Chapter 14
Sugarcane

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Abstract Sugarcane is one of the most important crops globally, providing most of the world's sugar and bioenergy (ethanol and electricity). This contribution has been underpinned by the successful introgression of genes from wild germplasm, particularly from *Saccharum spontaneum*, by breeders in the early 1900s. This introgression resulted in a step change in the vigour, ratoon growth (i.e. regrowth after harvest), and adaptation to adverse environments, compared with the existing *S. officinarum* varieties. Introgression of other *S. spontaneum* clones and other species (particularly *Erianthus* spp. and *Miscanthus*) related to sugarcane in a range of sugarcane breeding programmes around the world is continuing, and based on current reports it is expected to continue to contribute incrementally to gains in breeding programmes and cultivar performance. However, the low sugar content of most wild relatives means that several cycles of backcrossing (to commercial type sugarcane) and interim selection are required, and this makes investment in these programmes lengthy, costly, difficult, and risky. Technological advancements in GM research have been impressive in sugarcane with a variety of methods to introduce and express genes in sugarcane now available. So far no commercially successful outcomes of this technology have occurred, but some major programmes are currently underway aiming to develop commercial cultivars. Targets include herbicide tolerance, stem borer resistance, and production of foreign compounds (e.g. alternative sugars).

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14.1 Introduction

Sugarcane is a crop of major economic importance produced in many countries in the tropics and subtropics and is harvested in greater quantities than any other crop globally (http://faostat.fao.org). Over 100 countries grow sugarcane commercially, the top three being Brazil, India, and China. Over 1.5 billion tonnes of sugarcane is harvested and processed annually. World sugarcane production has approximately doubled over the last 25 years largely due to the expansion of sugarcane cultivation in Brazil. This global expansion has been driven by both a long-term trend increase in sugar consumption of around 2 % per annum and the use of sugarcane for ethanol production in Brazil.

A major objective of sugarcane improvement programmes has been and remains to increase levels of commercially extractable sucrose content in cane. In the production of raw sugar, high levels of fibre and soluble impurities in cane cause losses through increased juice extraction costs and loss of sucrose in processing. Because of this, the first commercially produced sugarcane varieties of the species *Saccharum officinarum* had high sucrose content and low fibre and impurity levels. As described below, a major progression in sugarcane improvement arose with introgression of components of the *S. spontaneum* genome and other species into *S. officinarum* cultivars. *S. spontaneum* provided sources of disease resistance, vigour, ratooning ability, and better yields under abiotic stresses (Fig. 14.1).

Current highest priority objectives of most modern sugarcane breeding programmes include maintaining or improving disease and pest resistance and improving commercially extractable sugar content, cane yield, and ratooning performance. Most breeding programmes focus on parental material based on known original clones and well-defined (average of eight to nine) prior cycles of breeding and selection. Most parental materials in sugarcane breeding programmes around the world are derived from a limited number of ancestors produced during the initial interspecific hybridisation of the early 1900s described above. Some concerns about the narrow sampling of ancestral clones in modern sugarcane breeding programmes have been expressed by sugarcane breeders (Roach 1989). This prompted periodic attempts by breeders at introgression of related species as described below, with mixed success. More recently researchers in some institutes, as in many crop species, have turned to introduce alien genes into sugarcane cultivars through genetic engineering approaches, particularly for sucrose accumulation, pest resistance, and herbicide resistance. The aim of this chapter is to provide an overview of past and current efforts to introgress alien genes into sugarcane improvement programmes, either using sexual hybridisation with wild species or through genetic engineering approaches. A brief overview of sugarcane genetics and breeding and the early

Fig. 14.1 Modern sugarcane (*top*) cultivars are derived from *Saccharum officinarum* (*below left*) and the wild species *S. spontaneum* (*below right*)

introgression of wild canes into the original sugarcane cultivars are given in Sects. 14.2 and 14.3.1. Following this, more recent efforts and research aimed at further introgression of favourable alien genes from related species are described in Sects. 14.3–14.5. In Sect. 14.6, an overview of genetic engineering approaches taken to date in sugarcane improvement programmes is described. Lastly in Sect. 14.7 some perspectives on potential future approaches are provided.

14.2 Biology and Breeding

14.2.1 Taxonomy

The taxonomy of sugarcane and its relatives remains complex and controversial (Kellogg 2012). Sugarcane cultivars belong to the genus *Saccharum*, within the tribe *Andropogoneae*. This tribe is frequently polyploid, and its speciation and evolution are unclear in many cases, with the monophyletic status of many genera being questioned in some studies (Hodkinson et al. 2002). Further it is difficult to define taxonomic boundaries because of frequent interspecific and inter-generic hybridisation. Mukherjee (1957) first used the term "*Saccharum* complex" to describe an interbreeding group implicated in the origin of sugarcane and was adopted by other sugarcane breeders and geneticists, including Daniels and Roach (1987) who provided a comprehensive review of the members of this complex. Apart from *Saccharum*, the related genera include *Erianthus*, *Miscanthus*, *Narenga*, and *Sclerostachya*.

Traditionally six species were recognised within the genus *Saccharum* (Naidu and Sreenivasan 1987), with two of these growing in the wild (*S. spontaneum* and *S. robustum*) and the other four (*S. officinarum*, *S. barberi*, *S. sinense*, *S. edule*) existing primarily in cultivation.

Earliest sugarcane industries in the world grew varieties of *S. officinarum*. This species is typically characterised by high sugar content, low fibre content, thick stalks, and broad leaves. It originated in New Guinea and/or nearby Melanesian or Polynesian islands (Mukherjee 1957; Brandes 1958) and is believed to have evolved from *S. robustum* (Grivet et al. 2006).

Modern cultivated sugarcane varieties are complex interspecific hybrids primarily involving *S. officinarum* and *S. spontaneum* and in most cases involving some contributions from *S. robustum*, *S. sinense*, *S. barberi*, and possibly other related genera (Daniels and Roach 1987). *S. barberi* and *S. sinense* comprise the ancient landraces of India and China, respectively, and could have developed from interspecific hybrids involving *S. officinarum* and *S. spontaneum* (D'Hont et al. 2002) with possible introgression from other genera (Brown et al. 2007).

S. spontaneum ($2n = 36–128$) is distributed widely in the tropics to the subtropics in Asia and Africa in a wide diversity of habitats. It is a highly variable species that is found in the wild and varies in appearance from short bushy types to large

stemmed clones over 5 m in height. Most *S. spontaneum* clones have thin stalks and leaves, low sugar content, and high fibre content.

The other wild species in the genus, *S. robustum*, has some characteristics similar to *S. officinarum* but with generally lower sugar content and more variability (Daniels and Roach 1987). *S. edule* is a species characterised by an aborted inflorescence and therefore cannot be used for breeding.

