20.5	58	146	>300
<i>S. brachypodum</i>	3.5	35	70	150 fl	210 fl	86	4	26.5	45	62.5	>300
<i>S. bulbosum</i>	3.0	30	55	120 fl	170 fl	95	22	67	89	–	–
<i>S. exstans</i> (p)	3.5	12	45 fl	70 fl	95 fl	65	10	17	47	113	235
<i>S. intrans</i> (p)	3.5	5	20 p	20 p	85.0	>160	–	–	–	–	–
<i>S. laxiflorum</i>	5.5	60	60 fl	90 fl	95 fl	61	md	16.5	44	94	214
<i>S. leiocladum</i>	5.5	27	30	35	85.0	141	1	–	–	–	–
<i>S. matarankense</i>	4.5	58	88 fl	130 fl	150 fl	81	2	5	23	53	69
<i>S. nitidum</i>	4.0	25	48	60	90	>160	–	–	–	–	–
<i>S. plumosum</i>	8.0	8	15	15	60	>160	–	–	–	–	–
<i>S. propinqueum</i>	3.0	45	50	100	240 fl	106	19	35	–	–	>300
<i>S. stipoideum</i>	4.0	45	70	60 fl	130 fl	91	18	60	112	–	–
<i>S. timorese</i>	3.0	45	100 fl	100 fl	145 fl	69	md	3	14	18	63

\* DTT, Days till transplant: seeds were germinated on damp filter paper; once a strong radicle and the first coleoptile had emerged they were transplanted to potting mix.

md, Missing data, (p), prostrate growth habit; fl, height was measured to the top of flag leaf or seed head.

A dash indicates that delayed onset of flowering caused the trial to be terminated before counts could be made.

microscope to determine if novel variations existed in the undomesticated species (Shapter *et al.*, 2007).

The endosperm of cultivated *S. bicolor* is described as having two distinct regions or layers. The floury central endosperm (Fig. 4A) contains simple round or lenticular starch granules in a discontinuous protein matrix with few if any protein bodies present. The vitreous or corneous outer endosperm (Fig. 4B) is characterized by polygonal starch granules, 4–25 µm in diameter, the surface of which is typically indented from the protein bodies that are part of the continuous protein matrix surrounding the granules. Variations to the distribution and configuration of these two regions have been shown to alter the functional and putatively the nutritional value of sorghum flours and other foods (Serna-Saldivar and Rooney, 1995; Lindeboom *et al.*, 2004; Tesso *et al.*, 2006).

The undomesticated *Sorghum* species showed varied distribution of protein bodies throughout the endosperm (Fig. 4C–H). Similarly, variation in the starch granule size and shape was also noted (Shapter *et al.*, 2007). Some of the undomesticated species had distinctly

smaller, more spherical granules throughout the endosperm (Fig. 4D). Importantly, several species showed native channelling of the starch granules and pores on their surface (Fig. 4C) which have been shown to improve the digestion of sorghum starches (Fannon *et al.*, 2003, 2004; Benmoussa *et al.*, 2006). One species appeared to have sections of the endosperm with small rice-like starch granules, usually only seen in the sub-aleurone layer in *S. bicolor* (Shapter *et al.*, 2007). Several wild species also maintained a single morphology across the entire endosperm, rather than the two layers seen in *S. bicolor*. Amongst these differences some species retained the characteristic morphology of the *S. bicolor* vitreous layer (Fig. 4F).

The sub-aleurone of *S. bicolor* is described as being 15–30 µm wide and is an area of very small starch granules and denser protein matrix, the endosperm proper (Fig. 5A and B).

In the *Para-Sorghum* and *Stiposorghum* species examined, areas of the sub-aleurone have a striated appearance (Fig. 5C) not previously reported in microscopy studies (F. M. Shapter *et al.*, unpubl. res.). Investigation of these areas under high magnification showed what appeared to be a much denser protein matrix, embedded with spherical-shaped bodies reminiscent of protein bodies. Within this layer, small starch granules typical of sub-aleurone starch granules are interspersed (Fig. 5D and E). More investigation is needed to confirm if this layer is proteinaceous. From an adaptive point of view, the development of a highly proteinaceous layer directly below the aleurone would provide a rich nitrogen source for the germinating seedling when establishing itself in low nitrogen soils, typical of northern Australia where many of these undomesticated species are endemic. Protein/starch interactions in sorghum have been shown to decrease starch digestibility, especially after cooking (Duodo *et al.*, 2003). The occurrence of increased protein content in the endosperm may therefore result in a further decrease in starch digestibility



FIG. 2. Growth trial of *Sorghum* species at seedling stage. Note the broader leaf (far left) of the *Eu-sorghum*, *S. propinqueum* compared with the *Para-Sorghum*, *Stiposorghum* and *Heterosorghum* species.

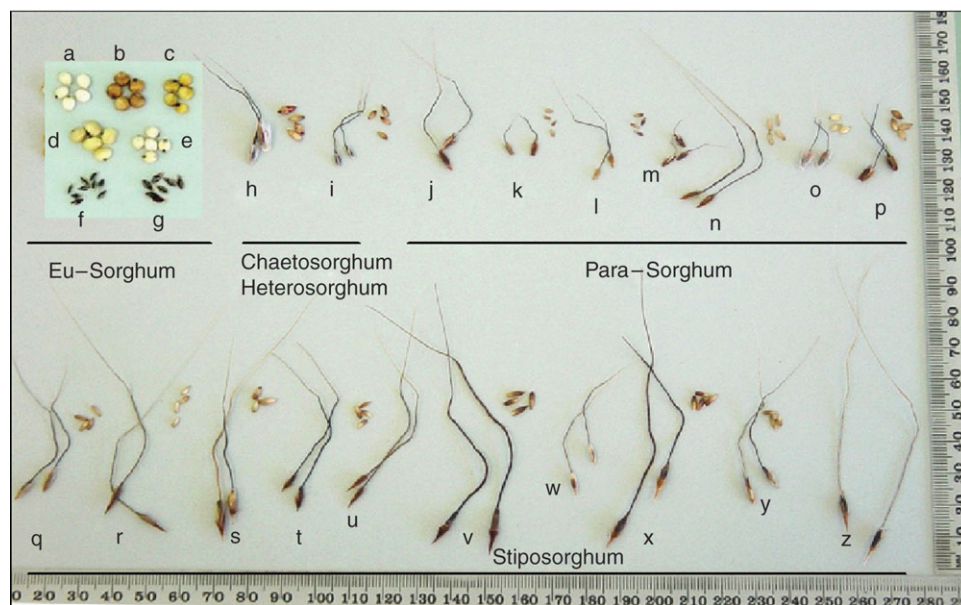


FIG. 3. Variation in *Sorghum* species seed and caryopsis morphology and size. Letters on the figure denote different species: a–e, *S. bicolor* caryopsis AusTRCF 322649, 322618, 322620, 322666 and 322611, respectively; f, *S. propinquum*; g, *S. halepense*; h, *S. macrospermum* 322277 seed and caryopsis; i, *S. laxiflorum* 302503 seed and caryopsis; j, *S. grande* 302580 seed; k, *S. leiocladum* 300170 seed and caryopsis; l, *S. matarankense* 302521 seed and caryopsis; m, *S. nitidum* 302539 seed; n, *S. timorensis* 302660 seed and caryopsis; o, *S. purpureo-sericeum* 321134 seed and caryopsis; p, *S. versicolor* 321126 seed and caryopsis; q, *S. amplum* 302623 seed and caryopsis; r, *S. angustum* 302604 seed and caryopsis; s, *S. brachypodium* 302480 seed and caryopsis; t, *S. bulbosum* 302646 seed and caryopsis; u, *S. ecarinatum* 302661 seed; v, *S. exstans* 302577 seed and caryopsis; w, *S. interjectum* 302563 seed; x, *S. intrans* 302390 seed and caryopsis; y, *S. plumosum* 302489 seed and caryopsis; z, *S. stipoideum* 302644 seed.

which has utility for raising the glycaemic index of foods for Western diets.

#### Hybridizing potential of undomesticated sorghum species

Modern sorghum breeding programmes have not used species outside of section *Eu-Sorghum* as sources of genetic diversity due to a lack of information regarding the genetic relationships between the species. Recent phylogenetic analysis of all 25 *Sorghum* species based on the three gene sequences ITS1, *ndhF* and *Adh1* has identified *S. macrospermum* and *S. laxiflorum* as the undomesticated species outside of *Eu-Sorghum* most closely related to cultivated sorghum varieties (Dillon *et al.*, 2007). The relationships identified can now act as a guide for plant breeders.

