

Spatial assessment of *Saccharum* species hybrids and wild relatives in eastern South Africa

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ABSTRACT

Sugarcane (*Saccharum* hybrids) belongs to the Andropogoneae in the Poaceae. The grass family is known to have provided the world's most economically important crops. In assessing the risk of cultivating genetically modified (GM) grass crops in South Africa, gene flow studies have to be conducted prior to the approval or release of such crops into the environment as hybridisation may occur between crop plants and wild relatives if certain barriers to gene flow are crossed. The aim of the study was to conduct a spatial assessment of *Saccharum* and its relatives in eastern South Africa and to assess potential gene flow, which in turn will inform the way forward for risk assessments. Eleven *Saccharum* wild relative species were selected for analyses based on their presence in the sugar producing region of South Africa: four species in the Saccharinae and seven in the Sorghinae. Spatial, temporal and gene flow assessments of wild relatives were conducted: prevalence, spatial overlap, proximity, dispersal potential, flowering times, hybridisation potential and relatedness. Field surveys, herbarium distribution records and literature were used to assess these factors and to determine the gene flow likelihood. A total of 815 herbarium specimens were sourced from 11 suitable herbaria and they were supplemented by 34 observations during field visits to sugarcane cultivation areas. The presence of all target species was confirmed in sugarcane areas. *Imperata cylindrica* (L.) Raeusch., *Sorghum arundinaceum* (Desv.) Stapf and *Miscanthidium capense* (Nees) Mabb. scored the highest likelihood for prevalence, flowering time and spatial overlap with sugarcane. Although *I. cylindrica* and *S. arundinaceum* generally ranked the highest for spatial and temporal assessments, they were not important candidates for gene flow potential from sugarcane, since they were not considered as reproductively compatible due to their low scoring on the relatedness assessment. *Cleistachne sorghoides* Benth., *Miscanthidium capense*, *Miscanthidium junceum* (Stapf) Pilg. and *Sarga versicolor* (Andersson) Spangler scored higher as close relatives of sugarcane in the study area. *Miscanthidium* species ranked highest for gene flow potential and were the only target species that were flagged by this study as having a high likelihood for gene flow with sugarcane. This is supported by the more recent divergent age from sugarcane that falls within the period considered to be optimal for hybridisation within Saccharinae species. When considering the likelihood scores of all species, the regions with the highest likelihood for gene flow were associated with coastal and southern-inland KwaZulu-Natal. These areas should be avoided when cultivating GM sugarcane should it be approved in the future, or in-depth risk assessments should be conducted before release. This study recommends that future studies be done to assess pollen compatibility and viability for sugarcane and related species (*Miscanthidium capense* and *M. junceum*) as part of a risk assessment, as some gene flow barriers, such as proximity and flowering time, was shown to be crossed in this study.

Keywords: Eastern South Africa; Gene flow likelihood; Genetically Modified (GM); Spatial assessment; Sugarcane; Taxonomy; Wild and weedy crop relatives

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SOLI DEO GLORIA

Behold, I will do a new thing; now it shall spring forth; shall you not know it? I will even make a way in the wilderness, and rivers in the desert.

Isaiah 43:19

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CHAPTER 1: INTRODUCTION

1.1 Background

Plants have a variety of uses such as food, medicine, fodder, ornamental, firewood and soil erosion control (Lira *et al.* 2009). Globally, the Poaceae (grass family) is the fifth largest family of flowering plants after the Asteraceae, Fabaceae, Orchidaceae and Rubiaceae (The Plant List 2013), with its members occurring on all habitable continents (Kellogg 1998). This family encompasses the world's most important food sources and provide cereals for human consumption and animal feed (Wang *et al.* 2015). Research on the taxonomy and ecology of this family is essential for understanding the staple crops that feed mankind (Kellogg 1998). Major cereals such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) are all members of the grass family (Kellogg 1998).

The economic value of the Poaceae family also stretches beyond food security (Ahmad *et al.* 2009). Ecological restoration incorporates grasses since they improve the functionality of mine dumps and control erosion of tailings (Mendez and Maier 2008). Two grass species, *Chrysopogon zizanioides* (L.) Roberty and *Cynodon dactylon* (L.) Pers., are effectively used for the rehabilitation of mine tailings (Li *et al.* 2016). Other Poaceae species are suitable for use in sustainable agricultural practices, such as the “push-pull” system, where *Pennisetum purpureum* Schumach. is used as a trap crop for certain pests of maize (Midega *et al.* 2009; Van den Berg and Van Hamburg 2015).

Domesticated crops are distributed across the world and this is one of the beneficial relationships between humankind and plants (Purugganan and Fuller 2009). Land-use, alien invasive plants and climate change are the main threats to germplasm of these crop plants and their wild relatives (Ford-Lloyd *et al.* 2011). Conservation assessments of crop wild relatives are essential, as they are genetically or taxonomically closely related to crops (Fielder *et al.* 2015) and harbour potentially valuable genetic resources that might be advantageous to agriculture (Hajjar and Hodgkin 2007). There exist challenges to conserve these wild relatives, because the majority of these plants are located outside protected areas (Heywood *et al.* 2007).

Maize, sugarcane (*Saccharum officinarum* L.) and sorghum (*Sorghum bicolor* (L.) Moench.), which belong to the Andropogoneae tribe in the Poaceae, are considered the world's most economically important crops (Welker *et al.* 2015). Sorghum and sugarcane are regarded as close relatives of one another (Paterson *et al.* 2004), and their wild relatives have been used in breeding programmes to enhance cultivars of these crops (Dillon *et al.* 2007).

Risk assessment studies are required prior to the approval and release of Genetically Modified (GM) crops. A limited number of risk assessment studies have been undertaken on potential gene flow between cultivated and wild *Sorghum* spp., as well as sugarcane crops and its wild relatives (Schmidt and Bothma 2006; Tesso *et al.* 2008; Rabbi *et al.* 2011; Bonnett *et al.* 2008, 2010). Irrespective of the studies on this subject, there is a lack of information, especially in the African context, about their reproductive systems and the likely introgression of transgenes into wild relatives, as well as the ecological and economic effects (Schmidt and Bothma 2006).

1.2 Motivation

Numerous environmental risks are associated with gene flow from GM crops such as maize, canola (*Brassica napus* L.), sorghum and sugarcane with herbicide tolerant, insect resistant, disease resistant, and stress tolerant transgenes (Andow and Zwahlen 2005; Snow and Palma 1997). These risks are enhanced when crops have the ability to hybridise with their wild relatives (Ellstrand *et al.* 1999; Senior and Dale 2002). Of the 13 most important crops in the world that are cultivated for human consumption, sugarcane was reported as one of 12 crop species that could hybridise with their wild relatives within the agro-ecosystem (Ellstrand *et al.* 1999). It is known that significant gene flow may occur when crops are cultivated in close proximity to their related wild relatives (Schmidt and Bothma 2006). However, the presence of a compatible wild species in cultivation areas does not necessarily mean there would be gene flow (Bonnett *et al.* 2008), because gene flow barriers must be crossed for gene flow to occur (McGeoch *et al.* 2009).

In assessing the environmental risk of cultivating GM sugarcane, it is important to conduct a spatial risk assessment and to estimate potential gene flow prior to the approval or release of such a GM crop into the agricultural environment (Bonnett *et al.* 2008; 2010; Cheavegatti-Gianotto *et al.* 2011). It is also necessary to investigate which cultivated or wild species can hybridise with the crop when assessing potential gene flow and its effects (Lu and Snow 2005). Plant species that should be considered when assessing potential for spontaneous hybridisation are those that are related to the crop, growing in close proximity or are weeds of the crop, including those growing outside areas of cultivation (Bonnet *et al.* 2008). No scientific studies have been conducted on gene flow potential of sugarcane in South Africa. A spatial risk assessment and estimation of gene flow potential is required for sugarcane, since:

- there is a lack of spatial data on *Saccharum* hybrids and wild relatives in South Africa. Only some work has been done on *Sorghum* species (closely related within the Andropogoneae, subtribe Sorghinae) in Gauteng by Schmidt and Bothma (2006).
- natural hybridisation with sugarcane can only occur close to the pollen-source as a result of its pollen having low viability (Moore 1976; Venkatraman 1922). The proximity of wild and weedy

Saccharum relatives to sugarcane fields could lead to potential gene flow and hybridisation, as well as between transgenic and non-transgenic relatives within *Saccharum*.

1.3 Aim and Objectives

1.3.1 Aim

The aim of the study was to conduct a spatial assessment of *Saccharum* and its relatives in eastern South Africa and to assess potential gene flow.

1.3.2 Objectives

The objectives of this study were to:

- 1.3.2.1 Map the sugarcane cultivation area of eastern South Africa;
- 1.3.2.2 Determine which close relatives of *Saccharum* occur in the sugarcane cultivation area of eastern South Africa;
- 1.3.2.3 Assess potential gene flow from *Saccharum* hybrids to their wild relatives in the sugarcane cultivation area of eastern South Africa by:
 - assessing dispersal potential, prevalence, spatial overlap and proximity within sugarcane production areas,
 - assessing relatedness and overlap in flowering time of *Saccharum* hybrids and their wild relatives.

1.4 Dissertation outline

Chapter 2: Literature Review

This chapter reviews the benefits of and threats to biodiversity in South Africa. An overview is given of the origin and taxonomy of the *Saccharum* genus. Various services of *Saccharum* species to humans and the environment are discussed. A brief discussion of GM crops is provided as well as an overview of the relevant concepts that are of main importance to this study. These concepts are: genetically modified sugarcane, risk assessment, risks to biodiversity and risk analyses. Background knowledge of these concepts are essential, since this study assesses the risks that are associated with cultivation of GM sugarcane before it might be approved for commercial release in South Africa.

Chapter 3: Materials and Methods

This methodological chapter presents the criteria that were used to select the target species for this *Saccharum* spatial assessment. General methodologies that were used for this study are described in detail. It also provides and motivates the use of separate techniques for data

analyses. Analytical approaches were formulated to specifically consider how the spatial risk assessment was conducted and how potential gene flow was assessed.

Chapter 4: Study Area

This chapter focuses on the factors that are associated with the cultivation of sugarcane in eastern South Africa. Past and current agricultural activities in the provinces where the study was undertaken were considered. This chapter describes the biomes, bioregions and conservation areas that are found within the Quarter Degree grid Squares (QDS) of commercial sugarcane regions. Climatic conditions of the study area are also provided.

Chapter 5: Taxonomy and Distribution

This chapter presents findings regarding the taxonomy of target species selected for this study. Nomenclature, morphology, distribution patterns and habitat preferences of Saccharinae (*Imperata cylindrica*, *Microstegium nudum*, *Miscanthidium capense* and *M. junceum*) and Sorghinae (*Cleistachne sorghoides*, *Sarga versicolor*, *Sorghastrum nudipes*, *S. stipoides*, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense*) are discussed.

Chapter 6: Spatial and gene flow assessments

This chapter considers the relatedness of *Saccharum* wild relatives to each other, and to *Saccharum* hybrids. Gene flow likelihood factors are defined, described and systematically scored at each separate sub-section. Outcomes of calculated prevalence, spatial overlap, proximity, dispersal and potential gene flow from *Saccharum* hybrids to their wild relatives are presented. Findings on *Saccharum* relatedness to wild relatives and flowering times of *Saccharum* hybrids compared to wild relatives are also considered. A scoring system (likelihood scores) for spatial, temporal and gene flow assessments is tested to assess each species and to estimate the level of gene flow likelihood in sugarcane production areas.

Chapter 7: Conclusions and recommendations

The last chapter comprises of the major research findings, recommendations and proposes future research.

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CHAPTER 2: LITERATURE STUDY

2.1 Biodiversity and its benefits

Vandermeer and Perfecto (1995) defined biodiversity as 'All species of plants, animals and micro-organisms existing and interacting within an ecosystem'. Biodiversity can be characterized and described at certain levels of organization (e.g. genetic, individual and population) to provide a conceptual framework for monitoring and assessing its status (Noss 1990). Information on what should be protected in terms of biodiversity is of importance (Klopper *et al.* 2002) so that relevant conservation efforts are guided for protecting biodiversity of the above-mentioned levels (Fairbanks and Benn 2000). For example, the Red List of South African plants which is mandated to evaluate the conservation status of South African plants (Raimondo 2011). South Africa is a signatory to the Convention on Biological Diversity (CBD) (Raimondo 2011) which was established to conserve biological diversity and to promote its sustainable use (Cardinale *et al.* 2012).

The African continent is known for its rich biological diversity (Klopper *et al.* 2002). South Africa is considered as one of the megadiverse countries, ranking third worldwide (Mittermeier and Mittermeier 1997). The main ecological services of agro-ecosystems such as productivity, crop protection and soil fertility can be sustained by management of agricultural biological diversity (Altieri 1999). Regions that were prioritised by conservation assessments due to their unique biodiversity are often linked with ecosystem hotspots (Egoh *et al.* 2009). Four recognised ecosystem services provided by biodiversity are provisioning services (e.g. medicinal plants and water supply), supporting ecosystem services (e.g. maize and rice), regulating services (e.g. water regulation and pollination), and cultural ecosystem services (e.g. tourism and heritage sites) (Egoh *et al.* 2012).

More than one hundred species of crops are cultivated across the world. Nearly all of these have been domesticated from their ancestral plant species which are still growing in natural environments (Asante 2008). Wild relatives of domesticated crops are components of biodiversity that are of value to man since the useful traits they possess (e.g. drought tolerance) can be used in modern agriculture to mitigate these stresses (Mwadzingeni *et al.* 2017).

2.2 Threats to biodiversity

2.2.1 Biological invasions

Biodiversity can be threatened by species that are introduced to areas from their native ranges elsewhere due to human activity (Pyšek and Richardson 2010). For example, *Opuntia* species were introduced as food source into South Africa during the early 19th century (Brutsch and

Zimmermann 1993). Invasive plant species in South Africa were mostly introduced to be cultivated for agricultural practices, forestry and horticultural purposes (Le Maitre *et al.* 2004), and their invasion became a serious environmental problem in the country (Richardson and Wilgen 2004). These invasive species often have a competitive advantage over indigenous vegetation in dominance of the landscape, because they can potentially reduce diversity of flora and fauna, and some unpalatable perennial alien grasses were found to be avoided by grazers (Milton 2004). Foxcroft *et al.* (2008) reported the abundance of these plant species increased even in protected areas that are aimed at preserving biodiversity. Speara *et al.* (2013) reported that high numbers of alien species are associated with conservation areas that are in close proximity to high-density human settlements in South Africa.