Irvine (1999) challenged this traditional division of *Saccharum* into six species arguing that there is little basis for the separation and that the six species should more properly consist of two: one being *S. spontaneum* and the other comprising the five other species to be called *S. officinarum* (Irvine 1999).

14.2.2 Genetics

The genome of modern sugarcane is widely recognised as being the most complex of all important crop species (Grivet and Arruda 2001). A major feature of the sugarcane genome is its high degree of ploidy and aneuploidy and two (although not entirely separate—see below) subsets of chromosomes arising from the two main progenitor species, *S. officinarum* and *S. spontaneum*. *S. officinarum*, the dominant progenitor of sugarcane, is an octoploid with a basic chromosome number of $x=10$ (D'Hont et al. 1996, 1998). The other main contributor to the sugarcane genome, *S. spontaneum*, has a basic chromosome number of $x=8$ (D'Hont et al. 1996, 1998) and a range of cytotypes from $2n=36$ to $2n=128$, with five major cytotypes of $2n=64$, 80, 96, 112, and 128 (Panje and Babu 1960). Modern sugarcane cultivars which are derivatives of interspecific hybridisation involving these two species are complex aneu-polyploids, comprising 70–80 % of *S. officinarum*, 10–20 % of *S. spontaneum*, and 5–20 % of recombinant chromosomes (D'Hont et al. 1996; Piperidis and D'Hont 2001).

An interesting feature of crosses between *S. officinarum* and *S. spontaneum* is the transmission of $2n$ chromosomes from *S. officinarum* (Bremer 1923), which contributes to the high ploidy of modern cultivars. From a review of literature and breeder's experience, this phenomena seems to occur when *S. officinarum* as a female parent is crossed with any clone containing some *S. spontaneum* chromosomes.

The high level of polyploidy of sugarcane contributes to a very large genome size: the non-replicated size of *S. officinarum* is estimated at 7,440 Mb (Grivet and Arruda 2001), while the monoploid genome size of 930 Mbp is comparable with a range of diploid crops.

Genetic linkage maps are difficult to construct because of the high level of polyploidy. The usage of single-dose markers (i.e. markers present in a single copy and therefore that segregate 1:1 in gametes) provided an avenue for construction of linkage maps based on this subset of markers (Wu et al. 1992). Current sugarcane maps containing in excess of 1,000 linked markers are available (Hoarau et al. 2001; Aitken et al. 2005) but are still incomplete and unsaturated. Presence of multiple copies of some linkage groups may make completion using genetic mapping

difficult because of the dependence of low copy number markers (single- or double-dose markers) using current approaches. The meiosis of sugarcane cultivars mainly involves bivalent pairing (Price 1963). Genetic linkage maps of *S. officinarum* and modern cultivars have indicated random pairing of chromosomes combined with some preferential pairing (e.g. Aitken et al. 2005, 2007a, b; Hoarau et al. 2001).

A range of QTL mapping studies have been performed in sugarcane (listed and reviewed by Pastina et al. 2010). Analyses on a range of traits, including brix, sucrose content, fibre, cane yield, and disease resistance have been conducted. The general result reported is of multiple small QTL explaining proportions of variation (r^2) from 2 to 22 %. However, the values in the higher ranges are in studies with relatively small numbers of genotypes and are most likely overestimates, considering the methods used. Only a small number of traits having a major effect controlled by a single gene have been reported in sugarcane, with examples provided by D'Hont et al. (2010) and Costet et al. (2012).

14.2.3 Brief History of Breeding

The development of plantation and factory-based sugarcane industries in the 1800s and up to the early 1900s were based on clones of *S. officinarum*, mainly because of the superior processing attributes of these clones. This included low fibre content and low levels of impurities in juice (non-sucrose-dissolved solids). The fertility of sugarcane was discovered in the late 1800s, and following this, breeding programmes were quickly developed. Historically, three main phases have been identified in professionally directed sugarcane breeding programmes conducted since the late nineteenth century (Roach 1989). The first was intraspecific hybridisation of selected *S. officinarum* clones. Generally the goal was more disease-resistant forms of *S. officinarum* with good factory qualities and improved yield. Apart from the favourable processing attributes of *S. officinarum*, clones from this species are also usually characterised by poor vigour under abiotic stresses, poor ratooning ability, and susceptibility to a range of diseases.

The second phase which occurred between 1912 and around the 1930s was interspecific hybridisation involving mainly *S. officinarum* × *S. spontaneum* clones and is described in more detail in Sect. 14.3.1. It was also found that resulting hybrids were generally more vigorous, were more tolerant to a range of environmental stresses, and had stronger ratooning, compared with the prior *S. officinarum* cultivars. Following initial interspecific hybridisation, breeders found it necessary to backcross the hybrids to *S. officinarum* to "dilute" the undesirable characters in the wild canes, particularly low sugar content. This backcrossing process is termed "nobilisation" by sugarcane breeders.

The third phase, beginning around the 1930–1940s and continuing until today, involved exploiting the material produced in the second phase. This has involved intercrossing among the original hybrids and recurrent crossing and selection among progeny with increasingly larger populations. Most crosses made in

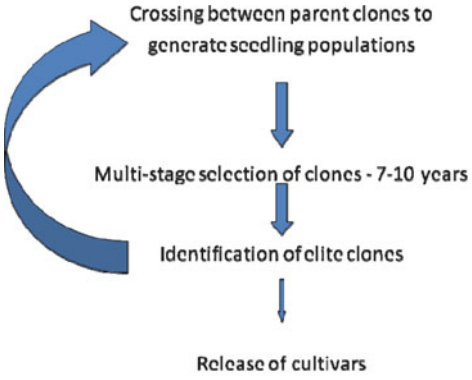

Fig. 14.2 General scheme of operations in most commercial sugarcane breeding programmes

breeding programmes throughout the world today are based on a relatively small number of ancestors derived from the early interspecific hybrids produced in the second phase.

14.2.4 Objectives and Structure of Breeding Programmes

There are currently around 40 significant sugarcane breeding programmes around the world, mostly specifically targeting sugarcane industries within individual countries (Machado 2001). The three traits of highest overall importance are resistance to prevailing diseases and pests, high commercially extractable sucrose content, and high cane yield in plant and ratoon crops. Some programmes also pursue traits associated with ease of harvesting and crop management (e.g. fast canopy cover to control weeds). A detailed account of many important aspects of sugarcane breeding, including important practical aspects, was provided by Heinz (1987). Most programmes follow the general scheme outlined in Fig. 14.2.

A heavy weighting in selection has been applied to commercially extractable sugar content in sugarcane breeding programmes and is justified for two reasons. First, any given incremental contribution of higher sugar content on sugar yields is economically more valuable than the same contribution by cane yield because increases in cane yield also give rise to higher harvesting, transport, and milling costs, in contrast to minimal marginal costs associated with increased sugar content (Jackson et al. 2000). Second, sugar content usually has a higher degree of genetic determination than cane yield (Skinner et al. 1987; Jackson and McRae 2001).