Most of the undomesticated *Sorghum* species fall within the tertiary genepool, making gene transfer to domesticated species very difficult due to strong sterility barriers (Harlan and de Wet, 1971). The nature of the sterility barriers in *Sorghum* have recently been identified as pollen–pistil incompatibilities whereby the pollen of undomesticated species behaves abnormally in the pistils of *S. bicolor*, resulting in no hybrid embryo formation (Hodnett *et al.*, 2005). As a result, pollen rarely grew beyond the stigma of *S. bicolor*; however, a single embryo was formed using *S. macrospermum* pollen. The embryo of this *S. bicolor* × *S. macrospermum* cross was rescued and raised through tissue culture, with the seedling verified as a hybrid based upon cytological and morphological characteristics (Price *et al.*, 2005b).

Although a hybrid embryo was formed and able to be rescued via tissue culture, pollen–pistil incompatibilities make this an extremely rare occurrence. Methods of increasing the frequency of hybridization are required to successfully utilize the undomesticated *Sorghum* species. An *S. bicolor* accession was discovered containing a recessive gene (inhibition of alien pollen = *iap*) that allowed maize (*Zea mays* L.) pollen tubes to grow through *S. bicolor* pistils (Laurie and Bennett, 1989). This *S. bicolor* accession can successfully override the pollen–pistil incompatibilities between *S. bicolor* and undomesticated *Sorghum* species and lead to the production of hybrid embryos and plants (Price *et al.*, 2006). Hybrids between *S. bicolor* × *S. macrospermum* were obtained from germinated seeds, while the hybrids between *S. bicolor* × *S. angustum* and *S. bicolor* × *S. nitidum* were recovered through embryo rescue and tissue culture. The hybrid nature of these seedlings was again confirmed by the presence of genomes from both parental species that could be readily identified based upon chromosome size and number (Price *et al.*, 2006).

Introgression of the undomesticated *S. macrospermum* genome with cultivated *S. bicolor* has been tracked using FISH (fluorescent *in situ* hybridization) (Kuhlman *et al.*, 2006). FISH discriminated between the chromosomes of the two parent species, and confirmed through bivalent formation and allosyndetic pairing that recombination was occurring. Progeny of this novel hybrid when backcrossed to *S. bicolor* expressed altered fertility, again confirming that introgression from the undomesticated parent has



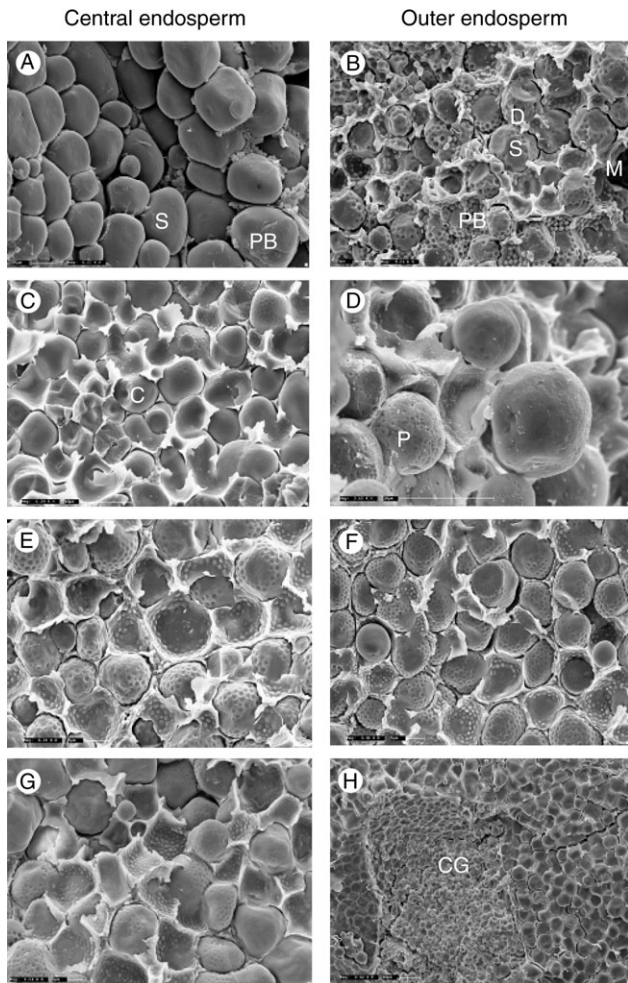


FIG. 4. The left-hand column shows the variation in the central endosperm and the right-hand column compares the outer layers. (A, B) Representative images of *S. bicolor*, showing the standard floury and vitreous endosperm, respectively. (C–H) Images from outside the *Eu-Sorghums* are representative of the variations observed across the species. PB, Protein bodies; M, matrix; S, starch granule; D, indentations left by protein bodies; C, channels; P, pores; CG, small polygonal starch granules forming compound granules.

occurred (Kuhlman *et al.*, 2006). The analysis of the amount of DNA introgressed from the undomesticated *S. macrospermum* is currently being undertaken using AFLPs (L. C. Kuhlman *et al.*, unpubl. res.).

The identification and use of the *iap S. bicolor* accession has enabled the successful introgression of genes from undomesticated *Sorghum* species into cultivated sorghum, and is the first step towards accessing these unique unexploited genes for both biotic and abiotic stresses and agronomic traits. The potential for improving the yield productivity through these traits in commercial sorghum varieties is now a reality.

#### The role of genomics in improving domesticated *S. bicolor*

*Sorghum bicolor*, a diploid, has a relatively small genome (735 Mbp), which although larger than rice

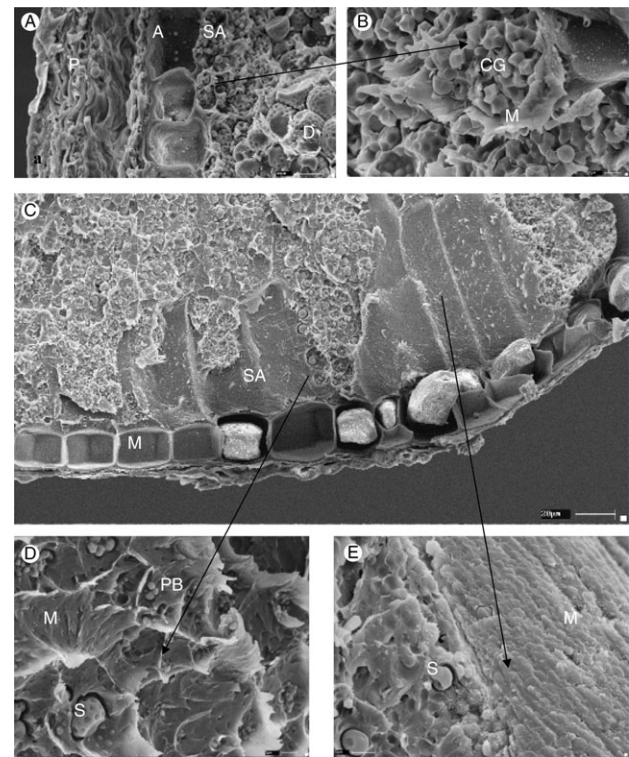


FIG. 5. Novel sub-aleurone morphology of the *Para-Sorghums* and *Stiposorghums*: (A) the characteristic *S. bicolor* outer endosperm and pericarp; (B) an increased magnification of the sub-aleurone layer itself; (C) a representative image of the novel morphology found in the undomesticated sorghums; (D) and (E) magnified features of the morphology shown in (C). SA, Sub-aleurone layer; A, aleurone; PB, protein bodies; M, matrix; S, starch granule; D, indentations left by protein bodies; P, pericarp; CG, small polygonal starch granules forming compound granules.

(389 Mbp) is smaller than the other important cereals (wheat 16 900 Mbp, maize 2600 Mbp). The last genome duplication event for the *S. bicolor* genome seems to have occurred much earlier than the divergence of the major cereal crops from a common ancestor (Paterson *et al.*, 2004). Completion of the whole genome sequencing project in 2007 will exponentially increase the sequence data available for *Sorghum* and will provide valuable information on cereal domestication in the African continent, an event that appears to have occurred independently of other continents though by similar reinforced selective pressures (Paterson *et al.*, 2004). In a way, the sorghum genome sequencing will close a biographic triangle into the knowledge of the polymorphism shared before the divergence of these important grasses and ultimately in the understanding of the evolution in cereals crops between Africa, America and Asia (Kresovich *et al.*, 2005). The tenets of colinearity and microlinearity of grass genomes mean that our knowledge of other cereals and their evolutionary ties will also greatly improve. Due to their economic and scientific value, cereal genomes have been studied over the last 15 years using highly advanced technologies. The similarity at the DNA level makes it possible to use comparative genetics to look for particular genes of unknown sequence

between the genomes with the aim of using that information to develop new varieties or discovering new genes that could have a potential impact on traits that are of global importance (e.g. food quality, drought resistance).