2.2.2 Urbanization

The needs of the human society contribute to the rate that urban areas increase in size (Neke and Du Plessis 2004) and such dynamics consequently lead to diminishing natural resources and replace habitats of indigenous species in urban areas (Czech *et al.* 2000). Pollution, poaching, disease transmission from domestic animals to wildlife and introduction of alien species to conservation areas are biodiversity threats that are associated with urbanization in developing countries, more especially when urban areas are adjacent to protected areas (McDonald *et al.* 2009). Human preferences, habitat fragmentation and transformation in urban developments are contributory aspects to plant homogeneity in urban areas (Williams *et al.* 2009). Urban settlements and its related activities resulted in the transformation of grasslands in South Africa, making urbanization one of the leading environmental disturbances of biodiversity in South African grassy biomes (Neke and Du Plessis 2004).

2.2.3 Agriculture

Many human activities are threatening biodiversity (Fairbanks and Benn 2000), as their habitats are exploited by humans (Ammann 2005). In South Africa, agricultural activities are considered a leading threat to habitat loss of natural resources and plant species (Raimondo 2011). In addition to this, Neke and Du Plessis (2004) stated that 29.2% of South African grasslands have already been transformed by agricultural activities. A study conducted by Ament and Cumming (2016) reported an increase of cultivated land cover in South Africa, that has been converted from areas that were previously covered by natural, previously uncultivated land. Some of these regions are known for their agricultural potential and extend to areas that are close to conservation areas (Ament and Cumming 2016). A study by Scharlemann *et al.* (2004) suggested that areas of high biodiversity value should be protected from being converted to agricultural land, especially if wellbeing of people is sustained by these areas. These abovementioned studies also indicated that conversion of natural areas into agricultural land will increase in the future.

Germplasm erosion in feral species is an ecological problem and provides a challenge for genetic plant conservation (Arriola and Ellstrand 1997). Introgression of transgenes into genomes of wild relatives may occur because of gene flow from genetically modified crops with competitive fitness traits to genetic pools of wild populations (Ellstrand 1992; Barnaud *et al.* 2008; Aduana *et al.* 2013). Gene introgression has been reported from GM crops to their wild relatives in canola (Légère 2005), maize (Eschenbach *et al.* 2008) sorghum (Barnaud *et al.* 2008) and sunflower (Cantamutto and Poverene 2007). The effect of gene escape from cultivated GM crops can occur outside of cultivated areas (Andow and Hilbeck 2004; Johnston *et al.* 2004; Chapman and Burke 2006), such as dispersal of volunteer canola plants outside of cultivated fields (Colbach 2008). Increased weediness of wild relatives that “received” certain traits from GM crops is also known to influence the gene pool of the agro-ecosystem (Johnston *et al.* 2004; Tesso *et al.* 2008). The movement of herbicide resistance traits through gene flow to wild relatives from GM crops is another environmental threat and has been reported in beet, canola and maize (Squire *et al.* 2008). Gene flow from GM crop varieties is also reported to have potential to cause unintended effects resulting from these traits in their wild relatives (Barton and Dracup 2000; Birch *et al.* 2004; Malone *et al.* 2004; Raybould 2004).

2.3 *Saccharum* taxonomy and origin

The accepted circumscription of the *Saccharum* genus is that of Jeswiet (1925). Modern sugarcane varieties (*Saccharum* spp.) that are commercially cultivated are complex interspecific hybrids which originated from crosses between Noble cane (*S. officinarum* L.) and Wild cane (*S. spontaneum* L.) (Edmé *et al.* 2005; Dillon *et al.* 2007). Commercial sugarcane varieties are tall, perennial grasses with a high concentration of sucrose (Cheavegatti-Gianotto *et al.* 2011). *Saccharum officinarum* is believed to have originated from Polynesia (Roach and Daniels 1987). The grass generally has a chromosome number of $2n=80$ (Grivet *et al.* 2004). It is cultivated for its thick sugar-rich stalks (Dillon *et al.* 2007) which are characterized by a bright colour (Grivet *et al.* 2004). India is the center of origin for *S. spontaneum* (Roach and Daniels 1987). This grass has a chromosome complement ranging between $2n=40$ and $2n=128$ (Grivet *et al.* 2004) and has thin stalks with very low sugar content. This species has a wide distribution which is ascribed to its diverse morphologies and ability to adapt to harsh environmental conditions (Panje and Babu 1960). The two above-mentioned *Saccharum* species were crossed to produce modern sugarcane varieties in order to address current agronomic needs associated with this crop (Amalraj and Balasundaram 2006; Cheavegatti-Gianotto *et al.* 2011).

Sugarcane (*Saccharum* spp.) is classified within Panicoideae subfamily, Andropogoneae tribe and Saccharinae subtribe of the grass family (Poaceae) (Skendzic *et al.* 2007; Estep *et al.* 2014; Soreng *et al.* 2015; Welker *et al.* 2015; Lloyd Evans and Joshi 2016). Members of Andropogoneae are morphologically diverse and the tribe is comprised of about 1200 species in 90 genera (Estep

et al. 2014). Mukherjee (1957) defined the informal taxonomic group 'Saccharum complex' to classify *Saccharum* and its closely related genera, namely *Erianthus* Nees, *Miscanthus* Nees, *Narenga* (Balansa) and *Sclerostachya* (Balansa). Sugarcane taxonomy is challenging compared to that of most other domesticated plants because of the broad hybridisation and high mutation rate which lead to significant cultivar diversity for agronomic variation (Amalraj and Balasundaram 2006).

2.4 Importance of *Saccharum* species

Approximately 70% of sugar yield across the world is produced from sugarcane, making it one of the most valuable crop species (Hameed *et al.* 2016). Sugarcane is economically viable in many countries due to its high profitability (Tarimo 1998; Viswanathan *et al.* 2008; Hameed *et al.* 2016). The inclusion of *Saccharum* species in research programmes aimed at addressing the needs of the feed stock and bioenergy sectors has increased in recent years (Dal-Bianco *et al.* 2012). *Saccharum spontaneum* was one of the crops that have been selected for bioenergy feedstock and it was reported that its drought tolerance traits made it the most beneficial and appropriate *Saccharum* species to use in these industries (Da Silva 2017). Bagasse is a sugarcane by-product which is used for making paper in addition to its use in bioelectricity generation (Pandey *et al.* 2000). Another cane by-product is molasses, which is known to be an inexpensive product (Calabia and Tokiwa 2007) used in ethanol production (Nguyen and Gheewala 2008).

2.5 Genetically Modified Crops

Genetically modified crop plants have been modified through gene technology to contain genes that provide certain desired traits to plants. These transgenes were not previously present in these crop species before being transferred to target crops by means of genetic engineering processes (Asante 2008). The use of biotechnology in the form of genetically modified crops can provide solutions to current food security challenges and those that may develop in the future (Christou and Twyman 2004). New crop cultivars and hybrids are cultivated in agricultural areas for the following benefits as listed by Asante (2008): enhanced yield of useful parts, greater resistance to diseases and insects, adaptation to different agro-ecological conditions, greater physiological efficiency and improved nutritional content.

Traits that are introduced into transgenic crops are mainly for agronomic purposes such as insect resistance and herbicide tolerance (Marvier and Van Acker 2005). These traits reduce the likelihood that these crops will suffer large losses when subjected to specific biotic and abiotic stress conditions, and under certain conditions, may also lead to reduced use of agro-chemicals (Aerni 2005).

On the African continent, genetically engineered crops are commercially grown only in South Africa and Sudan, although Burkina Faso and Egypt also started cultivation of such crops in recent years (James 2012; 2016). South Africa is ranked ninth in the world based on the amount of land cover that is cultivated with GM crops after the United States of America, Brazil, Argentina, Canada, India, Paraguay, Pakistan and China (James 2016). It leads all African countries in terms of adopting and producing these crops, as well as the regulatory processes regarding biosafety risk assessments. GM maize, cotton and soybean have been approved for general environmental release in South Africa and is widely cultivated in the country (Cooke and Downie 2010; Adenle 2011; James 2012, 2016; Wafula *et al.* 2012).

The use of genetically engineered food is a challenge in Africa due to trust issues regarding human health, regardless of the type of information that is provided on these crops or the sources that promote these food products (Asante 2008). This is in spite of the fact that these foods have been tested and found to be safe for human consumption (Belcher *et al.* 2005). Some examples in African where GM crops were removed from the market place are Egypt, where GM maize was banned due to safety concerns. In Burkina Faso GM cotton cultivation was stopped due to poor cotton lint quality (James 2016). In a study conducted by Aerni (2005) on public perception regarding GM crops, most people indicated that they did not believe that genetically engineered crops will contribute to solving future food security on the continent.

2.6 Genetically Modified *Saccharum*

Plant breeding through biotechnology is commonly used to develop new improved varieties that will meet the increased demand for sugarcane in the future (Dal-Bianco *et al.* 2012). There are several research projects aimed at engineering the genetic components of sugarcane to improve its agronomic performance under different biotic and abiotic stress conditions (Bonnet *et al.* 2008), for example, improved nitrogen use efficiency, insect resistance and herbicide tolerance, increased sugar yield, biomass and agronomic performances, drought-, virus-, disease- and pest resistance (Arruda 2012; Meyer and Snyman 2013; Snyman *et al.* 2015; Da Silva 2017). The overarching aim of these mentioned projects is to develop stress tolerant sugar cane varieties that will require less production inputs, enabling farmers to reduce production costs and increase production for generation of bioenergy (Arruda 2012).

Initiatives to develop transgenic sugarcane in South Africa are headed by the South African Sugarcane Research Institute (SASRI). These projects include improved nitrogen use efficiency (Snyman *et al.* 2015) and insect resistance and herbicide tolerance (Meyer and Snyman 2013). The only other country in the world where GM sugar cane has been developed and approved for cultivation is Brazil (Mano 2017). There varieties with stem borer resistance have been developed (Canal Rural 2016).

2.7 Risk Assessment

In South Africa, risk assessments are conducted as part of the regulatory measures to assess the risks that might be associated with GM plant varieties. These risk assessments are conducted prior to approving GM crops for commercial release into the environment (Jaffe 2004). The risk assessment process is important for protecting human health as well as the environment, since each genetically engineered crop species might pose potential risks to either human beings or the environment (Jaffe 2004). There are not sufficient studies that assess the risks of cultivating genetically engineered crops (Belcher *et al.* 2005). A study by Kumschick and Richardson (2013) reported that there are more developments with risk assessments for plants compared to other organisms. Spatial risk assessments are part of the legal requirements of the South African environmental framework (DAFF 2011). It is therefore important to estimate the potential gene flow that may occur between crops and wild relatives before transgenic crops are approved for cultivation since hybridisation can occur between a crop plant and their wild relatives if a number of barriers to gene flow are crossed (Johnston *et al.* 2004; Légère 2005; McGeoch *et al.* 2009; Rabbi *et al.* 2011; Bøhn *et al.* 2016).

Careful consideration is needed during the risk assessment of GM crops, the regulating process and the approval for release in certain environments (Raybould and Macdonald 2018). It is especially the occurrence of possible unintended and non-target effects that could arise from gene escape from cultivated GM species (Raybould 2004). It has been reported that transgenes can spread from GM crops to related species, either through natural processes or through human activities (Ellstrand *et al.* 1999; Akinbo *et al.* 2015; Bøhn *et al.* 2016). Risk assessments of transgenic plants should therefore take into account such potential risks that might unintentionally be hazardous to the environment and people (Marvier and Van Acker 2005). A study conducted in Zambia by Bøhn *et al.* (2016) showed that sharing, recycling and transporting of maize seeds to other regions within the country increased the rate of gene flow through gene contamination. Another example of transgene flow was reported by Iversen *et al.* (2014) in the Eastern Cape Province of South Africa where transgenes were detected in open pollinated maize varieties.

2.8 Risks to biodiversity

There exist ecological uncertainties on how natural ecosystems will be affected if transgenes escape from agricultural areas (Crawley *et al.* 2001; Eschenbach *et al.* 2008). The extent of traits that are transferred to transgenic crops such as agronomic or insecticidal traits (e.g Bt genes) may have various interactions with other species in the ecosystems due to supplemented genetic materials (Barton and Dracup 2000). Transgenic crops can reduce genetic pool diversity of a plant species due to human induced selection programs that are guided by few crop varieties of specific interest (Ammann 2005).

The potential ecological risks from transgenic crops include that their pollen may be dispersed to their wild relatives, resulting in transgene transfer and out-crossing (Sharma 2009). Depending on the specific trait, outcrossed wild relatives may have competitive advantages which may result in them becoming invasive or weedy plants (Crawley *et al.* 2001). Hybridisation between GM crops with herbicide tolerance traits and their wild relatives may lead to creation of herbicide resistant weeds (Verma *et al.* 2011), for example, *Sorghum halepense* x *S. bicolor* hybrids became difficult to control with herbicides in sorghum fields in parts of South America (Morrell *et al.* 2005). Therefore, cultivation of new plant varieties and GM crops in new areas may possibly have unusual persistence or invasiveness (Cantamutto and Poverence 2007; Kumschick and Richardson 2013). Introgression can therefore occur if cultivated crops are close enough to their related wild or weedy relatives and flowering synchrony is present, sharing of common pollination mechanisms and if they are sexually compatible with fertile species (Arriola and Ellstrand 1997; Chapman and Burke 2006; Schmidt and Bothma 2006; Eschenbach *et al.* 2008; Tesso *et al.* 2008; McGeoch *et al.* 2009; Melloni *et al.* 2013).

Poor stewardship of GM crop material may lead to mixing of non-transgenic crops, eventually resulting in the development of pest resistance (Iversen *et al.* 2014). It is not possible to remove certain gene traits once they escaped into wild relatives from the cultivated crop (Iversen *et al.* 2014). In some cases, if contamination of transgenes is experienced, such farms will only be allowed to produce transgenic forms of that certain crop, unless contaminated plants are eradicated (Belcher *et al.* 2005). The level of risks from genetically modified crops to hybridise with their relatives are managed by not allowing approved transgenic crops to be commercially cultivated in some regions, especially in cases where there were high levels of uncertainties (Marvier and Van Acker 2005).