High fibre content in cane impacts adversely on sugar production systems through increased loss of sucrose in bagasse and on milling rate by slowing it down. However, in future production systems producing energy (electricity or ethanol) from fibre, production of additional fibre above that needed for factory processing energy requirements may have a positive value. This may have important implications for sugarcane introgression breeding programmes in that early generation progeny

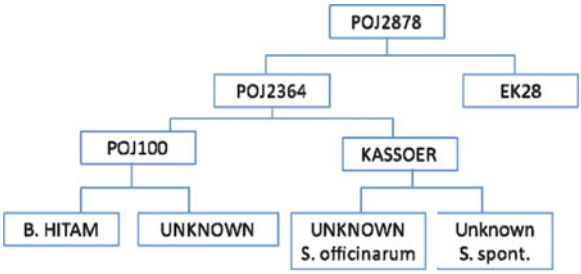

Fig. 14.3 Pedigree of clone "POJ2878" (from Mangelsdorf 1960) "*B Hitam*" (full name *Bandjermasin Hitam*) is a *S. officinarum* clone, which was open pollinated to produce the cultivar POJ100. POJ100 was crossed with the naturally occurring hybrid between *S. officinarum* and *S. spontaneum*, Kassoer, to produce POJ2363 which was crossed with the *S. officinarum* cultivar EK28

will have higher relative economic value than in production systems targeting only sucrose production, and therefore introgression of new traits from wild canes may be easier than in the past.

14.3 Introgression from *S. spontaneum*

14.3.1 Early Introgression

The introgression of genome components from *S. barberi* and *S. spontaneum* into *S. officinarum* in sugarcane breeding programmes in the early 1900s represents one of the most important and successful examples of introgression of wild relatives in any major crop species. Introgression of *S. spontaneum* into *S. officinarum* using deliberate hybridisation and selection within sugarcane breeding programmes occurred in both Indonesia and India.

In Indonesia, obtaining resistance to the diseases "serah" and sugarcane mosaic virus from *S. spontaneum*-related clones was an important motivating factor contributing to the early interspecific breeding efforts, and this was successful. However it was found that the interspecific hybrids and derivatives were also more vigorous and adaptable to environmental stresses and had better ratooning ability compared with the best *S. officinarum* clones.

The most famous clone produced from the early introgression programme in Java was POJ2878 (Fig. 14.3), also known as the "Java wonder cane". Development of this clone is described by Mangelsdorf (1960). In 1893 the breeder J.H. Wakker grew seed from open pollination of the typical *S. officinarum* clone "Bandjermasin Hitam", and from this population, he selected the variety POJ100 which became a major commercial variety in Java. In 1911, a sugarcane pathologist, Miss G. Wilbrink, became interested in the clone Kassoer as a source of resistance for serah disease which was important in the Java sugar industry up to that time. Kassoer was

later shown to be a natural hybrid between *S. officinarum* and *S. spontaneum*. Wilbrink crossed POJ100 with Kassoer and amongst this population a clone 'POJ2364' was selected. This clone although was immune to Serah disease, but had too low in sugar content for commercial production. In 1917 the breeder J. Jesweit crossed POJ2364 with the important *S. officinarum* cultivar EK28 and obtained several promising clones. In subsequent years he generated more clones from this cross, and from a cross made in 1921 he selected POJ2878. The superiority and reported rate of extension of this variety seem to be astonishing; within 8 years this variety occupied over 400,000 acres or 90 % of the production area in Indonesia, with reportedly a 35 % yield advantage over the varieties it replaced (Mangelsdorf 1960). This clone is exceptional also in its use in subsequent breeding efforts and is in the ancestry of most commercial cultivars around the world today.

In 1919, Dr. Elmer Brandes of the Office of Sugar Plant Investigations, Bureau of Plant Industry, USA, identified sugarcane mosaic virus in Louisiana (USA) and began to identify varieties resistant to the disease. Resistant POJ varieties were imported to the Southdown plantation, near Houma, Louisiana, through Washington, D.C. in 1922 and 1923. Local lore described the varieties as the "Please Oh Jesus" (POJ) varieties, because they were seen as one of the last hopes for a nearly bankrupt industry with heavy mosaic pressure. By 1924, the varieties were recognised as the salvation of the local industry, and the newly imported varieties (POJ234, 36, and 213) were described as "… a prosperous but self-centered oasis in a mosaic desert …" (Abbott 1971). Many other sugarcane industries around the world experienced similar results with the introduction of the POJ varieties, and local breeding programmes started using these varieties as parental material.

In India in 1912 the first recorded deliberate cross between *S. officinarum* and *S. spontaneum* was made at the Sugarcane Breeding Institute, Coimbatore, under the leadership of Dr. C.A. Barber and Sir T.S. Venkataraman (Nair 2008). This cross between Vellai (*S. officinarum*) and a wild *S. spontaneum* produced the interspecific hybrid Co205, which was released as a commercial cultivar in the Punjab province of northern India in 1918. This clone reportedly yielded 50 % more than the *S. barberi* cultivars it replaced in that region (Thuljaram Rao 1987, cited in Selvi et al. 2005). This clone was clearly better adapted to the challenging climatic conditions in the subtropical region it was released to. Other interspecific hybrids were also produced in India at around the same time and were crossed with *S. officinarum* clones and other hybrids between *S. officinarum* and either *S. spontaneum* or *S. barberi*, including POJ clones introduced from Java. These led to other important cultivars suited to production environments both in India and worldwide such as Co281 and Co290. These in turn led to further significant cultivars and parents such as Co331, Co419, Co421, and Co475, many of which also feature in ancestries of many important sugarcane cultivars worldwide today.

Between 1919 and 1939, the idea of using wild species in breeding programmes became more defined, and a serious effort was made to assemble a collection of sugarcane from around the world, both from breeding institutions and from indigenous regions (Brandes et al. 1939). Stated eloquently by Brandes (1935), "The pursuit of knowledge and the hope that such researches may eventually lead to production of

crop plans of economic importance is the double stimulus which prompts the attempts to secure and study these hybrids. The expenditure of effort and money in crossing the large thick-stemmed, tropical sugarcanes with the slender, unprepossessing wild cane *Saccharum spontaneum* has already paid enormous dividends". The development of commercial sugarcane is an example of the usefulness of germplasm enhancement programmes and gains that can be made through breeding with related wild species.

14.3.2 Later (Post 1960) Introgression of S. spontaneum

Following the successes and rapid genetic gains in cane yield, ratooning performance, and adaptation to marginal (especially cool) environments from the initial introgression of *S. spontaneum* in sugarcane breeding programmes, sugarcane breeders continued to exploit the early interspecific clones developed and their selected progeny. This involved intercrossing among the original hybrids and recurrent crossing and selection among progeny with increasingly larger populations, following the general process outlined in Fig. 14.1.