The genetic diversity existing within and between Australian *Sorghum* species was recently evaluated using simple sequence repeats (SSRs) (Dillon *et al.*, 2005). SSRs were sourced from the cultivated *S. bicolor* (Brown *et al.*, 1996; Taramino *et al.*, 1997; Kong *et al.*, 2000) to determine diversity in these closely related taxa. This method has successfully evaluated diversity in the related species of many crop groups (e.g. Peakall *et al.*, 1998; Hernández *et al.*, 2001; Chen *et al.*, 2002; Scott *et al.*, 2003; González-Martínez *et al.*, 2004; Sudupak, 2004). This evaluation of the Australian species has shown significantly higher levels of genetic diversity both between (inter-) and within (intra-) species compared with the intra-specific diversity of *S. bicolor* varieties. The relatively high transfer rate of *S. bicolor*-derived SSRs to the wild species and their high level of diversity suggests that these SSRs are an efficient, highly informative source of molecular markers for the undomesticated *Sorghum* species.

#### Screening for novel genetic variation in *S. bicolor*

Mutations, both natural and artificially induced, provide an alternate source of genetic diversity. Mutants have long been a valuable resource in plant breeding (van Harten, 1998) and, in recent times, in plant genomics research (Henikoff and Comai, 2003; Till *et al.*, 2003; Henikoff *et al.*, 2004). However, the method employed (irradiation or chemical) to induce a mutated population will affect its usefulness and application for genomics research. A review of the comprehensive International Atomic Energy Agency's Mutant Varieties Database (<http://www-mvd.iaea.org/MVD/default.htm>) shows only 15 induced sorghum mutant accessions amongst more than 2500 registered mutants.

As a result of the random nature of mutation induction, by physical and chemical means, each individual in a population will contain a unique range of gene mutations. This provides a powerful resource for genome analysis employing recent molecular technologies. It is well established that the ultimate goal in DNA research is to ascertain the DNA sequence of a gene. However, the existing technology for genotyping has become a powerful way to avoid the sequencing step or at least for reducing dramatically the number of samples needed to be sequenced. Analysis of DNA polymorphism in natural and mutated populations is more efficient with the use of capillary electrophoresis (Szantai *et al.*, 2005; Davies *et al.*, 2006) which has the advantages of improved efficiency, sensitivity and throughput (Tang *et al.*, 2004) when compared with gel electrophoresis (Vouk *et al.*, 2000; Cordeiro *et al.*, 2006b). Additionally, the use of capillary electrophoresis has the advantage of reducing costs and time through multiplexing (Kan *et al.*, 2004).

Gamma irradiation and EMS (ethyl-methane-sulfonate) mutation protocols have been optimized for selected

*S. bicolor* populations to generate random changes in the sorghum genome. The second generation of plants was screened to assess the amount of polymorphism that has been generated and now mutations can be identified in candidate genes by utilizing an approach to genetic analysis called TILLING (Targeting Induced Local Lesions IN Genomes), which was first applied in plants by McCallum *et al.* (2000). A significant body of scientific literature is now available on this technique (Comai and Henikoff, 2006).

TILLING allows for genotypic screening for allelic variations prior to commencing with the more costly and labour-intensive phenotyping (Henikoff *et al.*, 2004). EMS-induced TILLING populations have been produced for the major cereal crops: wheat (Slade *et al.*, 2005), rice (Wu *et al.*, 2005), barley (Caldwell *et al.*, 2004), maize (Till *et al.*, 2004) and sorghum (in the authors' laboratory). TILLING is fast becoming a mainstream technology for mutation characterization (Comai and Henikoff, 2006) and for analysing single nucleotide polymorphisms (SNP) (Cordeiro *et al.*, 2006a). A very sensitive high-throughput screening method based on capillary electrophoresis has been developed (Cross *et al.*, 2007) using Endonucleolytic Mutation Analysis by Internal Labelling (EMAIL) to greatly improve the effectiveness of this new reverse genetics approach to crop improvement.

#### THE GENOME OF SUGARCANE AND ITS WILD RELATIVES

Sugarcane is an important vegetatively propagated crop which is cultivated for its sugar-rich stalks. It contributes an estimated 75% of the world's sucrose supply with its mature stem capable of accumulating 12–16% of its fresh weight and approx. 50% of its dry weight as sucrose (Bull and Glasziou, 1963). Sugarcane originated in South-east Asia and New Guinea (Lebot, 1999). Modern cultivated sugarcane (*Saccharum* spp.) is a hybrid complex originating from crosses between *S. officinarum* L. and *S. spontaneum* L., and in some lineages *S. sinense* Roxb., or *S. barberi* Jesw (Edme *et al.*, 2005). Limited numbers of clones, and hence genetic variation, of the two major progenitors have been captured by commercial breeding programmes. Sugarcane, like sorghum, is a relatively recently domesticated species with little of the available genetic diversity having been incorporated or actively analysed for introgression into domesticated varieties. Breeding programmes in the early 1900s focused on hybridization of *S. officinarum* clones but soon progressed to inter-specific crosses incorporating *S. spontaneum*. This resulted in improved agronomic traits, such as ratooning and disease resistance, but required a backcrossing programme to *S. officinarum*, called 'nobilization', to elevate the sucrose content (Roach, 1989; Edme *et al.*, 2005). Since then the majority of breeding programmes have focused on inter-crossing between the hybrids, though in recent decades the larger increases in genetic gain have been made by incorporating more diverse germplasm into the cultivated backgrounds (Edme *et al.*, 2005).



### Taxonomy of sugarcane

Sugarcane belongs to the genus *Saccharum*, first established by Linnaeus in *Species Plantarum* in 1753 with two species: *S. officinarum* and *S. spicalum* L. The original classification of Linnaeus' has since been revised to contain six species: *S. officinarum*, known as the noble cane; *S. spontaneum* L., *S. robustum* E.W. Brandes & Jeswiet ex Grassl, and *S. edule* Hassk., classified as wild species; and *S. sinense* Roxb. and *S. barberi* Jeswiet, classified as ancient hybrids (Buzacott, 1965; Daniels and Roach, 1987; D'Hont and Layssac, 1998). The genus falls in the tribe Andropogoneae in the grass family, Poaceae, that includes other tropical grasses such as *Sorghum* and *Zea* (maize). Closely related to *Saccharum* are another four genera (*Erianthus* section *Ripidum*, *Miscanthus* section *Diandra*, *Narenga* and *Sclerostachya*) that purportedly readily interbreed, forming the 'Saccharum complex' (Daniels and Roach, 1987). They have in common a high level of polyploidy and aneuploidy (unbalanced number of chromosomes) that creates a challenge for both the taxonomist and molecular biologist (Daniels and Roach, 1987; Sreenivasan *et al.*, 1987).

### The sugarcane genome

The complexity and size of the sugarcane genome is a major limitation in genetic improvement. Whilst continued selective breeding for enhanced sucrose accumulation has been able to achieve over half of the yield increase in the past 50 years, it has been reported as having reached a plateau due to limits to the gene pool exploited in traditional breeding programmes (Mariotti, 2002). Individual research programmes, however, have been shown to still be making significant annual genetic gains by maintaining a diverse gene pool (Edme *et al.*, 2005). The employment of new technologies to assist in the association of traits with genetic markers and genetic maps can aid in achieving further yield increases in breeding programmes.

Most sugarcane cultivars contain more than 100 chromosomes which can be assigned to eight homology groups (Rossi *et al.*, 2003; Aitken *et al.*, 2005). Over the past two decades, studies utilizing various molecular techniques to unravel the complexity of this important crop species have provided a greater understanding of its complex genetic make-up (Bonierbale *et al.*, 1988; Wu *et al.*, 1992; D'Hont, 1994; Sills *et al.*, 1995; Grivet *et al.*, 1996; Ming *et al.*, 2001; Rossi *et al.*, 2003). Significant achievements include milestones that demonstrate the use of single (markers present on one chromosome only) and double dose (marker present on two chromosomes) markers for mapping and QTL analysis (Ming *et al.*, 2001, 2002; Hoarau *et al.*, 2002; Aitken *et al.*, 2004), and large-scale EST sequencing projects by SUCEST-Sugar Cane EST Genome Project (Vettore *et al.*, 2001), SASRI-South African Sugar Research Institute (Carson and Botha, 2000), UGA-University of Georgia, USA (Ma *et al.*, 2004), and CSIRO- Australia's Commonwealth Scientific and Industrial Research Organization (Casu *et al.*, 2004). Unfortunately, despite these achievements,

the pace of progress with sugarcane genomics has lagged behind that achieved with other agricultural crops (Ramsay *et al.*, 2000; Delseny *et al.*, 2001; Mullet *et al.*, 2002).