2.9 Risk analysis

The Department of Agriculture, Fisheries and Forestry (DAFF) of South Africa is responsible for managing the risks associated with introduced organisms as well as the registration of genetically engineered crops by assessing their possible consequences (DAFF 2011). During risk assessments the probable advantages and possible disadvantages of genetically engineered crops in the receiving environment are assessed based on scientific principles and guidance from governing authorities (Barton and Dracup 2000; Jansen van Rijssen *et al.* 2015). Risk assessments are incorporated in the governing measures of states that adopt transgenic products. This is done in order to monitor possible gene flow to wild and weedy relatives; the potential of the crop to become weedy in the agro-ecosystems; and the un-intended impacts non-target organisms (Barton and Dracup 2000).

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CHAPTER 3: MATERIALS AND METHODS

3.1 Criteria used to select target species

A literature search was used to generate a list of Andropogoneae that are closely related to *Saccharum* and with a high potential to hybridise with sugarcane. Wild relatives which diverged from *Saccharum* less than 7.3 million years ago (based on chloroplast sequence chronograms) were identified from a global phylogeny based on chloroplast genomes/regions for the Poaceae (Skendzic *et al.* 2007; Soreng *et al.* 2015; Lloyd Evans and Joshi 2016). Eleven species from the Sorghinae and Saccharinae subtribes of the Andropogoneae were selected for spatial analyses based on their presence within the sugarcane cultivation region of South Africa. Of these four species belong to the Saccharinae and seven to the Sorghinae (Retief and Herman 1997; Van Oudtshoorn 1999; OECD 2013; The Plant List 2013; Fish *et al.* 2015; Soreng *et al.* 2015). Grass nomenclature applied in this study followed The Plant List (2013).

3.2 General methodologies applied for assessing taxonomy, distribution and conducting spatial and gene flow assessments

3.2.1 Sourced herbarium specimens and field collections for herbarium voucher specimens of *Saccharum* wild relatives

Herbarium records provides valuable distribution data of plants (Smith *et al.* 2003; Aikio *et al.* 2010; Greve *et al.* 2016), including wild relatives of crop species in cultivated areas in South Africa (McGeoch *et al.* 2009). Herbarium specimens were sourced from the following 11 South African herbaria: A.P. Goossens Herbarium (PUC), Bews Herbarium (NU) Buffelskloof Private Nature Reserve Herbarium (BNRH), C.E. Moss Herbarium (J), Geo Potts Herbarium (BLFU), H.G.W.J. Schweickhardt Herbarium (PRU), KwaZulu-Natal Herbarium (NH), McGregor Museum Herbarium (KMG), Mpumalanga Parks Board Herbarium (LYD), Pretoria National Herbarium (PRE) and University of Zululand Herbarium (ZULU). These herbaria were chosen based on their suitability of housing the target species that were collected in the eastern South Africa.

A minimum of two pictures were taken per specimen during herbarium visits (Figure 3.1 B and Figure 3.1 C). The first pictures were aimed at depicting the whole specimen to capture if the specimen contained inflorescences (eg. Figure 3.1 B). The second picture were taken in close-up to capture information provided on the herbarium (eg. Figure 3.1 C). More than two photographs of one specimen was taken where there was more than one label on the specimen or in cases where specimens contained multiple herbarium sheets. Only specimens of target species that were collected within the study area were sampled. In cases where the locality or region was not clearly indicated to be within study areas, such specimens were also photographed. These photos were

kept after their localities were later checked and found to fall within study area otherwise they were discarded.

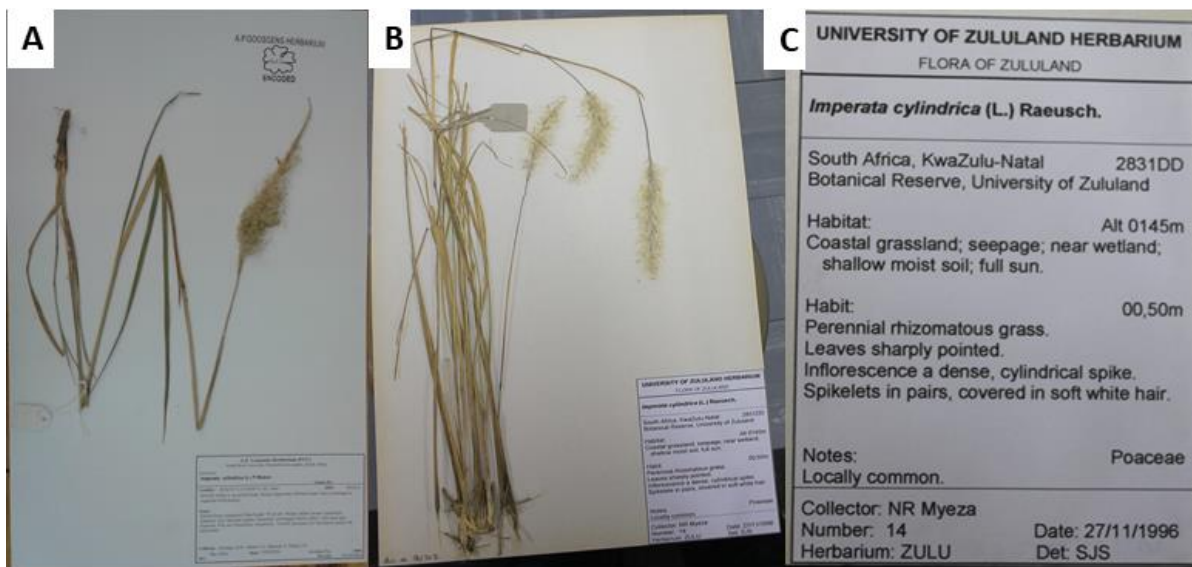


Figure 3.1. Herbarium voucher specimen of *Imperata cylindrica* (A) collected during field visits of this study in KwaZulu-Natal province. Sourced herbarium specimens of *Imperata cylindrica* from University of Zululand herbarium (ZULU) depicting flowering material (B). Herbarium label information of the previously mentioned specimen (C).

All the pictures were downloaded and saved to generate a *Saccharum* assessment database in Microsoft Excel spreadsheets. These pictures were then sorted per herbarium and species. This digitised *Saccharum* assessment database consisted of the following fields which were relevant for further analyses: Species name, Herbaria, Grid, GPS, Altitude, City/Town, Province, Collector, Collector's number, Date, Flowering, Habitat and Notes (Simon and Proenca 2000; Schmidt 2007; Minicante *et al.* 2017). Specimen data were used to populate the previously mentioned fields. Collector's numbers were used to identify duplicates of specimens. Single entry of duplicates was kept for the database.

Sourced specimens of which quarter-degree squares (QDS) ($\pm 621 \text{ km}^2$) information were provided, were captured in the database. Some of these specimens only had locality or place name (without QDS) in which case this information was georeferenced by internet browser to find the closest town or city which was subsequently used to generate QDS for such records (Williams and Crouch 2017). Specimens without QDS and locality notes were not considered for distribution mapping. The information available for such specimens were however used for other analyses such as habitats, proximity and flowering times if this type of data were available.

Specimen data were used to pinpoint localities and habitat types where selected wild relatives of *Saccharum* hybrids have been collected in the past and were known to occur (The Plant List 2013; Fish *et al.* 2015; Williams and Crouch 2017. This was easily achieved by sourced specimens with

Global Positioning System (GPS) coordinates and/or precise descriptions of localities on the specimens. At the targeted sites, sugarcane field margins were examined for the target species, especially in disturbed and waterlogged areas which are the preferred habitats of sugarcane relatives (Retief and Herman 1997; Van Oudtshoorn 1999).

Collections were made during the peak flowering periods of wild relatives in 2016 and 2017 as guided by the literature and data obtained from the herbarium specimens. Specimens were pressed on site and later dried in the drying room of the plant systematics department in A.P. Goossens herbarium, North-West University (Potchefstroom campus). Duplicates were collected for each collection. One duplicate was incorporated in PUC, while its duplicate was used to verify the identification in PRE and later sent to other herbaria as gift voucher specimens. Field data of collected species were recorded and accessioned into Botanical Research and Herbarium Management System (BRAHMS) database at PUC. Accessioned data were also used to generate herbarium labels for each specimen (Figure 3.1 A). All herbarium specimens were mounted and stored in PUC after they were verified for identification at PRE.

Herbarium distribution records (Figure 3.1 A) of the new collections were added to the master database to construct a distribution map per species with ArcGIS (student edition version 10.3, Esri, USA) to confirm their presence in sugarcane cultivation areas). The resultant database was used to conduct a gap analysis for the study area to identify areas where little information was available regarding the occurrence of wild relatives (Simon and Proença 2000; Smith *et al.* 2003; Aikio *et al.* 2010; Fish *et al.* 2015). The database was also used for producing a map highlighting areas of gene flow likelihood from *Saccharum* hybrids to their wild relatives in sugarcane production areas of eastern South Africa.

3.2.2 Phylogeography assessment of *Saccharum* wild relatives

The qualitative assessment to determine the likelihood of wild relatives which co-occur with cultivated sugarcane, and which may enhance gene flow potential, was based on the following factors: prevalence, spatial overlap, proximity, distribution potential, gene flow potential, and flowering times (Ellstrand *et al.* 1999; Chapman and Burke 2006; Schmidt and Bothma 2006; Tesso *et al.* 2008; McGeoch *et al.* 2009; Andriessen 2015). All target species were assessed and ranked per factor. Species with the highest rank was scored 11 and species with lowest rank was scored a value of 1. The ranking scale was based on the number of target species selected for this study. In cases where no information was available for a species, it could not be ranked and was scored 0 (no evidence equates to no ranking). It would be inaccurate to rank species without data, as it would inflate the likelihood scores for the areas where these species were found.

Assessments at the QDS scale have been practiced for conservation assessments in South Africa (Robertson and Barker 2006; Parusnath *et al.* 2017), such as spatial assessments for gene flow studies from cultivated crops to their related species (McGeoch *et al.* 2009). The above-mentioned

approach was also adapted in the current study to assess the geographic distributions of *Saccharum* wild relatives in the study area. Therefore, the study area was divided into QDS (Mararakanye *et al.* 2017; Williams and Crouch 2017) to provide mapping units for the spatial assessment. Sugarcane production areas in the Limpopo, Mpumalanga and KwaZulu-Natal provinces were obtained from the 2015 National Land Cover dataset.

The layer of study province was then overlaid with a grid of QDS using ArcGIS (student edition version 10.3, Esri, USA) to provide 113 mapping units for the spatial assessment (Robertson and Barker 2006). A layer of Land Cover data with 1:50 000 QDS boundaries containing sugarcane fields was used to create a study area layer of 113 sugarcane cultivation grids in ArcGIS. A map of commercial sugarcane cultivation was produced and used during the spatial and gene flow assessments. Some of these QDS overlapped with Mozambique and Swaziland, but no data was available for these areas. It should be noted that while wild relatives of *Saccharum* may be present in those jurisdictions, it did not form part of this study.

3.3 Analytical approaches applied for assessing the taxonomy and distribution of sugarcane and their wild relatives

Spatial assessment was conducted based on the following factors: scientific names and synonyms of *Saccharum* wild relatives, morphology of *Saccharum* hybrids and their wild relatives, distribution of *Saccharum* wild relatives in eastern South Africa and habitats of *Saccharum* wild relatives in eastern South Africa (Retief and Herman 1997; Van Oudtshoorn 1999; The Plant List 2013; Fish *et al.* 2015). The above-mentioned factors were considered during the analyses of the phytogeography of the studied species across the study area.

3.3.1 Scientific names and synonyms of *Saccharum* wild relatives

A literature search was conducted to find the names (scientific and synonyms) that were known and used for the target species. The synonyms associated with scientific (accepted) name at the time of the study were linked with the accepted name of that species. All the names linked with the accepted name was used for the target species throughout this study. This was done in order not to miss specimens during the herbarium visits. The Plant List (2013) (A working of all plant species) was used as a reference source for scientific names and synonyms since it is known to have the latest nomenclature for the selected *Saccharum* wild relatives. Numerous South African literature sources were also used in addition to The Plant List (2013) for presenting and for discussing the names of *Saccharum* wild relatives.

3.3.2 Morphologies of *Saccharum* wild relatives compared with *Saccharum* hybrids

Literature sources describing the morphologies of sugarcane were used to provide the relevant information on sugarcane. South African literature was used to describe sugarcane relatives selected for this study and these sources were also used to compare the morphologies of target species with that of sugarcane. Sourced herbarium specimens and *Saccharum* hybrids and their relatives collected during this study were also used to describe their morphology.

3.3.3 Distribution of *Saccharum* wild relatives in eastern South Africa

The knowledge of documented species in their region of occurrence has been supported by field observations and herbarium specimen data (Schmidt *et al.* 2005; Jaca and Mkhize 2018). Such data has been digitised in South Africa studies to map the spatial distribution patterns of plant richness using QDS (Mararakanye *et al.* 2017; Williams and Crouch 2017) as conducted in this study.

The master *Saccharum* assessment database was loaded onto ArcGIS in a Microsoft Excel format. The layer of study area containing sugarcane QDS with a layer of species names from the database were used to create spatial maps. A map of each target species was generated by selecting each species from the main database in ArcGIS, which showed their distribution across the study area.

3.3.4 Habitats of *Saccharum* wild relatives in eastern South Africa

The master *Saccharum* assessment database was mainly used to provide the habitats of *Saccharum* wild relatives in eastern South Africa. Information on habitats of the target species from sourced herbarium specimens, field observations and collections made during this study was compiled in the database. South African literature was also used to provide the known habitats of each target species and these sources were mainly used for discussing the habitats of target species. Habitats of target species were also compared with habitats of sugarcane in the study area to evaluate which target species are known to co-occur with sugarcane.