Following the generation of these original hybrids, few efforts were made in several subsequent decades to broaden the genetic base of sugarcane. However, around the 1960s breeders in several programmes across several countries recommenced significant crossing with basic *S. spontaneum* clones for several reasons:

1. A concern about the small number of ancestral clones on which sugarcane breeding programmes were based compared with the great diversity of materials available: Arceneaux (1967) and Price (1967) both reviewed the derivation of modern sugarcane varieties and emphasised the limited numbers of ancestor clones. Most crosses made in breeding programmes throughout the world today are still based on a relatively small number of ancestors derived from the early interspecific hybrids. In Louisiana, a new strain of mosaic virus (later designated as strain H) was first acknowledged in the 1950s. When it began to infect varieties considered resistant to other strains of the disease, it highlighted the need for increased genetic diversity in the breeding programme and new sources of variation (Abbott 1961; Breaux and Fanguy 1967).
2. An awareness that there are many desirable traits in clones in germplasm collections (e.g. drought tolerance, waterlogging tolerance), not yet captured in commercial varieties.
3. Some unease because the rate of genetic gain was slowing, with one hypothesis for this being that it was related to the narrow genetic base of sugarcane breeding programmes.

By the 1970s, the so-called base broadening programmes were present in Barbados, Australia (Macknade), Taiwan, India, China, and the USA (Hawaii and Louisiana) (Heinz 1987). Subsequently programmes commenced in other countries such as Thailand and Brazil. The focus of most of these programmes was, and

continues to be, introgressing traits from *S. spontaneum* into commercial sugarcane varieties. Traits of interest from *S. spontaneum* include dense plant cane stands, profuse tillering, good ratooning ability, disease resistance, stem borer resistance, and stress tolerance (e.g. flood, salt, drought, cold) (Duncleman and Breaux 1970, 1972; Walker 1972; Heinz 1987). The approach taken by the programmes to identify *S. spontaneum* breeding clones varied, with the USDA-ARS-Houma, LA, choosing to heavily screen parental material before crossing and the germplasm enhancement programme in Barbados deliberately choosing not to select wild clones in order to maximise variability.

In most cases, the *S. spontaneum* is used as the male parent because of its heavy pollen production, its ability to self-pollinate, and its status as a noxious weed. By using the species as a male, breeders are able to ensure that resulting seedlings are not a result of self-pollination of the *S. spontaneum* parent. The original sugarcane × *S. spontaneum* (or other wild species) cross is referred to as the F_1 generation. Selected F_1 clones are backcrossed to commercial sugarcane genotypes (generally high sucrose) to obtain the first backcross generation (BC_1). Selected BC_1 clones are again backcrossed to sugarcane giving rise to the second backcross generation (BC_2), and an iterative process is continued (sometimes through the BC_4 generation or higher) until desired commercial parental clones are obtained.

Some successes (i.e. release of productive new commercial varieties) have arisen from introgression breeding programmes incorporating new germplasm since the 1960s, but overall, the success rate has been mixed and sometimes poor. Roach (1984, 1989), based partly on direct experience with the CSR programme in Australia, listed several reasons for the failure of new introgression programmes to provide more productive varieties than equivalent effort devoted to improved breeding pools. These reasons were largely related to inferior traits in the wild donor clones and difficulties in selecting and combining the appropriate desirable portions of both the wild type and the recurrent parents during subsequent selection cycles. Another problem noted was the lack of cytological or genetic information to confirm the hybrid nature of initial clones derived from interspecific hybridisation and selected for further crossing. Grassl (1963) also highlighted the challenges of obtaining synchronous flowering. However, both these issues have largely been overcome in recent decades with the use of DNA markers and photoperiod treatments. In particular, the use of DNA markers has been of greater importance as illustrated in examples reported by Cai et al. (2005) and Aitken et al. (2007a, b) in the identification of true hybrids.

The overall major challenge associated with introgression of basic germplasm into highly selected and commercially adapted germplasm in sugarcane breeding is the same as in other crops: that the basic germplasm brings with it many undesirable traits which need to be selected against between cycles of crossing back to the highly bred and commercially superior parental material, while at the same time desirable traits and genes from the wild donor may be diluted or lost with successive generations. In the case of sugarcane, the major undesirable trait introduced with the use of wild canes is low sucrose content. Following initial crosses made with wild clones (e.g. *S. spontaneum*), generally two or more backcrosses to elite commercial

type parents and lengthy (up to 7 or 8 years) intervening field evaluation and selection programmes are traditionally conducted before commercial type progenies are developed (Miller and Tai 1992). Therefore the process of introgression in sugarcane using conventional breeding procedures is relatively long and risky, and this deters investment.

Despite the challenges, some important successes have been achieved in introgression programmes initiated since the 1960s. One highly successful example is the development of numerous important cultivars in Australia within the BSES Limited breeding programme developed from the wild *S. spontaneum* clone "Mandalay". This clone was first collected by A.J. Mangelsdorf in 1929 (Heinz 1980) and was induced to flower in synchrony with commercial parents by CSR Limited in Australia in 1962. The flowers were crossed with POJ2878 in 1962 by BSES breeders, and selected progenies were crossed with other commercial varieties to produce a series of major cultivars for the Australian sugarcane industry. Another successful example is the use of the *S. spontaneum* cultivar US56-15-8, collected from northern Thailand, and leading to a range of cultivars in Louisiana, including the major cultivar LCP85-384 (Milligan et al. 1994; Arro et al. 2006).

A range of reports on the use of *S. spontaneum* since the 1960s are present in the literature. Some common findings of these programmes are as follows:

- Early generations of progeny following initial crosses between *S. spontaneum* and sugarcane (*S. officinarum* or commercial hybrids) are characterised by levels of sucrose content too low for commercial production and high fibre levels. These levels progressively and rapidly increase in subsequent crosses (e.g. Hsu and Shih 1989; Mullins 1988).
- Some early-generation progenies derived from *S. spontaneum* have provided good biomass yields, particularly in ratoon crops (e.g. Terajima et al. 2007; Wang et al. 2008), although it should be noted that some studies have involved small plots in which competition effects may be important in affecting cane yield.
- As noted by Roach (1984) much breeding work involving *S. spontaneum* following the 1960s was conducted under the vague objective of "base broadening". This objective in itself has little value and may not provide a focused basis for achieving practical commercial results.
- Some *S. spontaneum* clones provide good sources of resistance to diseases such as sugarcane mosaic (Koike 1980), red rot (Hale et al. 2010), and sugarcane yellow leaf virus (Costet et al. 2012) and pests (e.g. Jackson and Dunckelman 1974; White et al. 2011).
- Some *S. spontaneum* clones provide good sources of resistance to environmental stresses such as cold tolerance (e.g. Irvine 1966; Tai and Miller 1986; Miller et al. 2005) and waterlogging tolerance (e.g. Srinivasan and Batcha 1962; Sookasthan et al. 1992).
- Some contradictory results have been reported regarding the heritability of traits from *S. spontaneum* parental clones. Roach (1977) reported results showing that sucrose level in *S. spontaneum* clones was an important predictor of progeny performance when crossed with sugarcane (*S. spontaneum* or commercial type clones),

while cane yield was a moderate predictor. Wang et al. (2008) found performance of *S. spontaneum* parent clones to be a poor predictor of progeny performance for both sucrose content and yield, although it was high for stalk number and stalk weight independently.