### Analysis of variation in the sugarcane genome

In 1997, an effort was made by the International Consortium for Sugarcane Biotechnology to develop and evaluate simple sequence repeats (SSRs) or microsatellite sequences as a marker system for sugarcane. Markers were developed from an enriched microsatellite library and were shown to have the capacity to distinguish between sugarcane genotypes due to their ability to detect large numbers of alleles (Cordeiro *et al.*, 2000). To date, this marker system has delivered a number of applications that have advanced both sugarcane research and breeding. Published applications include the mapping of alleles generated from 72 SSR primer pairs onto a genetic map constructed on the Australian hybrid cultivar, Q165<sup>A</sup> (Aitken *et al.*, 2005); validation of the introgression of genes into F<sub>1</sub> hybrids of crosses made between *S. spontaneum* and elite commercial clones (Pan *et al.*, 2004); the confirmation of fertile intergeneric F<sub>1</sub> hybrids of *S. officinarum* and *E. arundinaceus* as well as backcross (BC<sub>1</sub>) progeny from the F<sub>1</sub> to hybrid sugarcane (Cai *et al.*, 2005); and the use of the markers to register and confirm sugarcane varieties by the United States Department of Agriculture (USDA) (Tew *et al.*, 2003). SSR markers have also been used to draw useful information on the relationships between various members of the 'Saccharum complex' (Cordeiro *et al.*, 2003; Cai *et al.*, 2005) as well as relationships between clonal cultivars of hybrid canes (Pan *et al.*, 2003a). A fingerprint database of major Australian sugarcane cultivars has been developed using these markers (Piperidis *et al.*, 2001) as has molecular genotyping of elite clones produced by the USDA (Pan *et al.*, 2003a, b).

### High-throughput SNP genotyping

High-throughput genotyping technologies based on single nucleotide polymorphisms (SNPs) or small-scale insertion/deletions (indel) could become efficient alternative tools for traditional markers because of their greater abundance in the genome and ease of measurement. SNPs are being identified and rapidly mapped to provide a rich source of genetic information with the potential for allowing a greater insight into understanding the genetic complexity of many organisms. SNPs are present in high frequency in any genome, amenable to high throughput analysis and have the ability to reveal hidden polymorphisms where other methods fail (Bhattaramakki and Rafalski, 2001). In plants, a number of studies have been able to link SNPs with phenotypic traits of agronomic interest, such as the putative betaine aldehyde dehydrogenase gene responsible for the fragrance trait in rice (Bradbury *et al.*, 2005) and SNPs found in the starch synthase IIa gene associated with starch gelatinization temperature in rice (Waters *et al.*, 2005). These studies highlight the usefulness

of SNP markers, demonstrating both the abundance of this marker type and the potential causal association between a single nucleotide alteration and organism phenotype. A further major advantage of SNP markers is that they allow easy and unambiguous identification of alleles or haplotypes.

Whilst numerous technical methods have been developed for their detection (Gut, 2001), the majority are applicable mainly to diploid genomes where a simple presence/absence of either one or both of the alternative bases would indicate homozygosity or heterozygosity. Sugarcane, with its complex genome comprising an estimated 8–14 copies of every chromosome (Rossi *et al.*, 2003; Aitken *et al.*, 2004), can have up to 14 different alleles present, with individual alleles in varying numbers. Thus, the frequency of an SNP base at a gene locus will be determined by both the number of chromosomes carrying the gene, and the number of different alleles (or haplotypes) and frequency of each allele possessing each SNP base. Hence, any method used to detect SNPs at a particular locus in sugarcane must be able to determine the frequency of each SNP base in different genotypes, rather than simply detecting the presence and absence of SNPs. Such detection systems are generally more complex and expensive than simpler and more common methods used for detecting less complex genomes (Ross *et al.*, 1998; Ahmadian *et al.*, 2000; Alderborn *et al.*, 2000; Nurmi *et al.*, 2001; Storm *et al.*, 2003).

#### *Use of SNPs in sugarcane*

Currently, whilst there are only a limited number of papers describing the use of SNPs to understand the sugarcane genome, they point to this marker system as a valuable means of mapping candidate genes and for identifying the genetic basis of QTLs of agronomically important traits. These studies include a discussion on the ability of SNPs to: delineate a set of 64 ESTs into two groups that are likely to represent two gene family members of 6-phosphogluconate dehydrogenase (Grivet *et al.*, 2001); delineation of 178 ESTs into three paralogous genes to reveal the expression of an *Adh2* and two *Adh1* genes in sugarcane (Grivet *et al.*, 2003); the development of co-dominant cleaved amplified polymorphic sequence (CAPS) markers (Quint *et al.*, 2002); and to map several candidate genes and ESTs (McIntyre *et al.*, 2005).

In sugarcane, the proportional frequencies of each SNP base will vary depending on the number of alleles of the gene containing the SNP locus. The ability to capture this information accurately across several SNPs within a set of homo(eo)logous alleles can give an indication of the number of allele haplotypes present for a gene and potentially provide the haplotype sequences. This information could have implications for sugarcane breeding. High yield potential may be due to the presence of, or different number of copies of a specific allele(s) present at a gene locus, or possibly a combination of both. Knowledge of the sequence underlying each allele haplotype has the potential to allow allele-specific markers to be designed.

Quantitative methods to detect allele dosage in sugarcane are now possible with such techniques as pyrophosphate sequencing using the Pyrosequencer™ platform (Cordeiro *et al.*, 2006b) and mass-spectrometry using the Sequenom™ platform (Cordeiro *et al.*, 2006b). These methods have allowed the quantitative detection of frequencies of consensus to alternate SNP bases at any particular SNP locus. Utilizing a group of SNP markers developed to the same EST or gene, it becomes possible to infer the likely copy number of the EST or gene. This information then allows for possible haplotypes of a gene present in hybrid cane to be determined through statistical approaches (Cordeiro *et al.*, 2006b).

In theory, the association of SNP variations with either the presence or absence of different phenotypes among individuals or among individuals from different populations appears straightforward. This simplistic view does not account for the majority of base polymorphisms that do not result in any amino acid change. Determining the haplotypes is more important for predicting individual phenotypes than are the underlying SNPs. Determining haplotypes also allows the ability to infer the evolutionary history of a DNA region (Templeton *et al.*, 1988; Tishkoff *et al.*, 1998). However, difficulties are encountered in determining SNP haplotypes when inbred or homozygous individuals are not available (Rafalski, 2002) as is usually the case with sugarcane.

The ability to determine SNP base frequencies provides the means to determine the likely copy number of homo(eo)logous loci in sugarcane. Where chromosome counts have been performed for a genotype, this information can be used to support the inference of the most likely copy number of homo(eo)logous loci. Knowledge of the number of homo(eo)logous loci will assist in the deduction of the allelic composition of the locus in any particular sugarcane genotype. The ability to determine haplotypes also opens possibilities in unraveling the complexities of the sugarcane genome. By defining haplotypes in parents of crosses, it may be possible to deduce their segregation in progeny; or to determine allele dosage and composition in any particular genotype in relation to phenotypic performance. A further level of analysis is required to determine the level of expression of each of the haplotypes in this complex genome.

#### *Genetic mapping*

Genetic maps are widely used in plant breeding to identify genomic regions controlling traits of interest. Such information assists in understanding the genetic basis of the target trait, as well as providing DNA markers for use in marker-assisted breeding. In sugarcane, only markers that are present as a single copy in one parent and absent in the second [i.e. single-dose (SD) marker] can be incorporated into maps using populations of conventional size (approx. 250 progeny) (Wu *et al.*, 1992). In these populations, SD markers segregate in a 1 : 1 ratio.