3.4 Analytical approaches for assessing spatial and potential gene flow from *Saccharum* hybrids to their wild relatives

Gene flow potential was assessed based on the following factors: relatedness of *Saccharum* wild relatives to *Saccharum* hybrids and to one another, prevalence of *Saccharum* wild relatives in sugarcane cultivation areas, spatial overlap of *Saccharum* wild relatives with cultivated sugarcane, proximity of *Saccharum* wild relatives to cultivated *Saccharum* hybrids, potential gene flow (hybridisation) from *Saccharum* hybrids to their wild relatives, distribution potential of *Saccharum* wild relatives across the study area and flowering times of *Saccharum* hybrids and their wild

relatives. These factors were used to generate likelihood scores, which was then used for producing spatial map indicating various levels of likelihood for gene flow to occur from sugarcane to their wild relatives in commercial sugarcane areas of eastern South Africa.

3.4.1 Relatedness of *Saccharum* wild relatives to *Saccharum* hybrids and one another

The methodology used to study relatedness is described in detail by Snyman *et al.* (2018). The analysed phylogenetic tree and chronogram of *Saccharum* and their relatives was officially reproduced in this dissertation with kind permission granted by Dr Dyfed Lloyd Evans. The previously mentioned data was used to describe the relatedness between *Saccharum* hybrids and their relatives and to indicate how each target species relates to all other target species. Literature was used to discuss how all studied species relates to each other.

3.4.2 Prevalence *Saccharum* wild relatives in *Saccharum* cultivation areas

Prevalence refers to how common a species is within a given area (Manel *et al.* 2001). The QDS of sugarcane cultivation areas were used to calculate the prevalence of wild relatives, i.e. how common these relatives are in the study area. The numbers of individuals per species per QDS within the sugarcane cultivation area was determined and the proportion of individuals per species within each QDS was calculated. The same procedure was followed for QDS bordering sugarcane cultivation areas. These proportions were added to determine the proportional prevalence of each species in the study area. These prevalence values were then sorted from highest to lowest proportion of individuals per species within and bordering sugarcane QDS and scored. The following equation was used to calculate prevalence: $P_{\text{species}} = \sum p_{ri}$, where p_{ri} is proportion of individuals per species within and bordering sugarcane QDS.

3.4.3 Spatial overlap of *Saccharum* wild relatives with *Saccharum* cultivation areas

Spatial overlap is the notion of similarity in distribution patterns (or shared occurrences) (Hautier *et al.* 2017). It was calculated for each species by dividing the number of QDS that overlap with sugarcane cultivation areas with the total number of QDS for sugarcane cultivation areas. This derived a percentage of overlap per species. Species were ranked from highest to lowest based on overlap percentage, with the highest rank scoring 11 and lowest rank scoring 1. The following equation was used to calculate spatial overlap: $SO_{\text{species}} = x_1(100)/x_2$, where x_1 is number grids shared by wild relatives and sugarcane fields, and x_2 represents the total number of grids in which sugarcane is cultivated.

3.4.4 Proximity of *Saccharum* wild relatives to *Saccharum* hybrids in *Saccharum* cultivation areas

Pollen of graminoids can travel for long distance from the donor plant (Kawashima *et al.* 2011; Viljoen and Chetty 2011; Bøhn *et al.* 2016). The distance of 700 m was set as the cut-off for proximity measures because of gene flow introgression received from sorghum species to its relative in South African study by Schmidt and Bothma (2006). Sourced specimen data with the distance of 700 m from sugarcane fields were considered for proximity in this study. This distance was also used for during field work to search for wild relatives. The herbarium record database was used to construct a table of habitat notes per species and the presence or absence of wild relatives in the vicinity of sugarcane fields were noted. These records were combined with confirmations from the literature and field surveys. Species with more occurrences within the 700 m zone (high proximity) were ranked higher than species with few or no records in sugarcane fields and margins. Proximity was calculated using the following equation: $Py_{\text{species}} = fm + lt$, where **fm** is number of specimens collected from fields and margins per species and **lt** is the number of confirmations in the literature per species growing in sugarcane fields and margins.

3.4.5 Potential gene flow from *Saccharum* hybrids to their wild relatives

Prominent literature was consulted to assess gene flow potential, whereby printed evidence of reproductive compatibility and the formation of hybrids between commercial sugarcane with target related species were used to assess the likelihood of hybridisation (Parthasarathy 1948; Tai *et al.* 1991; Nair 1999; Burner *et al.* 2009; Chae *et al.* 2014; Gao *et al.* 2014; Lloyd Evans and Joshi 2016). The numbers of publications which reported hybridisation were recorded (Janaki-Ammal 1941; Terajima *et al.* 2007; Bonnett *et al.* 2008). Successes were scored if the publications reported formation of hybrid progeny (FitzJohn *et al.* 2007; McGeoch *et al.* 2009; OECD 2013) and ranked accordingly. In cases where literature recorded hybridisation evidence between *Saccharum* hybrids and wild relatives, the following approaches were taken: (i) if target species were reported to hybridise with *Saccharum* hybrids, the number of publications and successes were recorded and scored 1 per event; (ii) if species which did not occur in South Africa hybridised with *Saccharum* hybrids, but the genus is present in the sugar cane production area, the species from such genera were treated as reproductively compatible with commercial sugarcane and the number of publications and successes recorded and scored 0.5 per event. The wild relative-*Saccharum* crosses with most hybrids ranked the highest and species with fewer hybrids were ranked lower. The following equation was used to calculate gene flow potential: $GF_{\text{species}} = \sum sh_i$, where **sh_i** is the number of successful hybridisation events between *Saccharum* hybrids and their wild relative species.

3.4.6 Dispersal potential of *Saccharum* wild relatives across the study area

Weedy grasses are often spread by different modes of transport (Milton 2004). Transport networks therefore provide an indication of the potential for weedy relatives of sugarcane to spread, with denser networks implying higher chances for migrations. Road and railway networks were used to calculate the spatial dispersal potential of wild relatives across the study area. For each species the numbers of railway lines and roads per QDS were determined respectively. A map of each target species with QDS containing the abovementioned networks was used to count their numbers separately. Totals of QDS containing railways and roads per species were summed. Higher totals were considered indicative of a wild relative's ability to disperse and ranked as highest likelihood for the species to spread to sugarcane fields (Knispel *et al.* 2008). Dispersal potential was calculated using the following equation per species: $DP_{\text{species}} = \sum rl + \sum rd$, with rl the railway lines in QDS occupied by the species and rd the roads in QDS occupied by the species.

3.4.7 Flowering times of *Saccharum* hybrids and their wild relatives

Flowering times were assessed using literature, herbarium specimens and collections made during field surveys. Spatial distance of flowering crops and their relatives in cultivations has implications for pollen movement in gene flow studies (Bøhn *et al.* 2016). *Saccharum* hybrids flower from March to August in South Africa (Sithole and Singels 2013; Zhou 2013). Sourced herbarium specimens with flowering plant material (inflorescences), date of collection and descriptive localities area was used to generate data regarding their flowering periods (Simon and Proenca 2000; Greve *et al.* 2016). Sourced specimens without the above-mentioned data were not considered for analyses of flowering times. Information collected from herbaria were used to search for targeted field collections and sampling of species during their flowering period as shown in figure 3.1 (A). The species were added to the database to assess flowering times of *Saccharum* hybrids and their wild relatives. South African literature on flowering times were used to guide collections of *Saccharum* hybrids and their wild relatives (Retief and Herman 1997; Van Oudtshoorn 1999; Sithole and Singels 2013; Zhou 2013; Fish *et al.* 2015).

The overlapping percentages between the flowering period of *Saccharum* hybrids and each wild relative was calculated by dividing the number of overlapping flowering months with the total number of months of sugarcane flowering. The wild relatives with comparatively higher numbers of overlapping months were ranked the highest and species with less overlap were ranked lower. Flowering times were calculated using the following equation: $FT_{\text{species}} = n_1(100)/n_2$, where n_1 is the number of flowering months shared by wild relatives and sugarcane, and x_2 is the total number of months during which sugarcane flowers. While all non-target weedy grasses (not relatives of *Saccharum*) of which the flowering periods with *Saccharum* hybrids (synchronous flowering) in sugarcane fields were also collected and identified to species level, they were only listed in this

study as weeds that co-occurred with sugarcane. However, it should be noted that only target sugarcane wild relatives for this study were used in assessment of flowering times.

3.4.8 Likelihood scores of factors analysed for spatial assessment and potential gene flow from *Saccharum* hybrids to their wild relatives

Likelihood scores were calculated per species to determine which *Saccharum* relatives (based on relatedness, temporal and spatial assessment) might present a higher likelihood for gene flow. Factors were weighted equally for relatedness and spatial assessments (Butler *et al.* 2007). Relatedness was calculated from the phylogenetic classification and hybridisation events, while spatial assessment involved prevalence, spatial overlap, proximity, and dispersal potential. Thereafter, spatial, temporal (flowering time) and relatedness assessments were weighted 1:1:2 to come up with final likelihood score. This weighting was based on the assumption that gene flow and relatedness are not correlated due to reproductive barriers such as flowering time (Panova *et al.* 2006), and that gene flow likelihood is evenly dependent on temporal and spatial assessment factors. Relatedness was weighted more since it is the determining factor for successful gene flow when prevalence, spatial overlap, proximity, distribution potential or flowering time provide the required conditions for compatible pollen from one species to reach the stigma of another species.

Likelihood maps indicating various levels of potential for gene flow to occur between *Saccharum* hybrids and wild relatives within sugarcane production areas of eastern South Africa was constructed based on the factor scores per species and summed per grid. The following classes were used for assessing the likelihood for gene flow. QDS with sugarcane fields without wild relatives were assigned a score of 0–12, while QDS with sugarcane fields with varying abundances of wild relatives scored as follows: 13–43 (very low), 44–86 (low), 87–129 (high) and 130–172 (very high). For example, the lowest likelihood class for gene flow from sugar cane to wild relatives was found for *Sorghastrum nudipes* which scored 6 while there was no sugarcane QDS that contained only this wild relative species. These intervals were based on likelihood scores from spatial, temporal and relatedness assessments. The value of each QDS was calculated by adding the likelihood scores obtained per species from the above-mentioned assessments. The intervals were important to show the various levels of likelihood for gene flow to occur between sugarcane and wild relatives in the sugar production region of eastern South Africa.

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CHAPTER 4: STUDY AREA

4.1 Agricultural activities

In developing countries such as South Africa, agriculture is a keystone sector in the economy (Collett 2013). Agricultural practices influence the shaping of landscapes and patterns of biological diversity in agro-ecosystems (Torguebiau *et al.* 2012). Environmental conditions of South Africa allow for the cultivation of a wide variety of crops (Gbetibouo and Hassan 2005). Since the study area has high potential agricultural land (Collett 2013) these landscapes have been transformed to accommodate crop production and livestock as the main agricultural activities (Lawes *et al.* 2004; Torguebiau *et al.* 2012). The sugarcane industry provides vital income to the country's economy (SASA 2017), including the livelihoods of people (Kisaka-Iwayo and Obi 2012; Cockburn *et al.* 2014). Maize is also commonly grown (Torguebiau *et al.* 2012). Other agricultural products include avocado, orange, mango, banana, potato, beans and kikuyu pastures (Dlamini and Haynes 2004; Cockburn *et al.* 2014; Driver *et al.* 2015). Arid and semi-arid rangelands of the study area are mainly used for livestock and wildlife conservation (Rutherford and Powrie 2013).

4.2 Commercial cultivation of sugarcane

In eastern South Africa, sugarcane is produced in both small- and large-scale fields (Mbowa and Nieuwoudt 1998). Sugar cultivation requires lots of water usage, especially when irrigating sugarcane fields (DAFF 2014; SASA 2017). Insufficient irrigation are one of the challenges for small-scale sugar producers (Qongqo and Van Antwerpen 2000). Commercial sugarcane production areas of KwaZulu-Natal are Amatikulu, Dalton, Darnall, Eston, Felixton, Gledhow, Maidstone, Noodsberg, Pongola, Sezela, Umfolozi and Umzimkulu, and this crop is also produced in Komatipoort and Malalane in the Mpumalanga province (DAFF 2014). The choice of suitable sugarcane varieties and their performance in certain areas relies on the environmental conditions of such regions (Spaull *et al.* 2005). Nevertheless, production of sugarcane is also attributed to combinations of other factors such as the choice of land and climatic conditions (Collett 2013; DAFF 2014; SASA 2017).

4.3 Biomes, bioregions and conservation areas

4.3.1. Biomes

The Grassland and Savanna biomes occur throughout the study area with the latter the most prominent. The Indian Ocean Coastal Belt coincided with some of the sugarcane production areas of coastal KwaZulu-Natal (Figure 4.1). The Grassland biome of South Africa have been transformed for agricultural activities, urbanization and mining (Neke and Du Plessis 2004). Overgrazing during drought seasons has also contributed to transformation of grasslands with

palatable and unpalatable herbaceous species (O'Connor 1995). Savanna is primarily characterised by rainfall seasonality and is adapted to low precipitation (Lehmann *et al.* 2011). Indian Ocean Coastal Belt has low mean potential evaporation compared to Savanna (Rutherford *et al.* 2006). Crops, urbanization and alien invasive plant species are the main threats to these biomes (Fairbanks and Benn 2000; Mucina *et al.* 2006).

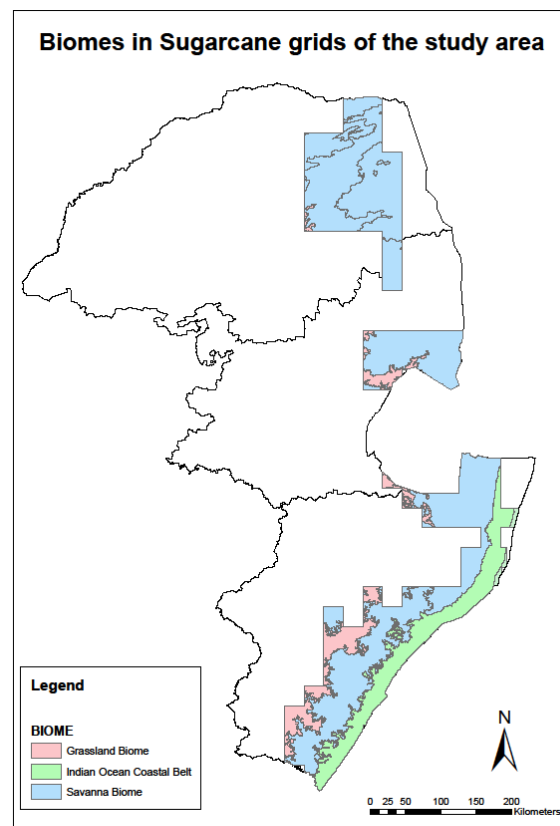


Figure 4.1. Biomes that occur in the study area. GIS layer of Quarter Degree Squares containing sugarcane fields were merged with a layer of eastern South Africa (Limpopo, Mpumalanga and KwaZulu-Natal) in ArcGIS. The mapped study area was overlaid with a layer of South African biomes.