- Although very preliminary, there are some signals that *S. spontaneum* clones sourced from the northern Thailand–Burma region could provide superior breeding material. Commercial successes in Australia and the USA have been obtained from clones selected in this region (as noted in references above). The impressive phenotypic characteristics of this material were highlighted by Sookasthan et al. (1992). Heinz (1980) noted the high yields of *S. spontaneum* clones collected from the northern tip of Thailand (19–20° latitude) and the apparent propensity to pass these favourable traits onto progeny. White et al. (2011) noted the significant contribution of *S. spontaneum* clones sourced from Thailand in sugarcane improvement.

14.4 Introgression of *Erianthus*

The genus *Erianthus* is related to *Saccharum* (sugarcane) and *Miscanthus* and is regarded by sugarcane breeders as a part of the *Saccharum* complex (Daniels and Roach 1987). The taxonomy of *Erianthus* is confusing, with some taxonomists regarding *Erianthus* as being within the genus *Saccharum*, although molecular studies indicate a relatively large difference with all other *Saccharum* species (Sobral et al. 1994; Hodkinson et al. 2002; Nair et al. 2005). There has been an interest among sugarcane breeders in utilising *Erianthus* in breeding programmes, particularly *Erianthus arundinaceus*, for many years. This is mainly due to the high level of vigour, drought and waterlogging resistance, good ratooning ability, and disease resistance attributed to this species. However, despite this reputation, it would appear that reports of well-controlled studies comparing performance of *Erianthus* with other *Saccharum* species and sugarcane cultivars are rare (Jackson and Henry 2011).

There have been several published reports of production of hybrids between *Saccharum* spp., especially *Saccharum officinarum* and sugarcane cultivars and *Erianthus arundinaceus* (D'Hont et al. 1995; Piperidis et al. 2000; Ram et al. 2001; Cai et al. 2005; Nair et al. 2006; Lalitha and Premachandran 2007), *Erianthus rockii* (Aitken et al. 2007a, b), and *Erianthus ravennae* (Janaki-Ammal 1941). We are aware of breeding programmes aiming to utilise *Erianthus* existing in a range of countries including China, India, the USA, Australia, and Thailand.

Several factors have limited the introgression of *Erianthus* in sugarcane breeding programmes to date, the major being the difficulty in producing fertile hybrids between sugarcane and *Erianthus*. This has been further complicated by difficulties in identifying true hybrids arising within seedlings from crosses between sugarcane (*Saccharum* spp.) and *Erianthus*. In many cases, putative hybrids have later been shown with DNA markers to be selfs or arising from pollen contamination (D'Hont et al. 1995, personal communication with sugarcane breeders). Further, while some

true hybrids have been produced, some breeders have reported difficulty in producing fertile crosses between these resulting hybrids and *Saccharum* (Piperidis et al. 2000). Difficulties in producing fertile hybrids, or any hybrids at all, may be attributed to the apparently relatively large genetic distance between *Saccharum* and *Erianthus*, even larger than for other genera such as *Miscanthus* (Sobral et al. 1994; Alix et al. 1998; Cai et al. 2005).

One successful example of progeny produced from hybrids between *Saccharum* and *Erianthus* was reported by Cai et al. (2005). However to date no commercial cultivars of sugarcane incorporating components of *Erianthus* have been reported to our knowledge.

Other factors suggested as potentially contributing to the lack of success of introgression of *Erianthus* genes into sugarcane breeding programmes include chromosome erosion during crossing and backcrossing and lack of recombination between the chromosomes of the two genera (D'Hont et al. 1995), although some recombinant chromosomes have been recently reported (Piperidis et al. 2012). Reduced transmission of chromosomes in crosses between *Saccharum* and *Erianthus* or hybrids and *Saccharum* was reported by D'Hont et al. (1995), Piperidis et al. (2000, 2010).

To date there have been few reports backed with statistically supported data demonstrating performance of *Saccharum*×*Erianthus* hybrids or their derivatives. Grassl (1972) reported vigorous and good-looking plants arising from a cross between *Erianthus kanashiroi* and *S. spontaneum*. Sugarcane clones produced by crossing an *Erianthus arundinaceus* clone with a *S. officinarum* clone were also reported by Ram et al. (2001) as having superior low temperature tolerance and red rot resistance.

14.5 Introgression of Other Species

Although its inclusion as part of the *Saccharum* complex has been debated, *Miscanthus* is a genus of interest to sugarcane breeders. The *Miscanthus* genus is distributed across Tahiti, Indonesia, China, Siberia, Japan, India, southern Africa, and Nepal (Adati and Shiotani 1962; Hodkinson et al. 2002). The genus is found in elevations ranging from sea level to 3,300 m in Taiwan (Lo et al. 1978) but is mainly found in upper elevations (1,500–2,500 m) (Paijmans 1976). Much like *S. spontaneum*, *Miscanthus* can be found growing in diverse environments including rocky, stony, dry, or wet and in full sun or shade (Lo and Su 1968). *Miscanthus sacchariflorus* is thought to be one of the species involved in the evolution of *S. sinense* (Grassl 1974), and phylogenetic analysis of cytoplasmic DNA suggests that *Miscanthus* and *Saccharum* hybridise naturally (Sobral et al. 1994). Traits of interest to sugarcane breeders from this genus include cold tolerance, downy mildew resistance, drought resistance, and smut resistance (Lo et al. 1978).

While hybrids with the genus are not common, some successful attempts have been documented. Tai et al. (1991) evaluated juice quality traits of hybrid and

backcross progeny of *Saccharum* and *Miscanthus* and found that mean sucrose content of F_2 and BC_1 progenies was higher than that of the F_1 hybrids, but stalk diameter remained small. Burner (1997) describes an $n+2n$ transmission when sugarcane was crossed to *M. sinensis* and $n+n$ transmission when crossed with *M. japonicas*. While no DNA-based markers were used in this study, chromosome transmission was documented, lending credence to the claim that successful hybridisations were made between *Saccharum* and *Miscanthus*.