The first maps of a cultivar were initiated on the selfed progeny of SP70-1006 (D'Hont, 1994). This map was later transferred and further developed on the cultivar R570

(Grivet *et al.*, 1996) using RFLP probes from maize and sugarcane. By 2001, the R570 map, as it had become commonly known, contained some 600 RFLP markers derived from a number of grass (Poaceae) species (D'Hont and Glaszmann, 2001). The markers on this map distribute over 98 cosegregation groups covering a total length of 2008 cM. A parallel mapping effort was also carried out to place 939 single-dose AFLP markers on R570, of which 887 were distributed into 120 cosegregation or linkage groups (Hoarau *et al.*, 2001). A more recent map has been developed on a cross between the Australian commercial variety Q165<sup>A</sup> ( $2n = 115$ ) with the *S. officinarum* clone IJ76-514 ( $2n = 80$ ) using a combination of AFLP and SSR markers. A total of 967 single dose markers were generated from the two marker systems, and 910 were distributed across 116 linkage groups covering a total map length of 9058.3 cM (Aitken *et al.*, 2005). Markers on these maps have all been generated through anonymous marker systems. However, the use of SNP markers are resulting in ESTs mapped onto the Q165<sup>A</sup> map.

#### *EcoTILLING for mapping ESTs*

Parallel to the development of quantitative SNP frequency scoring methods has been the adaptation of the EcoTILLING method for detecting and mapping sugarcane ESTs. TILLING utilizes the CelI mismatch-cleavage enzyme on heteroduplexed DNA strands with detection of end-labelled cleavage product (McCallum *et al.*, 2000). A variant of this method utilizes natural populations for the discovery of polymorphisms (SNPs, SSRs and indels), and is referred to as EcoTILLING (Comai *et al.*, 2004). Both methods as published rely largely on electrophoretic gels to separate and visualize the products. In sugarcane, this does not allow SNPs that occur on a single allele to be clearly detected. Modifying the protocol and moving the detection system to capillary electrophoresis has allowed the detection of single-dose SNPs in sugarcane to be identified (Cordeiro *et al.*, 2006b) and mapped (McIntyre *et al.*, 2006). Our early experience with family members of the sucrose phosphate synthase gene indicate straightforward detection of the presence of 5–11 SNPs in fragment lengths of genomic DNA between 300 bp and 400 bp in length. Neither prior knowledge of any SNP in the fragment nor the alignment of multiple ESTs are required to identify putative SNPs and their location. Whilst the method is as yet unable to indicate the frequency at which an individual SNP base is present, it has been demonstrated that the detected variation in base composition segregates as expected in progeny of mapping populations. Using the SPS gene family members as an example, the mapping of the gene family members through the EcoTILLING approach supports sequence information that three of the five gene family members may contain more than one gene, with each gene possessing from one to five alleles (McIntyre *et al.*, 2006). This observation will in time allow further unravelling of the complexities of the sugarcane genome.

#### *Sorghum genome information as a resource for sugarcane*

Sorghum is the closest cultivated relative of sugarcane. Sugarcane has a large genome that has duplicated at least twice since it diverged from sorghum, around 5 million years ago (Al-Janbi *et al.*, 1997). The extensive similarity in the gene order between these two genomes, where intercrosses are still possible (Ming *et al.*, 1998), makes sorghum the best model crop for the Androponeae tribe (Price *et al.*, 2005a) with the aim of understanding the extensive gene rearrangements and assisting the development of genetic maps in sugarcane.

Sequencing of *Sorghum* provides another model genome within the grasses, which particularly when utilized in conjunction with rice, will stimulate evolutionary understanding of the entire Poaceae. Sequencing will stimulate gene and allele discovery and crop improvement in *Sorghum* as it did in rice. Sugarcane genomics will be supported by the *Sorghum* sequence data. The sequences of *Sorghum* genes and to a lesser extent the location of genes in the genome should be useful in sugarcane.

Genetic resources for sorghum and sugarcane improvement have been enhanced by the application of genomic tools to analysis of wild relatives in the *Sorghum* and *Saccharum* genera. Mutant populations (including TILLING populations) of *Sorghum* expand the options for gene discovery and genetic manipulation. Protocols for EcoTILLING (Cordeiro *et al.*, 2006a) and quantitative SNP analysis in the complex sugarcane genome should be valuable tools for gene mapping, gene discovery and association genetics in sugarcane. The availability of a *Sorghum* genome sequence will further accelerate the potential to apply these techniques in both *Sorghum* and sugarcane. Gene discovery in this germplasm will also be supported by application of advances in expression profiling tools as has been applied to other crop species in the Poaceae (McIntosh *et al.*, 2007).

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#### LITERATURE CITED

- Abu Assar AH, Uptmoor R, Abdelmula AA, Salih M, Ordon F, Friedt W. 2005. Genetic variation in sorghum germplasm from Sudan, ICRISAT, and USA assessed by simple sequence repeats (SSRs). *Crop Science* 45: 1636–1644.
- Ahmadian A, Gharizadeh B, Gustafsson AC, Sterky F, Nyrén P, Uhlén M, *et al.* 2000. Single-nucleotide polymorphism analysis by pyrosequencing. *Analytical Biochemistry* 280: 103–110.
- Aitken K, Jackson P, Piperidis G, McIntyre L. 2004. QTL identified for yield components in a cross between a sugarcane (*Saccharum* spp.) cultivar Q165<sup>A</sup> and a *S. officinarum* clone IJ76-514. In: Fischer T, Turner N, Angus J, McIntyre L, Robertson M, Borrell A, *et al.*, eds. *New directions for a diverse planet. Proceedings for the 4th International Crop Science Congress*, Brisbane, Australia, 26 September to 1 October 2004.