4.3.2. Bioregions

Six bioregions overlap with the sugarcane fields of the study area (Figure 4.2). These include the Central Bushveld, Lowveld, Mopane and Sub-Escarpment Savanna bioregions within the Savanna Biome, and the Mesic Highveld Grassland and Sub-Escarpment Grassland bioregions within the Grassland Biome (Rutherford *et al.* 2006). Indian Ocean Coastal Belt biome is not further subdivided into bioregions. Rutherford *et al.* (2006) reported that the Central Bushveld Bioregion is the most diverse in terms of vegetation types, but is restricted to the northern part of the study area. The Lowveld bioregion co-occurs with sugarcane QDS in all three provinces (Figure 4.2) while the

Mopane Bioregion is predominant in the northern part of the study area (Rutherford *et al.* 2006). Sub-Escarpment Savanna is especially predominant in the southern part of the study area (Figure 4.2) inland to the Indian Ocean Coastal Belt (Rutherford *et al.* 2006). Mesic Highveld Grassland and Sub-Escarpment Grassland bioregions receive higher rainfall (Rutherford *et al.* 2006) but is only marginally associated with the sugar cultivation areas and occur only in the central and southern parts of the study area.

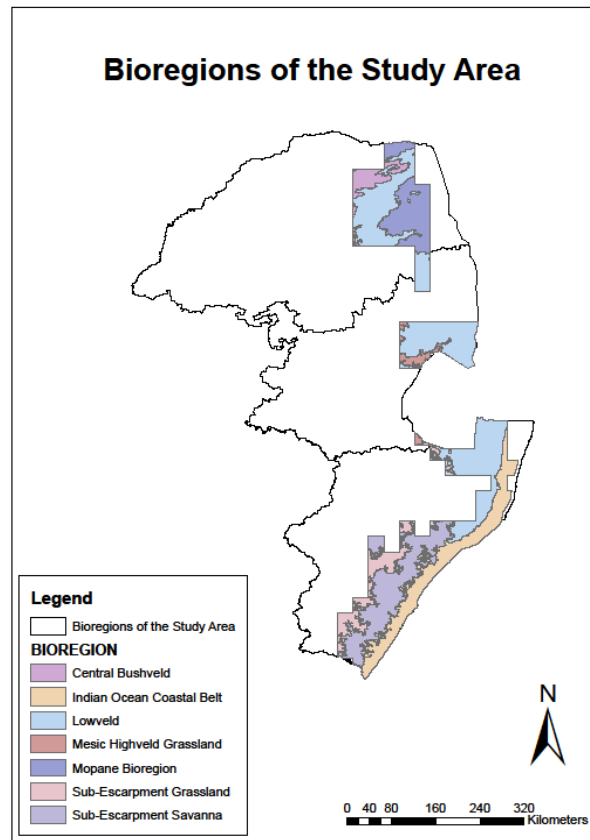


Figure 4.2. Bioregions that occur within the study area. GIS layer containing sugarcane fields was merged with a layer of eastern South Africa (Limpopo, Mpumalanga and KwaZulu-Natal) in ArcGIS. The study area was overlaid with a layer of South African bioregions.

4.3.3. Conservation areas

Eastern South Africa is well known for its rich biodiversity and conservation areas (Torguebiau *et al.* 2012; Minin *et al.* 2013; Driver *et al.* 2015). Protected areas are concerned with the continuation of long-term biological processes along representable geographic gradients (Fairbanks and Benn 2000). The conservation of crop wild relatives in both protected areas and outside should be practised due to the importance of their germplasm (Ford-Lloyd *et al.* 2011). Socio-economic activities adjacent to conservation areas are placing increased pressure on resources of protected areas (Crane 2006). A study by Goodman (2003) in KwaZulu-Natal showed that the poor setting of protected areas leads to transformation of nearby land for urbanization and activities such as

sugarcane cultivation. The St. Lucia System, Kruger to Canyons, Vhembe Biosphere Reserve, Lowveld National Botanical Garden, Ndumo Game Reserve and Turtle Beaches/Coral Reefs of Tongaland are six protected areas that co-occur with sugarcane QDS across the three provinces in the study area (Figure 4.3). Kruger to Canyon is in both Limpopo and Mpumalanga and covers 2.6 million hectares (Coetzer *et al.* 2010). Mining, agriculture (including permanent commercial sugarcane fields), human settlement, forestry and timber plantations are environmental threats to unprotected areas of this biosphere (Coetzer *et al.* 2010). The Ndumo Game Reserve is amongst conservation areas of biodiversity importance which are highly threatened in KwaZulu-Natal due to socio-economic activities (Goodman 2003).

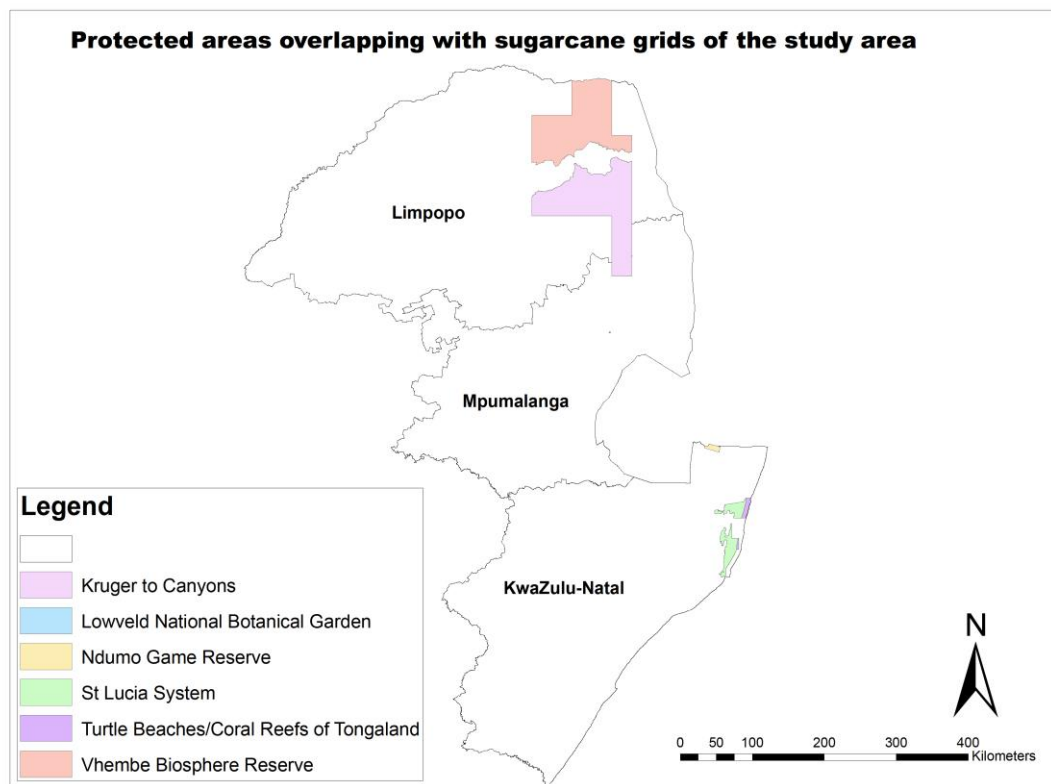


Figure 4.3. Protected areas overlapping with sugarcane grids in the study area. GIS layer of (QDS) containing sugarcane fields were merged with a layer of “study provinces (Limpopo, Mpumalanga and KwaZulu-Natal)” to create a layer of the study area in ArcGIS. The study area layer was overlaid with a layer of South African protected areas to highlight the protected areas that occur within the study area.

4.4 Climatic conditions

South Africa has diverse climatic conditions which contribute to different soils, terrains and topography where cultivation of crops is practiced (Collett 2013). Tropical and subtropical climate patterns of the study area are ideally suitable for cultivation of sugarcane (Fairbanks and Benn 2000; Dlamini and Haynes 2004; DAFF 2014; Gao *et al.* 2016). The subtropical climate of

KwaZulu-Natal results in variable coastal regions characterised by high temperatures, high humidity and high annual summer precipitation of up to 1200 mm (Fairbanks and Benn 2000). KwaZulu-Natal and Mpumalanga normally receive sufficient rainfall for sugarcane production besides the assistance provided by irrigation systems (DAFF 2014). Wetter months are from November to March and the dry season is from June to August in KwaZulu-Natal (Torguebiau *et al.* 2012). Variant rainfall patterns and unexpected fluctuating temperatures are seldom experienced in KwaZulu-Natal (Dube and Jury 2000; Torguebiau *et al.* 2012). Drastic climatic change such as droughts and insufficient precipitation has had negative effects on sugarcane production in KwaZulu-Natal and Mpumalanga (Singels *et al.* 2011; SASA 2017).

In the study area, sugarcane is ideally cultivated under the following conditions according to DAFF (2014) for rapid cane elongation during the main growth period: annual rainfall between 1100 and 1500 mm with a total of at least 1500 mm or made up with irrigation, a minimum of 600 mm of annual moisture, mean temperatures ranging between 20 and 35 °C, sunny, frost-free and high humidity (80–85 %).

4.5 References

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CHAPTER 5: TAXONOMY AND DISTRIBUTION

5.1 Scientific names of *Saccharum* wild relatives

5.1.1. *Cleistachne sorghoides* Benth.

Cleistachne sorghoides Benth. is an accepted scientific name, whereas *C. macrantha* Stapf and *C. stocksii* Hook.f. are synonyms of this grass species (The Plant List 2013). South African literature follow this nomenclature (Retief and Herman 1997; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). Victor (2004) only listed *C. macrantha* Stapf as a synonym of *C. sorghoides* Benth. in the national assessment (Red List). *Cleistachne stocksii* Hook.f. is generally not used as a synonym in South African publications.

5.1.2. *Imperata cylindrica* (L.) Raeusch.

Imperata cylindrica (L.) Raeusch. is the accepted scientific name (The Plant List 2013) and here twelve synonyms were listed, namely: *I. cylindrica* var. *africana* (Andersson) C.E.Hubb., *I. cylindrica* var. *condensata* (Steud.) Hack., *I. cylindrica* var. *cylindrica*, *I. cylindrica* var. *europaea* (Andersson) Asch. & Graebn., *I. cylindrica* subsp. *koenigii* (Retz.) Tzvelev, *I. cylindrica* var. *koenigii* (Retz.) Pilg., *I. cylindrica* var. *latifolia* (Hook.f.) C.E.Hubb., *I. cylindrica* var. *major* (Nees) C.E.Hubb., *I. cylindrica* f. *pallida* Honda, *I. cylindrica* var. *parviflora* Batt. & Trab., *I. cylindrica* var. *pedicellata* (Steud.) Debeaux and *I. cylindrica* var. *thunbergii* (Retz.) T.Durand & Schinz. *Imperata cylindrica* (L.) Raeusch. is also used as an accepted name in Southern Africa (Retief and Herman 1997; Van Oudtshoorn 1999; Bromilow 2001; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). Van Oudtshoorn (1999) mentioned that two synonyms of varieties, namely *I. cylindrica* (L.) Raeusch. var. *africana* (Andersson) C.E.Hubb. and *I. cylindrica* (L.) Raeusch. var. *major* (Nees) C.E.Hubb. were used in numerous South African studies (Retief and Herman 1997; Germishuizen and Meyer 2003; Fish and Victor 2006). *Imperata arundinacea* Cirillo, *I. arundinacea* Cirillo var. *africana* Andersson, *I. arundinacea* Cirillo var. *thunbergii* Hack. and *I. cylindrica* (L.) P.Beauv. are synonyms cited by Fish and Victor (2006) which were not listed by The Plant List (2013).

5.1.3. *Microstegium nudum* (Trin.) A.Camus

Microstegium nudum (Trin.) A.Camus is the accepted scientific name (The Plant List 2013) and here the following synonyms were listed: *Eulalia capensis* (Hochst.) Steud., *E. nuda* (Trin.) Kuntze, *Leptatherum nudum* (Trin.) C.Hui Chen, Kuoh & Veldkamp, *L. royleanum* Nees, *Microstegium arisanense* (Hayata) A.Camus, *M. capense* (Hochst.) A.Camus, *M. mayebaratum* Honda, *M. nudum* var. *boreale* (Ohwi) Ohwi, *M. nudum* subsp. *japonicum* (Miq.) Tzvelev, *M. nudum* var. *shimidzui* Honda, *Pollinia arisanensis* Hayata, *P. nuda* Trin. *P. nuda* var. *capensis* (Hochst.) Hack., and *Psilopogon capensis* Hochst. In accordance with The Plant List (2013), *M. nudum* (Trin.)

A.Camus is also used as an accepted name in South Africa (Retief and Herman 1997; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). *Microstegium capense* (Hochst.) A.Camus is the most popular synonym in South Africa (Retief and Herman 1997; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). Fish *et al.* (2015) also listed *Leptatherum nudum* (Trin.) C.Hui Chen, Kuoh & Veldkamp as a synonym although The Plant List (2013) has categorised this synonym as unresolved and it was therefore not considered as alternative name in this study.

5.1.4. *Miscanthidium capense* (Nees) Mabb.