Breeders in Taiwan have had an interest in breeding with *Miscanthus* since the 1950s. In 1953, a study was published on a cross between POJ2755 and *Miscanthus floridulus* (Chen 1953). One hundred and twenty-nine clones in their collection were screened for resistance to smut and downy mildew, and over 80 % were resistant to downy mildew. All these clones were resistant to smut (Lo et al. 1980). Downy mildew-resistant clones were used as males and crossed to commercial hybrids, resulting in 21 % of progeny that morphologically resembled *Miscanthus*. Selected progenies were downy mildew resistant, and a majority were highly resistant to smut. They had a brix that was higher than the *Miscanthus* parent (approaching that of sugarcane), a stalk diameter intermediate between the two parents, and populations that were greater than the sugarcane parent (Chen et al. 1980, 1982, 1983; Shen et al. 1981). In 1983, the research group reported that F_1 hybrids between sugarcane and *M. sinensis* or *M. floridulus* resulted in progeny with average pith, diameter, sucrose content, and tillering somewhere between the two parents. These were assumed to be true hybrids because of chromosome numbers ranging from $2n=70$ to 100 with irregular meiosis (Chen et al. 1983).

The recent emphasis on bioenergy production has corresponded with a renewed interest with intercrossing *Saccharum* and *Miscanthus*. The USDA-ARS in Houma, LA, has bred with *Miscanthus* in the past and has a few verified hybrids (unpublished). Recently, Texas A&M University has reported that hybrids between the two genera, named "Miscanes", have shown promise as donors for drought and cold tolerance. The group has verified the hybrid nature of the clones and continues to work with breeding them for bioenergy production (Park et al. 2011).

There have been several reports of hybridisation between sugarcane and bamboo, although hybridisation attempts resulted in very few viable seeds. Rao et al. (1967) reported the generation of four hybrid seeds from 960 crosses between *Bambusa arundinacea* (*B. bambos*) and *Saccharum*. When *Bambusa* was used as the male parent, no hybrids were obtained; however, when it was emasculated and used as the female parent, two seeds were produced from *S. spontaneum* and two from *S. robustum*, while the seed from the *S. spontaneum* only germinated.

Sorghum is another genera of interest to sugarcane breeders because it possesses drought tolerance, is widely adapted, and offers the potential to develop a hybrid crop that can be propagated through seed. Several attempts at hybridisation with *Sorghum* have been made over the last century. In 1930, Thomas and Venkatraman reported successful hybridisation between sugarcane and *Sorghum dura* Stapf, and in 1935, Bourne reported hybridisations between sugarcane and sweet sorghum, *Holcus sorghum* L. var. *saccharatus* (L.) Bailey. In both cases, hybrids were reported to be dwarf, with some albino types, and had little commercialisation potential.

Overall, 37 % of the 345 hybrids produced by Bourne survived, with only 3 % displaying enough vigour for further evaluation. The hybrids produced by Venkatram were said to mature in 5–6 months in comparison to the 9–10 months required for cane and despite their low yields were "high in sugar". (Brandes 1935). Another hybridisation between *Saccharum* and *Sorghum* was reported in 1999, but like previous attempts, recovery of viable seedlings was low, with only five seedlings recovered from 3,670 pollinated florets (Nair 1999).

Hybridisation between the two genera was reported recently (Hodnett et al. 2010). While past attempts at hybridisation met with poor seed set, a new approach was taken using an inbred line of *Sorghum bicolor* which was homozygous for the mutant *iap* (inhibition of alien pollen). The *iap* trait removes the reproductive isolation between sorghum and closely related taxa allowing for easy production of interspecific hybrids. Through the use of Tx3361 (the line containing the *iap* trait) as the male sterile female parent, 14,141 hybrid seeds were produced from 252 *Sorghum bicolour*×*Saccharum* crosses. Embryo rescue was used on the pollinated seed resulting in a seedling recovery rate of 33 %. Attempts to backcross the hybrids to sorghum have not been successful.

14.6 Transfer of Genes Through Genetic Engineering

As discussed above, sexual transmission of genes and traits is limited to relatively close relatives and members of wider Andropogoneae where sexual compatibility is allowed. However, the ability to introduce and express alien genetic materials into sugarcane by artificial methods allows possibilities of gene transfer from any other organism, even those from other kingdoms. The first demonstration of this process, called transformation or more broadly "genetic engineering", for sugarcane was the introduction of a bacterial gene conferring resistance to an antibiotic kanamycin into sugarcane protoplasts (Chen et al. 1987) by osmotic shock. Additional methods of genetic engineering used since then include electroporation (Chowdhury and Vasil 1992), biolistics (Franks and Birch 1991), and the soil bacterium, *Agrobacterium* (Arencibia et al. 1998).

The delivery and subsequent integration of the introduced genetic material is only the first step: to make introgression of alien genes useful, plants expressing them at the desired level need to be regenerated from the cells/tissues into which they were introduced. This has been done successfully in a variety of targets ranging from protoplasts to cells to different tissue types (Lakshmanan et al. 2005). Unlike the natural development of plants from axillary buds, regeneration of plants from a dedifferentiated transformed cell or tissue in an artificial sterile condition meant that their phenotype, including agronomic characteristics and yield, may vary from the mother plant (Lakshmanan 2006). Hence, identifying desirable genetically modified lines from a large population of transgenic plants is a critical step in crop genetic engineering. This involves not only the assessment of agronomics and yield of

genetically engineered lines but also the stable expression of introduced alien gene/traits(s) in different production environments and transmission to progenies in successive generations. This has been demonstrated by engineering herbicide tolerance and virus resistance traits simultaneously in sugarcane (Butterfield et al. 2002). However, it is important to note that expression of alien genes introduced transgenically may become silenced when grown in the field following successive vegetative propagation (Basnayake et al. 2012).

There are several types of alien genetic elements that have been introduced into sugarcane through transformation. Firstly, and most importantly, there are coding regions of genes, which confer the target trait/phenotype such as pest and disease resistance and herbicide tolerance. To make the coding region of gene express a desired phenotype the genetic message it carries will be transcribed into the messenger molecule RNA. Gene transcription is regulated by a promoter and terminator and sometimes enhancers. These regulatory elements also can be of foreign origin, and numerous such examples exist in sugarcane (Lakshmanan et al. 2005), starting from the very first creation of a genetically engineered sugarcane cell (Chen et al. 1987).

Since gene transfer through transformation is largely a mechanical process, both alien and native genes and/or its regulators in its original or modified forms can be introduced (Beyene et al. 2011). Also, in the same sugarcane line multiple genes controlled by genetic elements of diverse origin can be introduced. This becomes a necessity when a new metabolic pathway involving multiple genes needed for a target product is engineered into plants. Despite its technical complexity this has been achieved in sugarcane engineered to produce bioplastics (Mcqualter et al. 2004). More recently microRNAs controlling specific traits are being manipulated to modify phenotypes by altering the level of specific gene expression. For example, sugarcane microRNAs have been successfully used in tobacco (Begcy et al. 2012) and in sugarcane (Jung et al. 2012). With the explosion of genomics research in sugarcane and other crops application of native and alien microRNAs may become a significant tool for genetic modification in sugarcane.