- Aitken KS, Jackson PA, McIntyre CL. 2005. A combination of AFLP and SSR markers provides extensive map coverage and identification of homo(eo)logous linkage groups in a sugarcane cultivar. *Theoretical and Applied Genetics* 110: 789–801.
- Alderborn A, Kristofferson A, Hammerling U. 2000. Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing. *Genome Research* 10: 1249–1258.
- Al-Janbi SM, Honeycutt RJ, Peterson C, Sobral BWS. 1997. Phylogenetics analysis of organellar DNA sequences in the Andropogoneae: *Saccharum*. *Theoretical and Applied Genetics* 88: 933–944.
- Bapat DR, Mote UN. 1982. Sources of shootfly resistance in *Sorghum*. *Journal of the Maharashtra Agricultural University* 7: 238–240.
- Benmoussa M, Suhendra B, Aboubacar A, Hamaker BR. 2006. Distinctive sorghum starch granule morphologies appear to improve raw starch digestibility. *Starch – Starke* 58: 92–99.
- Bhatramakki D, Rafalski A. 2001. Discovery and application of single nucleotide polymorphism markers in plants. In: Henry RJ, ed. *Plant genotyping: the DNA fingerprinting of plants*. Wallingford: CAB International, 179–191.
- Bonierbale MW, Plaisted RL, Tanksely SD. 1988. RFLP maps based on common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* 120: 1095–1103.
- Borrell AK, Douglas ACL. 1997. Maintaining green leaf area in grain sorghum increased nitrogen uptake under post-anthesis drought. *International Sorghum and Millets Newsletter* 38: 89–91.
- Borrell AK, Bidinger FR, Sunitha K. 1999. Stay-green associated with yield in recombinant inbred sorghum lines varying in rate of leaf senescence. *International Sorghum and Millets Newsletter* 40: 31–33.
- Borrell AK, Hammer GL, Douglas ACL. 2000. Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. *Crop Science* 40: 1026–1037.
- Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH, et al. 2003. A high density genetic recombination map of sequence-tagged sites for *Sorghum*, as a framework for comparative structural and evolutionary genomics of tropical grasses. *Genetics* 165: 367–386.
- Bradbury LMT, Fitzgerald TL, Henry RJ, Jin QS, Waters DLE. 2005. The gene for fragrance in rice. *Plant Biotechnology Journal* 3: 363–370.
- Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, et al. 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. *Theoretical and Applied Genetics* 93: 190–198.
- Bull TA, Glasziou KT. 1963. The evolutionary significance of sugar accumulation in *Saccharum*. *Australian Journal of Biological Science* 16: 737–742.
- Buzacott JH. 1965. Cane varieties and breeding. In: Kim NJ, Mungomery RW, Hughes CG, eds. *Manual of cane growing*. Sydney: Angus and Robertson, 220–253.
- Cai Q, Aitken K, Deng HH, Chen XW, Fu C, Jackson PA, et al. 2005. Verification of the introgression of *Erianthus arundinaceus* germplasm into sugarcane using molecular markers. *Plant Breeding* 124: 322–328.
- Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R. 2004. A structured mutant population for forward and reverse genetics in barley (*Hordeum vulgare* L.). *The Plant Journal* 4: 143–150.
- Carson DL, Botha FC. 2000. Preliminary analysis of expressed sequence tags for sugarcane. *Crop Science* 40: 1769–1779.
- Casu RE, Dimmock CM, Chapman SC, Grof CPL, McIntyre CL, Bonnett GD, et al. 2004. Identification of differentially expressed transcripts from maturing stem of sugarcane by *in silico* analysis of stem expressed sequence tags and gene expression profiling. *Plant Molecular Biology* 54: 503–517.
- Chen X, Cho YG, McCouch SR. 2002. Sequence divergence of rice microsatellites in *Oryza* and other plant species. *Molecular Genetics and Genomics* 268: 331–343.
- Clayton WD, Renvoize SA. 1986. Genera *Graminum* grasses of the world. *Kew Bulletin Addition Series XIII*. London: Royal Botanic Gardens, Kew, 338–345.
- Comai L, Henikoff S. 2006. TILLING: practical single-nucleotide mutation discovery. *The Plant Journal* 45: 684–694.
- Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA, et al. 2004. Efficient discovery of DNA polymorphisms in natural populations by EcoTILLING. *The Plant Journal* 37: 778–786.
- Cordeiro GM, Taylor GO, Henry RJ. 2000. Characterisation of microsatellite markers from sugarcane (*Saccharum* sp.), a highly polyploid species. *Plant Science* 155: 161–168.
- Cordeiro GM, Pan YB, Henry RJ. 2003. Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. *Plant Science* 165: 181–189.
- Cordeiro GM, Elliott F, McIntyre CL, Casu RE, Henry RJ. 2006a. Characterisation of single nucleotide polymorphisms in sugarcane ESTs. *Theoretical and Applied Genetics* 113: 331–343.
- Cordeiro G, Elliott F, Henry R. 2006b. An Optimised Ecotilling protocol for polyploids or pooled samples using a capillary electrophoresis system. *Analytical Biochemistry* 355: 145–147.
- Cross M, Lee SL, Henry RJ. 2007. A novel detection strategy for scanning multiple mutations using CEL I. Paper presented at the *Plant and Animal Genomes Conference XV, Mutation Screening Workshop*, San Diego, USA, 13–17 January 2007.
- Daniels J, Roach BT. 1987. Taxonomy and evolution in sugarcane. In: Heinz DJ, ed. *Sugarcane improvement through breeding*. Amsterdam: Elsevier Press, 7–84.
- Davies H, Dicks E, Stephens P, Cox C, Teague J, Greenman C, et al. 2006. High throughput DNA sequence variant detection by conformation sensitive capillary electrophoresis and automated peak comparison. *Genomics* 87: 427–432.
- Delseny M, Salses J, Cooke R, Sallaud C, Regad F, Lagoda P, et al. 2001. Rice genomics: present and future. *Plant Physiology and Biochemistry* 39: 323–334.
- Deu M, Rattunde F, Chantreau J. 2006. A global view of genetic diversity in cultivated sorghums using a core collection. *Genome* 49: 168–180.
- D'Hont A. 1994. A molecular approach to unravelling the genetics of sugarcane, a complex polyploid of the Andropogoneae tribe. *Genome* 37: 222–230.
- D'Hont A, Glaszmann JC. 2001. Sugarcane genome analysis with molecular markers: a first decade of research. In: Hogarth DM, ed. *Proceedings of the International Society of Sugarcane Technologists XXVI Congress*. Brisbane: The Australian Society of Sugar Cane Technologists, 556–559.
- D'Hont A, Laissac M. 1998. Analysis of cultivars genome structure by molecular cytogenetics and the study of introgression mechanisms. France: Centre de coopération internationale en recherche agronomique pour le développement, Annual Crops Department (CIRAD-CA), 9–10.
- Dillon SL, Lawrence PK, Henry RJ. 2005. The new use of *Sorghum bicolor* derived SSR markers to evaluate genetic diversity in seventeen Australian *Sorghum* species. *Plant Genetic Resources* 3: 19–28.
- Dillon SL, Lawrence PK, Henry RJ, Price HJ. 2007. *Sorghum* resolved as a distinct genus based on combined ITS1, *ndhF* and *Adh1* analyses. *Plant Systematics and Evolution* (in press).
- Doggett H. 1970. *Sorghum*. London: Longman; published by Wiley, New York.
- Doggett H. 1988. *Sorghum*. 2nd edn. London: Longman; published by Wiley, New York.
- Duodo K, Taylor J, Belton P, Hamaker B. 2003. Factors affecting sorghum protein digestibility. *Journal of Cereal Science* 38: 117–131.
- Edme SJ, Miller JD, Glaz B, Tai PY-P, Comstock JC. 2005. Genetic contribution to yield in the Florida sugarcane industry across 33 years. *Crop Science* 45: 92–97.
- Ellis RH, Qi A, Craufurd PQ, Summerfield RJ, Roberts EH. 1997. Effects of photoperiod, temperature and asynchrony between thermoperiod and photoperiod on development to panicle initiation in *Sorghum*. *Annals of Botany* 79: 169–178.
- Fannon J, Gray J, Gunawan N, Huber K, BeMiller J. 2003. The channels of starch granules. *Food Science and Biotechnology* 12: 700–704.
- Fannon J, Gray J, Gunawan N, Huber K, BeMiller J. 2004. Heterogeneity of starch granules and the effect of granule channelization on starch modification. *Cellulose* 11: 247–254.
- Feltus FA, Hart GE, Schertz KF, Casa AM, Kresovich S, Abraham S, et al. 2006. Alignment of genetic maps and QTLs between inter- and