Miscanthus ecklonii (Nees) Mabb. is the accepted name (The Plant List 2013) for this species. *Miscanthidium capense* (Nees) Stapf was used as the preferred name in this study because of the latest phylogeny and chromosome counts; *Miscanthidium* has $x=15$, while *Miscanthus* has $x=19$ (Price 1965; Hoshino and Davidse 1988; Hodkinson *et al.* 2001; Chramiec-Głębik *et al.* 2012; Hodkinson *et al.* 2015). The following synonyms have been identified by The Plant List (2013): *Erianthus capensis* Nees, *E. capensis* var. *angustifolius* Nees, *E. capensis* var. *ecklonii* (Nees) Hack., *E. capensis* var. *villosa* Stapf, *E. capensis* var. *villosus* Stapf, *E. ecklonii* Nees, *E. sorghum* Nees, *Miscanthidium capense* (Nees) Stapf, *M. capense* var. *villosum* (Stapf) E.Phillips, *M. erectum* Stent & C.E.Hubb., *M. sorghum* (Nees) Stapf, *Miscanthus capensis* (Nees) Andersson, *M. sorghum* (Nees) Pilg., *Saccharum capense* (Nees) Steud., *S. ecklonii* (Nees) Steud. and *S. sorghum* (Nees) Steud. *Miscanthidium capense* as used in this study is currently regarded as a synonym (The Plant List 2013). Fish and Victor (2005a) and Fish *et al.* (2015) also reported *Miscanthus ecklonii* (Nees) Mabb. as the accepted name, even though other studies used *Miscanthus capensis* (Nees) Andersson (Van Oudtshoorn 1999; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006).

Miscanthidium capense (author not cited) was listed as the old name and a synonym of *Miscanthus capensis* (author not cited) by Van Oudtshoorn (1999). *Miscanthidium capensis* Nees var. *capensis*, *M. capense* Nees var. *villosa* Stapf, *M. erectum* Stent and *M. sorghum* Stent were not listed by The Plant List (2013) although both (Germishuizen and Meyer 2003; Germishuizen *et al.* 2006) listed these names as synonyms of *Miscanthus capensis* (Nees) Andersson. Further synonyms of *M. ecklonii* (Nees) Mabb. that were used in South Africa include: *Erianthus capensis* Nees, *E. capensis* Nees var. *villosa* Stapf, *E. ecklonii* Nees, *E. sorghum* Nees and *Miscanthus sorghum* (Nees) Pilg (Fish and Victor 2005a).

5.1.5. *Miscanthidium junceum* (Stapf) Pilg.

Miscanthus junceus (Stapf) Pilg. is the accepted name (The Plant List 2013) for this species. *Miscanthidium junceum* (Stapf) Pilg. was used as the preferred name in this study because of the latest phylogeny and chromosome counts (Price 1965; Hoshino and Davidse 1988; Hodkinson *et*

al. 2001; Chramiec-Głabik *et al.* 2012; Hodkison *et al.* 2015). The following existing synonyms are recorded for this grass: *Cleistachne teretifolia* Hack., *Erianthus junceus* Stapf, *E. teretifolius* Stapf., *Miscanthidium gossweileri* Stapf, *M. junceum* (Stapf) Stapf, *M. teretifolium* (Stapf) Stapf, *Miscanthus gossweileri* (Stapf) Pilg. and *M. teretifolius* (Stapf) Pilg. (The Plant List 2013). *Miscanthus junceus* (Stapf) Pilg. is an accepted name in South Africa (Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). *Miscanthidium junceum* (author not cited) was thought to be the old name and synonym of *Miscanthus junceus* (author not cited) in South African literature (Van Oudtshoorn 1999) which is found to be commonly applied (Retief and Herman 1997; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). Another recognised synonym in the country is *M. teretifolium* (Stapf) Stapf (Retief and Herman 1997; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015).

5.1.6. *Sarga versicolor* (Andersson) Spangler

Sorghum versicolor Andersson is the accepted name (The Plant List 2013) of the species. *Sarga versicolor* (Andersson) Spangler is a synonym of *Sorghum versicolor* Andersson and the preferred name for this study because it fits the phylogeny (Figure 6.1), and its base chromosome number of $x=5$ differs from *Sorghum* ($x=10$) (Spangler 2003; Anderson 2010). Other synonyms are *Andropogon serratus* var. *versicolor* (Andersson) Hack. and *Sorghum purpureosericeum* var. *trinervatum* Chiov. (The Plant List 2013). *Sorghum versicolor* Andersson is often the preferred name in South Africa (Retief and Herman 1997; Van Oudtshoorn 1999; Bromilow 2001; Henderson 2001; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). *Sarga versicolor* (Andersson) Spangler is the only documented synonym of *S. versicolor* Andersson in South Africa (Fish *et al.* 2015).

5.1.7. *Sorghastrum nudipes* Nash

Sorghastrum nudipes Nash is the accepted scientific name (The Plant list 2013), and here seven synonyms were identified, namely *Andropogon friesii* Pilg., *A. nutans* var. *angolensis* Rendle, *Sorghastrum friesii* (Pilg.) Pilg., *S. micratherum* (Stapf) Pilg., *Sorghum friesii* (Pilg.) C.E.Hubb., *S. micratherum* Stapf and *S. nutans* var. *angolense* Rendle. *Sorghastrum nudipes* Nash is widely applied in South Africa (Germishuizen *et al.* 2006; Fish *et al.* 2015). *S. friesii* (Pilg.) Pilg. was used in older works (Retief and Herman 1997; Germishuizen and Meyer 2003), but this name is now commonly acknowledged as a synonym (Germishuizen *et al.* 2006; Fish *et al.* 2015). Hence, *S. friesii* (Pilg.) Pilg. is the only synonym of *S. nudipes* Nash that is used in South African literature.

5.1.8. *Sorghastrum stipoides* (Kunth) Nash

Sorghastrum stipoides (Kunth) Nash is the accepted name and its synonyms are *S. stipoides* subsp. *agrostoides* (Speg.) Roseng., B.R.Arrill. & Izag. and *S. stipoides* subsp. *stipoides* (The Plant List 2013). *Sorghastrum rigidifolium* (Stapf) Chippind. is the only used synonym in South Africa (Retief and Herman 1997; Germishuizen and Meyer 2003; Fish *et al.* 2015), but not listed as synonym in The Plant List (2013).

5.1.9. *Sorghum arundinaceum* (Desv.) Stapf

Sorghum arundinaceum (Desv.) Stapf is an accepted name (The Plant List 2013). Synonyms are *Andropogon arundinaceus* Willd., *A. arundinaceus* var. *effusus* Hack., *A. halepensis* var. *kinshasanensis* Vanderyst, *A. sorghum* subsp. *abyssinicus* Piper, *A. sorghum* var. *aethiopicus* Hack., *A. sorghum* subsp. *effusus* (Hack.) Hitchc., *A. sorghum* var. *effusus* Hack., *A. sorghum* subsp. *verticilliflorus* (Steud.) Piper, *A. sorghum* subsp. *vogelianus* Piper, *A. stapfii* Hook.f., *A. verticilliflorus* Steud., *Holcus sorghum* subsp. *effusus* (Hack.) Hitchc., *H. sorghum* subsp. *verticilliflorus* (Steud.) Hitchc., *Rhaphis arundinacea* Desv., *Sorghum abyssinicum* (Piper) Stapf, *Sorghum aethiopicum* (Hack.) Rupr. ex Stapf, *S. aethiopicum* var. *brevifolium* Snowden, *S. bicolor* subsp. *arundinaceum* (Desv.) de Wet & J.R. Harlan, *S. bicolor* subsp. *verticilliflorum* (Steud.) de Wet ex Wiersema & J.Dahlb., *S. brevicarinatum* Snowden, *S. brevicarinatum* var. *swahilorum* Snowden, *S. castaneum* C.E.Hubb. & Snowden, *S. halepense* f. *aristatum* Rendle, *S. halepense* var. *effusum* (Hack.) Rendle, *S. halepense* var. *effusum* (Stapf) Burt Davy, *S. halepense* f. *submuticum* Hack., *S. lanceolatum* Stapf, *S. macrochaetum* Snowden, *S. panicoides* Stapf, *S. pugionifolium* Snowden, *S. somaliense* Snowden, *S. stapfii* (Hook.f.) C.E.C.Fisch., *S. usambarense* Snowden, *S. verticilliflorum* (Steud.) Stapf, *S. verticilliflorum* var. *infrequens* Snowden, *S. verticilliflorum* var. *ornatum* Snowden, and *S. vogelianum* (Piper) Stapf (The Plant List 2013).

Most of the above synonyms were not found to be used in South African literature. *S. bicolor* subsp. *arundinaceum* (The Plant List 2013) is used as the accepted name of *S. arundinaceum* (Desv.) Stapf in South Africa (Retief and Herman 1997; Van Oudtshoorn 1999; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015). Van Oudtshoorn (1999) listed *S. verticilliflorum* (not in-text) as the old name and synonym of *S. bicolor* subsp. *arundinaceum* (not in-text). *S. verticilliflorum* (Steud.) Stapf, a synonym provided by The Plant List (2013) is a commonly used synonym of *S. bicolor* subsp. *arundinaceum* in numerous South African publications (Retief and Herman 1997; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Bromilow 2001; Fish *et al.* 2015).

5.1.10. *Sorghum ×drummondii* (Nees ex Steud.) Millsp. & Chase

Sorghum ×drummondii (Nees ex Steud.) Millsp. & Chase is an accepted name (The Plant List 2013) and here eighteen synonyms are listed, namely *Andropogon sorghum* subsp. *drummondii* (Nees ex Steud.) Piper, *A. sorghum* var. *sudanensis* Piper, *Holcus sorghum* subsp. *drummondii* (Nees ex Steud.) Hitchc., *H. sorghum* var. *sudanensis* (Piper) Hitchc., *Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet ex Davidse, *S. drummondii* Nees ex Hack., and *S. vulgare* var. *drummondii* (Nees ex Steud.) Chiov.. *Sorghum ×drummondii* (Nees ex Steud.) Millsp. & Chase were used as an accepted name in Visser *et al.* (2017), although most South African studies prefer to use the synonym *S. bicolor* (L.) Moench subsp. *drummondii* (Steud.) de Wet (Retief and Herman 1997; Germishuizen and Meyer 2003; Fish and Victor 2005b; Germishuizen *et al.* 2006; Fish *et al.* 2015). *Sorghum sudanense* (Piper) Stapf is a synonym only documented in South African literature (Retief and Herman 1997; Germishuizen and Meyer 2003; Fish and Victor 2005b; Germishuizen *et al.* 2006; Fish *et al.* 2015). Van Oudtshoorn (1999) also reported *S. sudanense* (not in-text) as the old name and synonym of *S. bicolor* subsp. *drummondii* (not in-text). *Sorghum. drummondii* (Steud.) de Wet is hybrid derived from *S. bicolor* (crop plant) and *S. arundinaceum* (wild relative) (Dillon *et al.* 2001; Skendzic *et al.* 2007; Fish *et al.* 2015).

5.1.11. *Sorghum halepense* (L.) Pers.

Sorghum halepense (L.) Pers. is the accepted name (The Plant List 2013) and here fourteen synonyms are listed, namely *S. halepense* f. *aristatum* Rendle, *S. halepense* var. *effusum* (Hack.) Rendle, *S. halepense* var. *effusum* (Stapf) Burt Davy, *S. halepense* f. *halepense*, *S. halepense* var. *halepense*, *S. halepense* var. *latifolium* Willk. & Lange, *S. halepense* var. *mekongense* A.Camus, *S. halepense* f. *muticum* (Hack.) C.E.Hubb., *S. halepense* var. *muticum* (Hack.) Grossh., *S. halepense* var. *muticum* (Hack.) Hayek, *S. halepense* var. *propinquum* (Kunth) Ohwi, *S. halepense* var. *saccharatum* (L.) Goiran, *S. halepense* f. *submuticum* Hack. and *S. halepense* var. *sudanense* (Piper) Soó. *Sorghum halepense* (L.) Pers. is also used as the accepted name in South Africa (Retief and Herman 1997; Bromilow 2001; Henderson 2001; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015; Visser *et al.* 2017). *Sorghum alnum* Parodi was the only synonym of *S. halepense* (L.) Pers. provided in South African publications (Retief and Herman 1997; Henderson 2001; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015), which was not documented by The Plant List (2013). *Sorghum alnum* (author not cited) is the recognised as the old name of *S. halepense* (author not cited) (Van Oudtshoorn 1999).

5.2 Morphology of *Saccharum* wild relatives compared with *Saccharum* hybrids

5.2.1. *Cleistachne sorghoides* Benth.

Cleistachne sorghoides (Figure 5.1 A) is a robust and coarse grass which grows up to 2.5 m high (Retief and Herman 1997). It is an annual with stilt roots and the inflorescence is 40–400 mm long (Fish *et al.* 2015) (Table 5.1). Spikelets are all alike, pedicellate, 4–5 mm long not paired and they become glossy and dark when mature (Retief and Herman 1997). The upper lemma awn is 5–8 times the length of the lemma with hairy column and upper parts are scaberulous (Fish *et al.* 2015). The anther of *C. sorghoides* is up to 3.5 mm long (Fish *et al.* 2015). Various accounts (James 2004; Amalraj and Balasundaram 2006; Ahlawat 2008) described the general morphology of *Saccharum* species including *Saccharum officinarum* (Figures 5.2 E) and *S. spontaneum* (Figures 5.2 F).

The height of both *C. sorghoides* and sugarcane are similar as these plants have been described as tall grasses, which are known to grow to more than 2 m tall (Retief and Herman 1997; Amalraj and Balasundaram 2006; Pandey *et al.* 2015). Sugarcane is a perennial grass (Ahlawat 2008), unlike *C. sorghoides* which is annual (Fish *et al.* 2015). Sugarcane has a longer inflorescence (Ahlawat 2008), growing up to 60 cm tall (Pandey *et al.* 2015) (Table 5.1). Spikelets of sugarcane and *C. sorghoides* are similar in being pedicellate, although they differ in that sugarcane's spikelets are in pairs and that of *C. sorghoides* is sessile (Retief and Herman 1997; Amalraj and Balasundaram 2006) (Table 5.1). Sett and shoot roots are two types of roots present in sugarcane (Ahlawat 2008). Sugarcane and *C. sorghoides* have less similar characters such as their height and pedicelled spikelets. *C. sorghoides* is therefore amongst target species for this study with few shared similar morphological characteristics with those of sugarcane.