The fact that the fundamental features of genetic elements are the same across all organisms, genes from any source can be introduced into sugarcane. Table 14.1 shows examples from various phyla from which DNA has been introduced into sugarcane. However, sometimes the coding regions of genes are redesigned to optimise its expression in other species. The most common changes have been (1) codon optimisation to produce an enzyme in the correct configuration and (2) use of eukaryotic introns in prokaryotic genes to affect the expression in a eukaryote-like sugarcane.

DNA sequences originally isolated from sugarcane have been transformed back into sugarcane such as genes (e.g. polyphenol oxidase, Vickers et al. 2005a, b), promoters (Mudge et al. 2009), and targeting sequences (Jackson et al. 2007), but this is referred to as *cis*-genics and is not discussed here further.

Several examples of sugarcane plants engineered using alien genes are described below:

Table 14.1 Sources of some of the genes introduced into sugarcane through biotechnological methods

Phyla	Species, DNA, and trait	References
Virus	Sugarcane mosaic virus, coat protein gene (resistance)	Joyce et al. (1998)
	SrMv (sorghum mosaic virus) (resistance)	Ingelbrecht et al. (1999)
	Sugarcane yellow leaf virus (resistance)	Rangel et al. (2003)
	Fiji disease virus (resistance)	Mcqualter et al. (2001)
	Cauliflower mosaic virus promoter (gene expression)	Gallomeagher and Irvine (1993)
	Cauliflower mosaic virus terminator (gene expression enhancement)	Beyene et al. (2011)
	Cavendish banana streak badnavirus promoter (gene expression)	Petrasovits et al. (2012)
Bacteria	*Pseudomonas mesoacidophila* MX-45, trehalulose synthase (alternative sugar)	Hamerli and Birch (2011)
	Pantoea dispersa, albicidin dehydrogenase (resistance to leaf scald)	Zhang et al. (1999)
	Pantoea dispersa UQ68J isomaltulose synthase (alternative sugar)	Basnayake et al. (2012)
	Ralstonia eutropha phaA, *phaB*, *phaC* (polyhydroxybutyrate)	Petrasovits et al. (2007)
	Agrobacterium tumefaciens nopaline synthase terminator (gene expression)	Petrasovits et al. (2007)
Fungi	*Grifola frondosa*, trehalose synthase gene (drought tolerance)	Zhang et al. (2006)
Animals	*Aequorea victoria,* green fluorescent protein (selectable marker)	Elliott et al. (1999)
Human	Human-granulocyte macrophage colony-stimulating factor gene (pharmaceutical)	Wang et al. (2005)
Dicotyledons	Soybean proteinase inhibitor genes (insect resistance)	Falco and Silva-Filha (2003)
Monocotyledons	Maize ubiquitin promoter (gene expression)	Hamerli and Birch (2011)
	Maize chlorophyll a/b-binding protein	Petrasovits et al. (2012)
	Rice ubiquitin promoter (gene expression)	Petrasovits et al. (2012)

14.6.1 Protecting Against Sugarcane Leaf Scald

Leaf scald is a major bacterial disease caused by *Xanthomonas albilineans*, which produces the toxin albicidin. Engineered resistance to leaf scald was one of the first significant examples of exploiting alien genes for a commercially important trait (Zhang et al. 1999). Transgenic sugarcane plants that express an albicidin-detoxifying gene (*albD*) cloned from the bacterium *Pantoea dispersa* which is used as a biocontrol against leaf scald disease did not develop disease symptoms in inoculated leaves, whereas all non-transgenic control plants developed severe symptoms. Expression of *albD* gene also protected plants against systemic multiplication of the pathogen proving that a single gene-based detoxification strategy alone *is sufficient to confer resistance to both disease symptoms and control of pathogen in the host*.

14.6.2 Production of Alternative Sugars in Sugarcane

Use of sugarcane as a biofactory for alternative sugars attracted substantial interest in the past decade. As an example Basnayake et al. (2012) were successful to express isomaltulose synthase (IMS) gene in stem parenchyma vacuole of sugarcane in order to produce isomaltulose (IM), a low GI alternative sugar. Transgenic sugarcane plants of seven Australian cultivars expressing IMS gene when grown in the field accumulated IM up to 33 % of total sugar. However, a concomitant decrease in sucrose concentration resulted in no change in total sugar content in these IMS lines. Several cycles of field propagation and careful selection were needed to identify clones that yielded similar to the recipient non-transgenic lines. Clones with no apparent adverse effect of IM accumulation on growth and germination of setts were identified in the test population. However, there was some inconsistency in IM production in vegetatively propagated field-grown transgenic lines. Despite this observation, the results in general indicated good potential to develop sugarcane for commercial scale production of IM.

14.6.3 Weed and Mosaic Virus Control in One Shot

Transgenic sugarcane plants resistant to herbicide bialaphos and sorghum mosaic virus (SrMV) were produced by introducing alien genes *bar* (herbicide resistance) and *hut* (SrMV resistance) (Ingelbrecht et al. 1999). These lines were crossed with non-transgenic sugarcane varieties to study the segregation of the transgenes and trait expression in the progeny. Both transgenes were integrated in the genome as a linked insertion in one locus or they occurred in two independent, unlinked loci. Analysis of progeny of parent unlinked independent insertions indicated rearrangements in both loci. Most transgenic progenies containing the *bar* gene showed resistance to herbicide, while a high proportion of progenies were susceptible to SrMV. This pioneering study on transgene segregation in sugarcane demonstrated the viability of transgenic sugarcane parents in breeding programmes.

Whilst some plants have been tested in field trials, development of commercial sugarcane varieties from transgenic technology has yet to be realised. Several specific steps including additional technical, regulatory, and commercial aspects which are generally not part of a research activity need to be addressed to develop genetically modified (GM) commercial sugarcane cultivars.

The first technical issue is to reduce the number of transgene insertions. Ideally a potential commercial GM clone will have an intact single-copy transgene insertion event for simple integration during sexual transmission to other genetic backgrounds. The stability of trait expression across multiple vegetative generations under different crop production environments is another major consideration for commercial GM crop development. Further, transgene introduction must not have a negative impact on cane or sugar yield: early field trials indicated that this could be the case (Vickers et al. 2005a, b). Additionally, introduction of the alien gene should

not predispose the host to susceptibility to diseases and pests. The transgene should also perform consistently in different genetic backgrounds, a key requirement for successful introgression of the transgenic trait through breeding.

Regulatory agencies assess GM plants and the food originating from them for a range of potential hazards relating to the environment and human health. For the environment, the ability of the transgene to increase the weediness of the altered plant or any sexually compatible species is assessed as is the potential effect of the transgene product on the organisms it may come in contact with. These impacts are assessed on a case-by-case basis, but information required to define the baseline biology of sugarcane to assist assessment has been receiving attention (Bonnett et al. 2008; Office of Gene technology Regulator, Australia (OGTR) 2011; Cheavegatti-Gianotto et al. 2011). An Organisation for Economic Co-Operation and Development (OECD) consensus document on GM sugarcane is also being prepared. For regulation in food, a common practice is to assess the substantive equivalence of the new plant compared to the existing ones, and a recent document from the OECD has suggested about what components of sugarcane modified with alien genes should be tested for comparison (OECD 2011). In addition, the potential for the inserted DNA to produce toxins or allergens is assessed, and sometimes feeding studies are also conducted.