- intra-specific sorghum populations. *Theoretical and Applied Genetics* **112**: 1295–1305.
- Franzmann BA, Hardy AT. 1996.** Testing the host status of Australian indigenous sorghums for the sorghum midge. In: Foale MA, Henzell RG, Kneip JF, eds. *Proceedings of the Third Australian Sorghum Conference*. Tamworth, NSW, Australia, 365–367.
- Garber ED. 1950.** Cytotaxonomic studies in the genus *Sorghum*. *University of California Publications in Botany* **23**: 283–361.
- González-Martínez SC, Robledo-Arnuncio JJ, Collada C, Díaz A, Williams CG, Alía R, et al. 2004.** Cross-amplification and sequence variation of microsatellite loci in Eurasian hard pines. *Theoretical and Applied Genetics* **109**: 103–111.
- Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann J-C. 1996.** RFLP mapping in a highly polyploid and aneuploid interspecific hybrid. *Genetics* **142**: 987–1000.
- Grivet L, Glaszmann JC, Arruda P. 2001.** Sequence polymorphism from EST data in sugarcane: a fine analysis of 6-phosphogluconate dehydrogenase genes. *Genetics and Molecular Biology* **24**: 161–167.
- Grivet L, Glaszmann JC, Vincentz M, da Silva F, Arruda P. 2003.** ESTs as a source for sequence polymorphism discovery in sugarcane: example of the *Adh* genes. *Theoretical and Applied Genetics* **106**: 190–197.
- Gut IG. 2001.** Automation in genotyping of single nucleotide polymorphisms. *Human Mutation* **17**: 475–492.
- Harlan JR, de Wet MJM. 1971.** Toward a rational classification of cultivated sorghums. *Crop Science* **12**: 172–176.
- Harlan JR, de Wet MJM. 1972.** A simplified classification of cultivated plants. *Taxon* **20**: 509–517.
- van Harten AM. 1998.** *Mutation breeding theory and applications*. Cambridge: Cambridge University Press.
- Henikoff S, Comai L. 2003.** Single-nucleotide mutations for plant functional genomics. *Annual Review of Plant Biology* **54**: 375–401.
- Henikoff S, Till BJ, Comai L. 2004.** TILLING: traditional mutagenesis meets functional genomics. *Plant Physiology* **135**: 630–636.
- Hernández P, Dorado G, Laurie DA, Martin A, Snape JW. 2001.** Microsatellites and RFLP probes from maize are efficient sources of molecular markers for the biomass energy crop *Miscanthus*. *Theoretical and Applied Genetics* **102**: 616–622.
- Hoarau JY, Offmann B, D'Hont A, Risterucci AM, Roques D, Glaszmann JC, et al. 2001.** Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp.). I. Genome mapping with AFLP markers. *Theoretical and Applied Genetics* **103**: 84–97.
- Hoarau JY, Grivet L, Offmann B, Raboin LM, Diorflar JP, Payet J, et al. 2002.** Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp.). II. Detection of QTLs for yield components. *Theoretical and Applied Genetics* **105**: 1027–1037.
- Hodnett GL, Burson BL, Rooney WL, Dillon SL, Price HJ. 2005.** Pollen–pistil interactions result in reproductive isolation between *Sorghum bicolor* and divergent *Sorghum* species. *Crop Science* **45**: 1403–1409.
- House LR. 1985.** *A guide to sorghum breeding*, 2nd edn. Patancheru, India: International Crops Research Institute for the Semi-Arid tropics, 206.
- House LR, Osmanzai M, Gomez MI, Monyo ES, Gupta SC. 1995.** Agronomic principles. In: Dendy DAV, ed. *Sorghum and millets, chemistry and technology*: St Paul, MN: American Association of Cereal Chemists, 27–67.
- Jordan DR, Tao YZ, Godwin ID, Henzell RG, Cooper M, McIntyre CL. 1998.** Loss of genetic diversity associated with selection for resistance to sorghum midge in Australian sorghum. *Euphytica* **102**: 1–7.
- Kamala V, Singh SD, Bramel PJ, Rao DM. 2002.** Sources of resistance to downy mildew in wild and weedy sorghums. *Crop Science* **42**: 1357–1360.
- Kan CCPF, Doherty EAS, Barron AE. 2004.** DNA sequencing and genotyping in miniaturized electrophoresis systems. *Electrophoresis* **25**: 3564–3588.
- Karunakar RI, Narayana YD, Pande S, Mughogho LK, Singh SD. 1994.** Evaluation of wild and weedy sorghums for downy mildew resistance. *International Sorghum and Millets Newsletter* **35**: 104–106.
- Kayode APP, Linnemann AR, Nout MJR, Hounhouigan JD, Stomph TJ, Smulders MJM. 2006.** Diversity and food quality properties of farmers' varieties of sorghum from Benin. *Journal of the Science of Food and Agriculture* **86**: 1032–1039.
- Komolong B, Chakraborty S, Ryley M, Yates D. 2002.** Identity and genetic diversity of the sorghum ergot pathogen in Australia. *Australian Journal of Agricultural Research* **53**: 621–628.
- Kong L, Dong J, Hart GE. 2000.** Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple-sequence repeats (SSRs). *Theoretical and Applied Genetics* **101**: 438–448.
- Kresovich S, Barbazuk B, Bedell JA, Borrell A, Buell CR, Burke J, et al. 2005.** Toward sequencing the sorghum genome. A US National Science Foundation-sponsored Workshop Report. *Plant Physiology* **138**: 1898–1902.
- Kuhlman LC, Burson BL, Klein PE, Stelly DM, Rooney WL. 2006.** Interspecific sorghum breeding using *S. macrospermum*. In: *Proceedings of the ASA-CSSA-SSA 2006 International Meetings*, Indianapolis, 12–16 November 2006.
- Laurie D, Bennett MD. 1989.** Genetic variation in *Sorghum* for the inhibition of maize pollen tube growth. *Annals of Botany* **64**: 675–681.
- Lazarides M, Hacker JB, Andrew MH. 1991.** Taxonomy, cytology and ecology of indigenous Australian sorghums (*Sorghum* Moench: Andropogoneae: Poaceae). *Australian Systematic Botany* **4**: 591–635.
- Lebot V. 1999.** Biomolecular evidence for plant domestication in Sahul. *Genetic Resources and Crop Evolution* **46**: 619–628.
- Lindeboom N, Chang PR, Tyler RT. 2004.** Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. *Starch – Starke* **56**: 89–99.
- McCallum CM, Comai L, Greene EA, Henikoff S. 2000.** Targeting induced local lesions in genomes (TILLING) for plant functional genomics. *Plant Physiology* **123**: 439–442.
- McIntosh SR, Watson L, Bundock PC, Crawford AC, White J, Cordeiro GM, et al. 2007.** SAGE of the most abundant transcripts in the developing wheat Caryopsis. *Plant Biotechnology Journal* **5**: 69–83.
- McIntyre CL, Casu RE, Drenth J, Knight D, Whan VA, Croft BJ, et al. 2005.** Resistance gene analogues in sugarcane and sorghum and their association with quantitative trait loci for rust resistance. *Genome* **48**: 391–400.
- McIntyre CL, Jackson M, Cordeiro G, Amouyal O, Elliott F, Henry RJ, et al. 2006.** The identification and characterisation of alleles of sucrose phosphate synthase gene family III in sugarcane. *Molecular Breeding* **18**: 39–50.
- Ma HM, Schulze S, Lee S, Yang M, Mirkov E, Irvine J, et al. 2004.** An EST survey of the sugarcane transcriptome. *Theoretical and Applied Genetics* **108**: 851–863.
- Mariotti JA. 2002.** Selection for sugar cane yield and quality components in subtropical climates. Sugar Cane International, March/April, 22–26.
- Miller FR. 1980.** The breeding of sorghum. *Texas Agricultural Experiment Station* **1451**: 128–136.
- Miller FR. 1982.** Genetic and environmental response characteristics of sorghum. In: *Sorghum in the Eighties, Proceedings of International Symposium on Sorghum*, 2–7 November 1981. Patancheru, India: ICRISAT, 393–402.
- Ming R, Liu SC, Lin YR, da Silva J, Wilson W, Braga D, et al. 1998.** Detailed alignment of *Saccharum* and *Sorghum* chromosomes: comparative organization of closely related diploid and polyploid genomes. *Genetics* **150**: 1663–1682.
- Ming R, Liu S-C, Moore PH, Irvine JE, Paterson AH. 2001.** QTL analysis in a complex autopolyploid: genetic control of sugar content in sugarcane. *Genome Research* **11**: 2075–2084.
- Ming R, Del Monte TA, Hernandez E, Moore PH, Irvine JE, Paterson AH. 2002.** Comparative analysis of QTLs affecting plant height and flowering among closely-related diploid and polyploid genomes. *Genome* **45**: 794–803.
- Mullet JE, Klein RR, Klein PE. 2002.** *Sorghum bicolor* — an important species for comparative grass genomics and a source of beneficial genes for agriculture. *Current Opinion in Plant Biology* **5**: 118–121.
- Nurmi J, Kiviniemi M, Kujanpaa M, Sjooroo M, Ilonen J, Lovgren T. 2001.** High-throughput genetic analysis using time-resolved fluorometry and closed-tube detection. *Analytical Biochemistry* **299**: 211–217.