Table 5.1. Morphology of sugarcane and its target wild relatives studied in eastern South Africa. Literature was used to compare the life forms, height (species with the height of >2 m were classified as tall (T) and species <2 m in height were classified as short (S)), rhizomes and or roots, stems, inflorescences and leaves of sugarcane with its wild relatives. The following literature was used to generate this table: Retief and Herman 1997; Van Oudtshoorn 1999; Griffiee 2000; James 2004; Dangol 2005; Amalraj and Balasundaram 2006; Ahlawat 2008; De Sousa *et al.* 2013; Fish *et al.* 2015; Pandey *et al.* 2015; Chidambaram and Sivasubramaniam 2017; Da Silva 2017 and Prince and MacDonald 2017.

Species	Life forms: Annual (A), Perennial (P)	Height	Rhizomes and roots	Stems	Inflorescence	Leaves
Sugarcane	P	T	Sett and shoot roots present.	Stems with lateral branches. Nodes and internodes present.	Up to 60 cm. Paired and pedicellate spikelets. Panicle is hairy and silky white in colour, with size of 25–50 cm long.	Hairy leaf sheaths. Thick visible midrib. Leaf blades up to 45–200 x 0.21.5 cm.
<i>Cleistachne sorghoides</i>	A	T	Stilt roots.	Robust.	40–400 mm in size. Unpaired pedicellate spikelets, 4–5 mm long. Glossy and dark at maturity.	
<i>Imperata cylindrica</i>	P	S	Long, strong rhizomes.	Creeping stems. Unbranched culms. Smooth stems.	Dense, silky and hairy inflorescence. Silver-white panicle. Awnless spikelets, 3–6 mm long.	Leaves contain as prominent midrib. hardy leaves. Leaf blades up to 1500 mm long and 2- 12 mm wide.
<i>Microstegium nudum</i>	A	S		Creeping stems.	Spikelets are solitary or	Leaf blades are 80 x 2-7

					paired and they are 3.5–4.5 mm long.	mm.
<i>Miscanthidium capense</i>	P	T	Deep roots systems with rhizomes present.	Robust and unbranched stems with dark and hairy nodes.	Inflorescence is hairy and contracted with grey or brown panicle. Inflorescence is 200–450 mm long. Spikelets are 3–6 mm long.	Leaf blades are 90 x 16 mm.
<i>Miscanthidium junceum</i>	P	S	Rhizomes present.	Robust stems.	Inflorescence is a hairy contracted panicle and is 200–550 mm long. Spikelets are 3–5 mm long.	Leaf blades are 500–1000 mm long, up to 3 mm wide. Visible midrib.
<i>Sarga versicolor</i>	A or P	S		Loose stems with white spreading hairs around its nodes.	Inflorescence is a contracted and drooping panicle. Sessile spikelets are 4–7 mm long and pedicelled spikelets are 3–4 mm long.	Leaf blades have a prominent midrib and are 4–8 mm wide.
<i>Sorghastrum nudipes</i>	P	S		Stems with nodes.	Inflorescence is an open panicle. Paired spikelets 5–8 mm long.	Leaf blades up to 200 mm long with a width of 2 to 6 mm.
<i>Sorghastrum stipoides</i>	P	S		Stems with nodes.	Inflorescence to 200 mm long. Paired spikelets 4–7 mm long.	Leaf blades to 450 x 2–5 mm.
<i>Sorghum</i>	A or P	T	Rhizomes	Thick culms	Inflorescence	Broad

<i>arundinaceum</i>			absent.	with nodes.	either an open or contracted panicle. Spikelets sessile with pedicels 5–7 mm long.	leaves, 20–30 mm; with a conspicuous white midrib.
<i>Sorghum ×drummondii</i>	A	T	Rhizomes absent.	Thick culms with nodes.	Compact panicle; spikelets sessile, 6–7 mm long.	Leaves 8–15 mm wide with conspicuous white midrib.
<i>Sorghum halepense</i>	P	T	Long, strong rhizomes and sometimes found with prop roots.	Thick culms with nodes.	Open inflorescence. Spikelets with pedicelled spikelets 5–7 mm long and sessile spikelets to 5.7 mm long.	Leaves have white visible midrib.



Figure 5.1. Herbarium specimens of Sorghinae species from eastern South Africa: *Cleistachne sorghoides* (A), *Sarga versicolor* (B), *Sorghastrum nudipes* (C), *Sorghastrum stipoides* (D), *Sorghum arundinaceum* (E), *Sorghum x drummondii* (F) and *Sorghum halepense* (G).

5.2.2. *Imperata cylindrica* (L.) Raeusch.

Imperata cylindrica Figure 5.2 (A) is a perennial creeping grass which spreads with long rhizomes and can also form dense stands (Van Oudtshoorn 1999; Retief and Herman 1997). The plants are up to 1.2 m high (Retief and Herman 1997) (Table 5.1). It has a dense, silky and hairy inflorescence with a cylindrical silver-white panicle akin to a spike (Van Oudtshoorn 1999; Fish et al. 2015). The culms of *I. cylindrica* are not branched and the leaves contain a prominent midrib (Van Oudtshoorn 1999). Its leaves are stiff with a sharp tip, broad in the middle and become reddish in winter (Retief and Herman 1997; Van Oudtshoorn 1999). The leaf sheath is smooth and round, becoming fibrous at maturity (Van Oudtshoorn 1999). Leaf blades are up to 1500 x 2–12 mm (Retief and Herman 1997). The spikelets are 3–6 mm long and awnless, with the glumes as long as the spikelet and its anthers are 3.0–3.5 mm long (Fish et al. 2015) (Table 5.1).

The maximum height of *Imperata cylindrica* is less than 1.5 m, making it shorter than *Saccharum* species and these species are both perennial (Retief and Herman 1997; Amalraj and

Balasundaram 2006). Both these grasses have long rhizomes and *S. spontaneum* roots are similar to that of *I. cylindrica* (Van Oudtshoorn 1999; Pandey *et al.* 2015). The stem of *I. cylindrica* does not have nodes or lateral branches (tillers) like sugarcane (Retief and Herman 1997; Amalraj and Balasundaram 2006) (Table 5.1). Leaf sheaths of sugarcane are hairy unlike those of *I. cylindrica* which is smooth (Van Oudtshoorn 1999; Amalraj and Balasundaram 2006).

Leaves of sugarcane have a thick visible midrib similar to *I. cylindrica* (Van Oudtshoorn 1999; James 2004; Prince and MacDonald 2017) and the midrib is often whitish in some sugarcane varieties (Ahlawat 2008). Their leaves are also broader with sharp tips (Van Oudtshoorn 1999; James 2004). Sugarcane has leaf blades that are broad and hairy (Retief and Herman 1997; Griffie 2000; James 2004). The panicle of sugarcane is hairy, silky white in colour and terminal, usually 25–50 cm long (James 2004). Both *I. cylindrica* and sugarcane have similar leaves and inflorescences, and both are rhizomatous (Table 5.1). The above-mentioned species differ mainly in height and stem morphology and other morphological characteristics of *I. cylindrica* are very similar to those of with sugarcane.

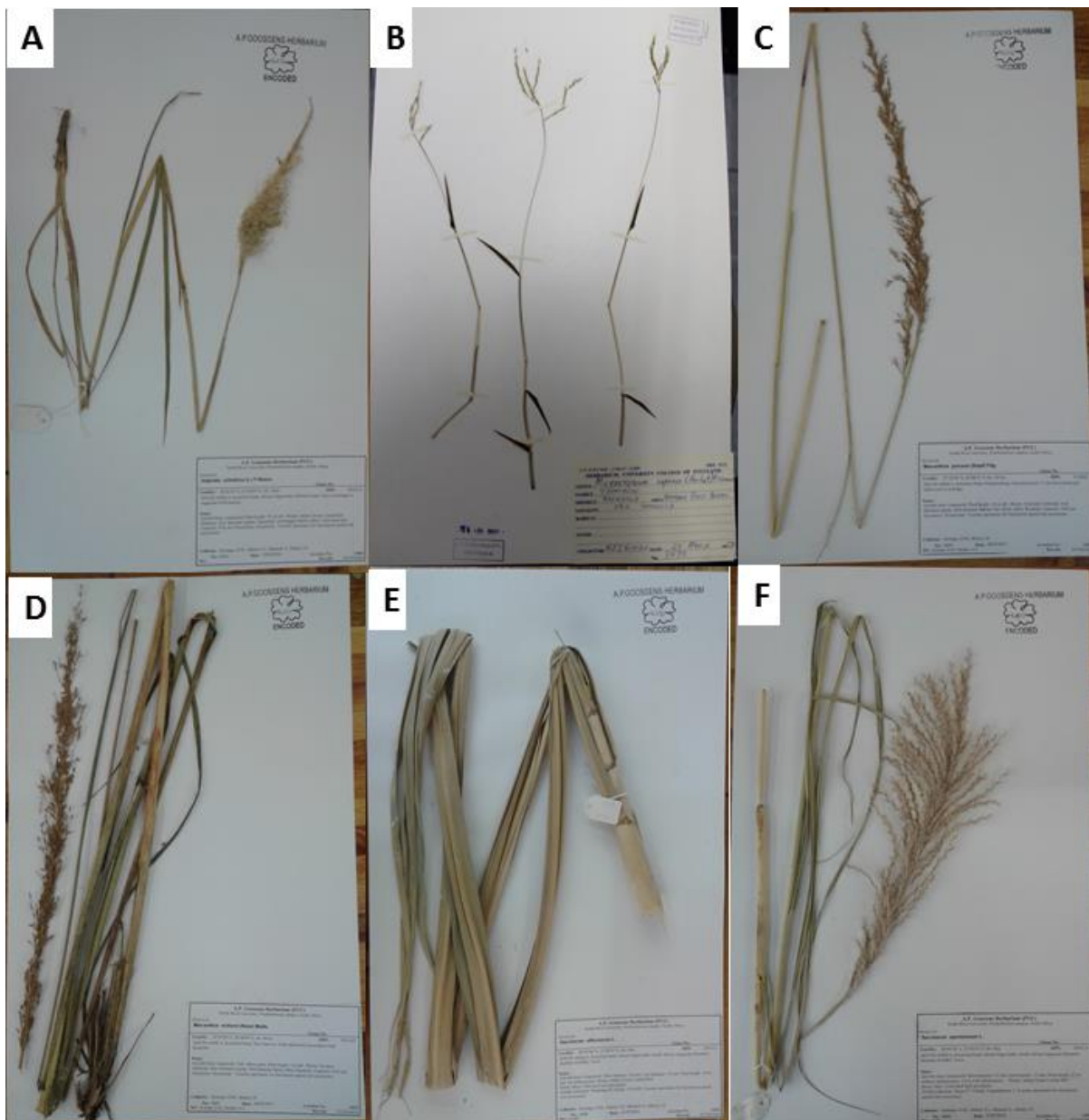


Figure 5.2. Herbarium specimens of Saccharinae species in eastern South Africa: *Imperata cylindrica* (A), *Microstegium nudum* (B), *Miscanthidium capense* (C), *Miscanthidium junceum* (D), *Saccharum officinarum* (E) and *Saccharum spontaneum* (F).

5.2.3. *Microstegium nudum* (Trin.) A.Camus

Microstegium nudum (Figure 5.2 B) is an annual grass, growing up to 600 mm tall and also trails on the ground forming tangled mats (Retief and Herman 1997). Its leaf blades are 80 x 2–7 mm (Fish *et al.* 2015). Inflorescences contains of 3–4 racemes and are either solitary or paired on a central axis with the lower glume of sessile spikelets concave at the back (Retief and Herman 1997) (Table 5.1). The spikelets are 3.5–4.5 mm long and the upper lemma has a capillary (Fish *et al.* 2015). The awns of *M. nudum* are glabrous and its anthers are from 0.8–1.9 mm long (Fish *et al.* 2015). Spikelets of sugarcane are all paired unlike those of *M. nudum* (Retief and Herman 1997; James 2004) (Table 5.1). *Microstegium nudum* is a very short grass when compared with

sugarcane (Retief and Herman 1997; Amalraj and Balasundaram 2006; Pandey *et al.* 2015; Prince and MacDonald 2017) (Table 5.1). Growth structures of *M. nudum* also differ in that it is ground dwelling, whereas sugarcane grows upright (Retief and Herman 1997; Amalraj and Balasundaram 2006) (Table 5.1). In contrast to *M. nudum* the life form of sugarcane is perennial (Retief and Herman 1997; Ahlawat 2008) (Table 5.1). Leaf blades of sugarcane are generally much broader than those of *M. nudum* (Fish *et al.* 2015; Pandey *et al.* 2015) (Table 5.1). From the above it is clear that *M. nudum* is morphological dissimilar to sugarcane.

5.2.4. *Miscanthidium capense* (Nees) Andersson

Miscanthidium capense (Figure 5.2 C) is a robust, tufted, perennial grass with rhizomes and is considered large to very large, growing up to 2.4 m tall (Van Oudtshoorn 1999; Fish *et al.* 2015). Its culms are not branched, and the hairy nodes are usually darkly coloured (Van Oudtshoorn 1999). The inflorescence is characterised by hairiness, a contracted grey or brown panicle and its 200–450 mm long (Van Oudtshoorn 1999; Fish *et al.* 2015) (Table 5.1). Leaf blades are 90 x 16 mm and are found to be expanded, folded or narrowed to the midrib only near the base (Fish *et al.* 2015). The spikelets are 3–6 mm long and the upper lemma is awnless, or if there are awns present, they are up to 7 mm long (Fish *et al.* 2015). *Miscanthidium capense* is similar to sugarcane with its perennial life form, tall habits and deep roots systems and the presence of rhizomes (Van Oudtshoorn 1999; James 2004; Fish *et al.* 2015; Pandey *et al.* 2015).

The above-mentioned grasses both have nodes, although they differ in that a series of internodes are present in sugarcane with nodes being found with leaves (Van Oudtshoorn 1999; Amalraj and Balasundaram 2006; Ahlawat 2008; Pandey *et al.* 2015) (Table 5.1). They are also characterised by having robust tall stems (Grivet *et al.* 2004; James 2004; Dangol 2005; Ahlawat 2008; Fish *et al.* 2015; Pandey *et al.* 2015; Chidambaram and Sivasubramaniam 2017). Leaf blades of sugarcane are hairy and normally found to be held in an erect position or drooping (Amalraj and Balasundaram 2006), up to 10 cm wide and narrowing towards the pointed tip (James 2004). Spikelets of sugarcane are longer (to 5 mm), paired and also always awnless compared to *M. capense* that is usually awnless but may only sometimes have awns present (Fish *et al.* 2015; Pandey *et al.* 2015) (Table 5.1). *Miscanthidium capense* is a target species that highly resembles sugarcane due to their similar morphology (height, growth form, roots, culms, leaves and inflorescences).