Whilst at the time of writing no sugarcane with an alien gene transferred by molecular techniques has yet been commercialised, it is likely to occur within the next few years. Significant investments are being made in sugarcane by companies that have brought other genetically modified crops to the market (Bonnett et al. 2010) which will increase the effort in commercialisation of sugarcane with alien genes transferred by molecular techniques. The high cost of regulating each individual event and the long time to breed new sugarcane cultivars will see increased discussion of whether regulation can be conducted on an individual gene construct basis (allowing different events arising from the use of the same assembly of alien gene elements into different background cultivars).

14.7 Conclusions and Future Prospects

The development of modern sugarcane cultivars has provided a great example of the successful introgression of genes from wild germplasm for enhancement of genetic gains from breeding. The introgression of parts of the wild cane *S. spontaneum* genome into the *S. officinarum* genome in the early 1900s provided improved disease resistance, better adaptation to environmental stresses, and much improved ratooning performance and had enormous impact on lowering the cost of sugar production worldwide thereafter.

Following the first interspecific hybrids, sugarcane breeding programmes continued to make large gains from this initial introgression for several decades, illustrated by clear superiority of successions of new varieties in many industries until at least the

1950s. However, based on anecdotal evidence it appears that after several decades of exploiting the newly introgressed materials, rates of genetic gain gradually slowed. This prompted concerns expressed by a range of sugarcane breeders around the 1960s and stimulated efforts to introgress more genetic diversity from wild canes, particularly *S. spontaneum*, into sugarcane breeding programmes. While there have been some successes from these more recent efforts, the gains have clearly not been as large as the initial introgression breeding, and there have been many cases where little or no commercial success has arisen from significant efforts.

Introgression of new wild cane genomes which is occurring in a range of sugarcane breeding programmes around the world is expected to continue into the future. Based on current reports this effort will probably provide for further incremental gains in profitability. However, because of the need to obtain high sugar content for commercial cultivars, and the very low sucrose content in wild canes, several cycles of backcrossing and selection are required, and this will continue to make investment in conventional approaches to introgression breeding lengthy, costly, difficult, and risky.

Berding and Roach (1987) and Roach (1992) amongst many others have lamented the lack of characterisation of clones in sugarcane-related germplasm collections and pointed to this as limiting wider and more effective use of material in collections. While some characterisation of clones in various collections has been reported (Roach 1986; Tai and Miller 1988; Balakrishnan et al. 2000), it is also questionable as to whether characterisation for some traits provides a useful indicator of breeding value. One line of argument is that for complex traits like yield wild species may contain mostly inferior traits and alleles but that at a smaller number of loci, some alleles with more favourable effects than in existing commercial materials may exist (Tanksley and McCouch 1997). The key challenge to the breeder is therefore retaining the favourable alleles while eliminating the rest during the backcrossing cycles. Identification of favourable alleles amongst unfavourable ones may benefit from DNA markers for QTL analysis (see below). This argument is consistent with some reports of poor-performing wild clones proving to produce the best progeny in two or more generations (Rao and Martin-Gardiner 2000).

Two developments in the future may improve contributions from introgression of wild germplasm in sugarcane breeding. Firstly, the likely increased value of the fibre component in sugarcane for energy production (electricity or biofuel) in future may tilt optimal selection indices in sugarcane breeding programmes slightly away from the current very high weighting to sucrose content and more towards total biomass production and fibre content. If this occurs then commercial cultivars with a higher proportion of the wild *S. spontaneum* genome may be possible, meaning that less cycles of backcrossing may be required and early-generation clones (F_1 or BC_1) or crosses among such clones may be commercially viable. Such a change would increase the accessibility and use of wild clones in sugarcane breeding programmes.

Secondly, as with all other crops, the application of DNA markers may also provide ways to better use wild germplasm in breeding programmes. Undertaking QTL

analysis in advanced backcrosses may provide a way to effectively identify the wanted and unwanted parts of the wild genome. Markers closely linked to the desirable and undesirable genome components could be selected for or against during subsequent breeding efforts.

The use of DNA markers for assisting in introgression breeding however provides some challenges in practical implementation, particularly in costs and time. Undertaking QTL analysis to detect markers linked to traits at low false discovery rates for most quantitative traits usually requires field phenotyping and genotyping of large (>500) populations. Generation of advanced backcross populations in sugarcane also usually takes several years. However, despite these costs such approaches may still provide an effective way to utilise wild genomes in sugarcane breeding more effectively than conventional approaches.

The large genome size of sugarcane and the high polyploidy add additional challenges to the application of DNA markers in sugarcane which need careful consideration. For example if the donor genome (e.g. *S. spontaneum*) has a high ploidy level, progeny in first or second backcross generations may not segregate in terms of the presence versus absence of chromosomes from a homology group. Such populations therefore may only provide analysis of variation due to alternative alleles from the donor germplasm, rather than test for the presence versus absence of any donor germplasm alleles at different loci. The large genome size of sugarcane also means that a large number (e.g. >3,000) of markers may be required to obtain comprehensive genome coverage, and with many marker systems used (e.g. AFLPs) this is very expensive. The development of newer marker systems such as SNP chips may help address this issue in the future in sugarcane. However obtaining sufficient phenotype data to undertake powerful QTL mapping for most important traits (e.g. sugar content and cane yield) will remain slow and expensive without some high-throughput technologies.

Technological advancements in GM sugarcane research have also been impressive with a variety of methods to introduce and express genes in sugarcane now available. However, as in many other crops, a major hurdle is recalcitrance of sugarcane genotypes. More research effort is needed to tackle this issue. Recently, the value of linearised minimal gene vectors carrying only the expression cassette for GM sugarcane research was recognised (Jackson et al. 2013), and it is expected to become a routine technology for sugarcane transformation. Development of synthetic mini chromosomes that offer the ability to target transgenes to a defined insertion position for predictable expression (Birchler et al. 2010) is likely to make a significant impact in GM technology especially in polyploids. Sugarcane, touted as a viable energy crop, is attracting large investments in developing commercial GM crops in various countries. The target of alien (GM) traits in the near to medium term will be those that are not readily available for conventional breeding, as occurred in other crops. These include herbicide tolerance, pest resistance (e.g. stem borer resistance), and production of foreign compounds (e.g. alternative sugars). However, it must be stressed that the high regulatory costs and intellectual property restrictions may restrict the development of commercial GM sugarcane to mostly large multinational companies for the foreseeable future.

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