- Pan Y-B, Cordeiro GM, Richard EP, Henry RJ. 2003a. Molecular genotyping of sugarcane clones with microsatellite DNA markers. *Maydica* **48**: 319–329.
- Pan Y-B, Miller JD, Schnell RJ, Richard J, RP, Wei Q. 2003b. Application of microsatellite and RAPD fingerprints in the Florida sugarcane variety program. *Sugar Cane International* **2003**: 19–28.
- Pan YB, Burner DM, Wei Q, Cordeiro GM, Legendre BL, Henry RJ. 2004. New *Saccharum* hybrids in *S. spontaneum* cytoplasm developed through a combination of conventional and molecular breeding approaches. *Plant Genetic Resources* **2**: 131–139.
- Paterson AH, Bowers JE, Chapman BA. 2004. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. *Proceedings of the National Academy of Sciences of the USA* **101**: 9903–9908.
- Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. *Molecular Biology and Evolution* **15**: 1275–1287.
- Piperidis G, Taylor GO, Smith GR. 2001. A microsatellite marker database for fingerprinting sugarcane clones. In: XXIV Proceedings of the International Society of Sugar Cane Technologists. Mackay: Australian Society of Sugar Cane Technologists, 632–633.
- Price HJ, Dillon SL, Hadnett G, Rooney WL, Ross L, Johnston JS. 2005a. Genome evolution in the genus *Sorghum* (Poaceae). *Annals of Botany* **95**: 219–227.
- Price HJ, Hodnett GL, Burson BL, Dillon SL, Rooney WL. 2005b. A *Sorghum bicolor* × *S. macrospermum* hybrid recovered by embryo rescue and culture. *Australian Journal of Botany* **53**: 579–582.
- Price HJ, Hodnett GL, Burson BL, Dillon SL, Stelly DM, Rooney WL. 2006. Genome dependent interspecific hybridisation of *Sorghum bicolor* (Poaceae). *Crop Science* **46**: 2617–2622.
- Quint M, Mihaljevic R, Dussle CM, Xu ML, Melchinger AE, Lubberstedt T. 2002. Development of RGA-CAPS markers and genetic mapping of candidate genes for sugarcane mosaic virus resistance in maize. *Theoretical and Applied Genetics* **105**: 355–363.
- Rai KN, Murty DS, Andrews DJ, Bramel-Cox PJ. 1999. Genetic enhancement of pearl millet and sorghum for the semi-arid tropics of Asia and Africa. *Genome* **42**: 617–628.
- Rafalski A. 2002. Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology* **5**: 94–100.
- Ramsay L, Macaulay M, degli Ivanisovich S, MacLean K, Cardle L, Fuller J, et al. 2000. A simple sequence repeat-based linkage map of barley. *Genetics* **156**: 1997–2005.
- Rao NPG, Rana BS. 1982. Selection in temperate and tropical crosses of sorghum. In: *Sorghum in the Eighties, Proceedings of International Symposium on Sorghum*, 2–7 November 1981. Patancheru, India: ICRISAT, 403–420.
- Reddy BBS, Ramesh S, Reddy PS. 2006. Sorghum genetic resources, cytogenetics and improvement. In: Singh RJ, Jauhar PP, eds. *Genetic resources, chromosome engineering, and crop improvement*. Vol. 2. *Cereals*. Boca Raton, FL: CRC Taylor and Francis, 309–363.
- Roach BT. 1989. Origin and improvement of the genetic base of sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists*, 34–47.
- Rooney WL, Smith CW. 2000. Techniques for developing new cultivars. In: Smith CW and Frederiksen RA, eds. *Sorghum, origin, history, technology and production*, New York, NY: John Wiley & Sons, 329–347.
- Rosenow DT, Clark LE. 1995. Drought and lodging resistance for a quality sorghum crop. In: *Proceedings of the Fiftieth Annual Corn and Sorghum Industry Research Conference, Chicago, IL*, 6–7 December, 82–97.
- Rosenow DT, Dahlberg JA. 2000. Collection, conversion and utilisation of sorghum. In: Smith CW, Frederiksen RA, eds. *Sorghum, origin, history, technology and production*. New York, NY: John Wiley, Sons, 309–328.
- Ross P, Hall L, Smirnow I, Haff L. 1998. High level multiplex genotyping by MALDI-TOF mass spectrometry. *Nature Biotechnology* **16**: 1347–1351.
- Rossi M, Araujo PG, Paulet F, Garsmeur O, Dias VM, Chen H, et al. 2003. Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. *Molecular Genetics and Genomics* **269**: 406–419.
- Scott LJ, Shepherd M, Henry RJ. 2003. Characterization of highly conserved microsatellite loci in *Araucaria cunninghamii* and related species. *Plant Systematics and Evolution* **236**: 115–123.
- Serna-Saldivar S, Rooney L. 1995. Structure and chemistry of sorghum and millets. In: Dendy DAV, ed. *Sorghum and millets, chemistry and technology*. St Paul, MN: American Association of Cereal Chemists 69–124.
- Shapter FM, Lee LS, Henry R. 2007. Endosperm and starch granule morphology in wild cereal relatives. *Plant Genetic Resources* (in press).
- Sharma HC. 1993. Host-plant resistance to insects in sorghum and its role in integrated pest management. *Crop Protection* **12**: 11–34.
- Sharma HC, Franzmann BA. 2001. Host-plant preference and oviposition responses of the sorghum midge, *Stenodiplosis sorghicola* (Coquillett) (Dipt., Cecidomyiidae) towards wild relatives of sorghum. *Journal of Applied Entomology* **125**: 109–114.
- Sills G, Bridges W, Al-Janabi S, Sobral BWS. 1995. Genetic analysis of agronomic traits in a cross between sugarcane (*Saccharum officinarum* L.) and its presumed progenitor (*S. robustum* Brandes, Jesw. Ex. Grassl). *Molecular Breeding* **1**: 355–363.
- Slade AJ, Fuerstenberg SI, Loeffler D, Steine MN, Facciotti D. 2005. A reverse genetic, non-transgenic approach to wheat crop improvement by TILLING. *Nature Biotechnology* **23**: 75–81.
- Smith CW, Frederiksen RA. 2000. *Sorghum: origin, history, technology, and production*. New York, NY: John Wiley and Sons, 824.
- Spangler RE. 2003. Taxonomy of *Sarga*, *Sorghum* and *Vacoparis* (Poaceae: Andropogoneae). *Australian Systematic Botany* **16**: 279–299.
- Sreenivasan TV, Ahloowalia BS, Heinz DJ. 1987. Cytogenetics. In: Heinz DJ, ed. *Sugarcane improvement through breeding*. Amsterdam: Elsevier, 211–253.
- Storm N, Darnhofer-Patel B, van den Boom D, Rodi CP. 2003. MALDI-TOF mass spectrometry-based SNP genotyping. *Methods in Molecular Biology* **212**: 241–262.
- Sudupak MA. 2004. Inter- and intra-species inter simple sequence repeat (ISSR) variations in the genus *Cicer*. *Euphytica* **135**: 229–238.
- Szantai E, Ronai Z, Szilagyi A, Sasvari-Szekely M, Guttman A. 2005. Haplotyping by capillary electrophoresis. *Journal of Chromatography* **1079**: 41–49.
- Tang T, Huang JZ, Zhong Y, Shi SH. 2004. High-throughput S-SAP by fluorescent multiplex PCR and capillary electrophoresis in plants. *Journal of Biotechnology* **114**: 59–68.
- Tao YZ, Hardy A, Drenth J, Henzell RG, Franzmann BA, Jordan DR, et al. 2003. Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. *Theoretical and Applied Genetics* **107**: 116–122.
- Taramino G, Tarchini R, Ferrario S, Lee M, Pé ME. 1997. Characterisation and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. *Theoretical and Applied Genetics* **95**: 66–72.
- Templeton AR, Sing CF, Kessler A, Humphries S. 1988. A cladistic analysis of phenotype associations with haplotypes inferred from restriction endonuclease mapping. II. The analysis of natural populations. *Genetics* **120**: 1145–1154.
- Tesso T, Ejeta G, Chandrashekar A, Huang CP, Tandjung A, Lewamy M, et al. 2006. A novel modified endosperm texture in a mutant high-protein digestibility/high-lysine grain sorghum (*Sorghum bicolor* (L.) Moench). *Cereal Chemistry* **83**: 194–201.
- Tew TL, White WH, Grisham MP, Dufrene EO Jr, Garrison DD, Veremis JC, et al. 2003. Registration of ‘HoCP 13 96–540’ Sugarcane. *Crop Science* **45**: 785–786.
- Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, et al. 2003. Large-scale discovery of induced point mutations with high-throughput TILLING. *Genome Research* **13**: 524–530.
- Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Young K, et al. 2004. Discovery of induced point mutations in maize genes by TILLING. *BMC Plant Biology* **4**: 12.
- Tishkoff SA, Goldman A, Calafell F, Speed WC, Deinard AS, Bonne-Tamir B, et al. 1998. A global haplotype analysis of the myotonic dystrophy locus: implications for the evolution of modern humans and for the origin of myotonic dystrophy mutations. *American Journal of Human Genetics* **62**: 1389–1402.
- USDA ARS. 2007. National Genetic Resources Program. *Germplasm Resources Information Network (GRIN)* [Online Database].



- Beltsville, MD: National Germplasm Resources Laboratory. URL: [http://www.ars-grin.gov/cgi-bin/npgs/html/tax\\_search.pl](http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl) (17 May 2007)
- Vettore AL, da Silva FR, Kemper EL, Arruda P. 2001.** The libraries that made SUCEST. *Genetic Molecular Biology* **24**: 1–7.
- Vouk K, Gazvoda B, Komel R. 2000.** Fluorescent multiplex PCR and capillary electrophoresis for analysis of PKD1 and PKD2 associated microsatellite markers. *BioTechniques* **29**: 1187–1190.
- Waters DLE, Henry RJ, Reinke RF, Fitzgerald MA. 2005.** Gelatinisation temperature of rice explained by polymorphisms in starch synthase. *Plant Biotechnology Journal* **4**: 115–122.
- Wright S. 1931.** Evolution in Mendelian populations. *Genetics* **16**: 97.
- Wu JL, Wu CJ, Lei CL, Baraoidan M, Bordeos A, Madamba MRS, et al. 2004.** Sorghum diversity evaluated by simple sequence repeat (SSR) markers and phenotypic performance. *Plant Production Science* **7**: 301–308.
- Wu JL, Wu CJ, Lei CL, Baraoidan M, Bordeos A, Madamba MRS, et al. 2005.** Chemical- and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. *Plant Molecular Biology* **59**: 85–97.
- Wu K, Burnquist W, Sorrels M, Tew TL, Moore P, Tanksley S. 1992.** The detection and estimation of linkage in polyploids using single-dose restriction fragments. *Theoretical and Applied Genetics* **83**: 294–300.