5.2.5. *Miscanthidium junceum* (Stapf) Pilg.

Miscanthidium junceum (Figure 5.4 D) is a perennial grass growing to 1.8 m tall (Retief and Herman 1997), with rhizomes present (Fish *et al.* 2015). It is a dense and robust grass forming large tufts (Van Oudtshoorn 1999). Its leaves are smooth and solid with white fibres and a non-hairy leaf sheath (Van Oudtshoorn 1999). Leaf blades are 500–1000 x 3 mm, terete is reduced to a yellow midrib (Retief and Herman 1997; Fish *et al.* 2015) (Table 5.1). The length of *M. junceum*'s

inflorescence is 200–550 mm and it is a hairy, contracted, light-brown panicle (Van Oudtshoorn 1999; Fish *et al.* 2015). Its spikelets are 3–5 mm long, the lower glume is hairy, while the upper lemma is awned and 2–10 mm long (Fish *et al.* 2015) (Table 5.1). Inflorescence of sugarcane is an open panicle, longer than those of *M. capense* (to 60 cm long), silky white in colour and hairy (Ahlawat 2008; Fish *et al.* 2015; Pandey *et al.* 2015; Prince and MacDonald 2017). Spikelets of sugarcane are awnless unlike those of *M. junceum* (Amalraj and Balasundarum 2006; Fish *et al.* 2015). *Miscanthidium junceum* is shorter than sugarcane being less than 2 m, although both have the same life form and are rhizomatous (Retief and Herman 1997; James 2004; Dangol 2005; De Sousa *et al.* 2013) (Table 5.1). Leaf blades of sugarcane are wider than those of *M. capense* (to 10 cm wide) and the leaves of these two species have prominent midribs (Retief and Herman 1997; James 2004; Fish *et al.* 2015) (Table 5.1). Unlike *M. capense*, sugarcane has hairy leaf sheaths (Van Oudtshoorn 1999; Chidambaram and Sivasubramaniam 2017; Prince and MacDonald 2017). *Miscanthidium junceum* was also found to be amongst target species with morphological characteristics similar to that of sugarcane.

5.2.6. *Sarga versicolor* (Andersson) Spangler

Sarga versicolor (Figure 5.3 G) is a tufted, short-lived perennial or annual grass (Retief and Herman 1997; Fish *et al.* 2015). It has few loose culms and grows up to 1.2 m tall (Retief and Herman 1997; Van Oudtshoorn 1999) (Table 5.1). White spreading hairs are present around the nodes (Van Oudtshoorn 1999). Leaf blades have prominent midrib and it is 4–8 mm wide (Retief and Herman 1997; Van Oudtshoorn 1999) (Table 5.1). The primary branches of the inflorescence are loose and whorled and is characteristically a contracted and drooping panicle (Van Oudtshoorn 1999; Fish *et al.* 2015). Sessile spikelets are 4–7 mm long and they are shiny and black when at maturity (Van Oudtshoorn 1999; Fish *et al.* 2015) (Table 5.1). The lower glume is elliptic-oblong, hard, reddish brown to black when mature, and glossy, glabrous to loosely hairy, whereas the bent and twisted upper lemma awn is 30–45 mm long (Fish *et al.* 2015). The pedicelled spikelets are linear to lanceolate, 3–4 mm long and without awns, with loose hairs and green in colour (Fish *et al.* 2015) (Table 5.1). The anther is 2–3.8 mm long (Fish *et al.* 2015). Sugarcane is taller than *S. versicolor* and these grasses both are perennials, although *S. versicolor* can also be annual at times (Retief and Herman 1997; Van Oudtshoorn 1999; James 2004) (Table 5.1). Nodes of sugarcane have lateral buds, whereas that of *S. versicolor* has been described to be with spreading hairs (Van Oudtshoorn 1999; James 2004; Ahlawat 2008). Visible midribs are present in the leaves of both species (Retief and Herman 1997; Van Oudtshoorn 1999; Pandey *et al.* 2015) (Table 5.1). Inflorescence of *S. versicolor* is contracted, whereas sugarcane has an open panicle (Van Oudtshoorn 1999; Ahlawat 2008). These grasses both have spikelets that are sessile and pedicellate, but in sugarcane they are without awns (Amalraj and Balasundarum 2006; Fish *et al.* 2015) (Table 5.1). *Sarga versicolor* was found to have many morphological characteristics shared with sugarcane.

5.2.7. *Sorghastrum nudipes* Nash

Sorghastrum nudipes (Figure 5.3 B) is a tufted perennial grass, growing up to 1.2 m tall (Retief and Herman 1997), with the lowest culm node to 5 mm long (Fish *et al.* 2015) (Table 5.1). The leaf blades are up to 200 x 2–6 mm, its base is expanded with a sheath mouth that is not appendaged and auricles are not present, its ligule is a fringed membrane, and the hairs are as long as the membrane (Retief and Herman 1997; Fish *et al.* 2015) (Table 5.1). Inflorescence of *S. nudipes* is an open panicle (Fish *et al.* 2015). The spikelets are 5–8 mm long, similar in appearance and each pair with one spikelet and an empty pedicel (Retief and Herman 1997; Fish *et al.* 2015). The upper lemma awn is straight, bent or twisted and 3–10 mm long; the anther is 2.5–3.5 mm long (Fish *et al.* 2015). Retief and Herman (1997) described *S. nudipes* as a tall grass although its crown height is generally shorter than sugarcane (Dangol 2005; Prince and MacDonald 2017). Life forms of the above-mentioned grasses are similar with nodes present (Retief and Herman 1997; Pandey *et al.* 2015) (Table 5.1). The inflorescence of these grasses has both been described as open panicles with paired spikelets (Ahlawat 2008; Fish *et al.* 2015). Inflorescence of sugarcane is awnless unlike those of *S. nudipes* (Fish *et al.* 2015; Pandey *et al.* 2015). Sugarcane has wider (up to 10 cm in width) leaf blades than those of *S. nudipes*, but the leaf sheaths of both are hairy (Retief and Herman 1997; James 2004; Chidambaram and Sivasubramaniam 2017; Prince and MacDonald 2017) (Table 5.1). Even though *S. nudipes* is shorter than sugarcane and with smaller leaf blades, overall these species have similar morphological characteristics.

5.2.8. *Sorghastrum stipoides* (Kunth) Nash

Sorghastrum stipoides (Figure 5.3 C) is a tufted perennial grass, growing to the height of 1.5 m (Retief and Herman 1997), with the lowest culm node to 10 mm long (Fish *et al.* 2015). The leaf blades are up to 450 x 2–5 mm, its base is tightly rolled, narrower than the middle portion of the blade and the sheath mouth contain erect appendages (Retief and Herman 1997; Fish *et al.* 2015) (Table 5.1). It has erect auricles and the ligule is a fringed membrane, with minute hairs that are shorter than the membrane (Fish *et al.* 2015). The inflorescence of *S. stipoides* is 200 mm long (Fish *et al.* 2015) (Table 5.1). The spikelets are paired and similar, and 4–7 mm long with each pair having one sessile and an empty pedicel (Fish *et al.* 2015). The upper lemma awn varies from 8–16 mm long and is never e straight, but is always bent and twisted. Anthers are 2–2.5 mm long (Fish *et al.* 2015). *Sorghastrum stipoides* is shorter than sugarcane and both grasses are perennials (Retief and Herman 1997; Pandey *et al.* 2015) (Table 5.1). Nodes are present in both *S. stipoides* and sugarcane (Retief and Herman 1997; James 2004; Pandey *et al.* 2015) (Table 5.1). The inflorescence of sugarcane is larger than that of *S. stipoides* and in both the spikelets are paired (James 2004; Amalraj and Balasundarum 2006; Fish *et al.* 2015). Spikelets of sugarcane are awnless unlike those of *S. stipoides* (Fish *et al.* 2015; Pandey *et al.* 2015) (Table 5.1). These grasses have hairy leaf sheaths even though leaf blades of sugarcane are wider than that those of *S. stipoides* (Retief and Herman 1997; James 2004; Chidambaram and Sivasubramaniam 2017;

Prince and MacDonald 2017) (Table 5.1). *Sorghastrum stipoides* is amongst the targeted wild relatives that has fewer morphological characteristics in common with sugarcane.

5.2.9. *Sorghum arundinaceum* (Desv.) Stapf

Sorghum arundinaceum (Figure 5.3 D) is a robust and tufted graminoid, classified as a short-lived perennial or annual (Retief and Herman 1997; Van Oudtshoorn 1999; Fish *et al.* 2015). It has thick culms and its nodes do not have spreading white hairs (Van Oudtshoorn 1999; Fish *et al.* 2015) (Table 5.1). It grows up to 2.5 m high and it has no rhizomes (Fish *et al.* 2015). It has broad leaves (20–30 mm) with a conspicuous white midrib (Retief and Herman 1997; Van Oudtshoorn 1999) (Table 5.1). The inflorescence is either an open or contracted panicle with loose ascending or spreading branches which are sometimes pendulous and its racemes are readily disarticulating (Van Oudtshoorn 1999; Fish *et al.* 2015). The sessile spikelets are pedicelled and 5–7 mm long, they are linear to lanceolate, awnless and deciduous (Fish *et al.* 2015). The lower glumes are densely to loosely hairy and the awn of the upper lemma are 10–16 mm (rarely 18 mm) long; it is further geniculate and partly twisted (Fish *et al.* 2015). Spikelets are normally pale greenish-yellow or reddish to purplish although they vary in colour when mature often being glabrous and glossy (Fish *et al.* 2015) (Table 5.1). The anthers are 2–3.5 mm long (Fish *et al.* 2015). Sugarcane is also described as a robust tall grass like *S. arundinaceum* and they both have tall stems with noticeable nodes (Van Oudtshoorn 1999; Grivet *et al.* 2004; Amalraj and Balasundaram 2006; Fish *et al.* 2015). The previously mentioned grasses have similar life forms even though *S. arundinaceum* are sometimes annual (Retief and Herman 1997; Van Oudtshoorn 1999; Fish *et al.* 2015; Pandey *et al.* 2015) (Table 5.1).

Sugarcane is known to have rhizomes whereas *S. arundinaceum* is not rhizomatous (Van Oudtshoorn 1999; Amalraj and Balasundaram 2006) (Table 5.1). Sugarcane has wider (up to 10 cm) leaf blades than those of *S. arundinaceum* (Retief and Herman 1997; James 2004) (Table 5.1). They also have noticeable midribs on their leaves (James 2004; Fish *et al.* 2015) (Table 5.1). Inflorescences of sugarcane and *S. arundinaceum* are similar in that they are awnless with sessile spikelets and with loose, droopy and open panicles, although the panicle of *S. arundinaceum* can become contracted (Van Oudtshoorn 1999; James 2004; Ahlawat 2008; Fish *et al.* 2015; Pandey *et al.* 2015; Prince and MacDonald 2017) (Table 5.1). *Sorghum arundinaceum* share most morphological characteristics with sugarcane.

5.2.10. *Sorghum ×drummondii* (Nees ex Steud.) Millsp. & Chase

Sorghum ×drummondii (Figure 5.3 F) is a robust, tufted, annual grass with thick culms and is not rhizomatous (Van Oudtshoorn 1999; Retief and Herman 1997) (Table 5.1). Its height is up to 3 m and the culm node lacks spreading white hairs (Retief and Herman 1997). The leaves are 8–15 mm wide with a conspicuous white midrib (Van Oudtshoorn 1999; Fish *et al.* 2015) (Table 5.1). Inflorescence of *S. ×drummondii* is a compact panicle with racemes that are tardily disarticulating

(Fish *et al.* 2015). The spikelets are 6–7 mm long, awnless and sessile (Retief and Herman 1997; Fish *et al.* 2015). Spikelets are persistent, pale greenish-yellow or reddish to purplish, glossy and glabrous, and the lower glume is loosely hairy with the upper lemma shortly lobed (Fish *et al.* 2015) (Table 5.1). The awn is to 16 mm long and the anthers 2.5–4 mm long (Fish *et al.* 2015). *Sorghum ×drummondii* is described as tall as sugarcane, but is annual, even though it is sometimes described to be a perennial grass (Retief and Herman 1997; Dangol 2005; Chidambaram and Sivasubramaniam 2017; Prince and MacDonald 2017) (Table 5.1). *Sorghum ×drummondii* and sugarcane has broad leaves, wide leaf sheaths and prominent midribs (Van Oudtshoorn 1999; James 2004). Sugarcane has an open panicle, unlike the compact panicle of *S. ×drummondii* (James 2004; Ahlawat 2008; Fish *et al.* 2015) (Table 5.1). They further differ in that *S. ×drummondii* does not have rhizomes like sugarcane and sugarcane spikelets are awnless (Van Oudtshoorn 1999; Amalraj and Balasundarum 2006; Fish *et al.* 2015; Pandey *et al.* 2015) (Table 5.1). The colour of sugarcane inflorescence is generally whitish, whereas *S. ×drummondii* range from pale greenish-yellow or reddish to purplish, with even more colours at maturity (Fish *et al.* 2015; Pandey *et al.* 2015) (Table 5.1). *Sorghum ×drummondii* was also found to have many similar morphological characteristics shared with sugarcane.

5.2.11. *Sorghum halepense* (L.) Pers.

Sorghum halepense (Figure 5.3 E) is an erect perennial tufted grass with thick culms (Van Oudtshoorn 1999) (Table 5.1). It is easily distinguished from other *Sorghum* species by its long, strong rhizomes and is sometimes found with prop roots (Retief and Herman 1997; Van Oudtshoorn 1999; Fish *et al.* 2015). It is a tall grass, growing to 2.5 m high (Fish *et al.* 2015) and its nodes are without spreading white hairs (Fish *et al.* 2015) (Table 5.1). The leaves have a conspicuous, white midrib (Van Oudtshoorn 1999), with leaf blades of 200–600 x 10–30 mm (Retief and Herman 1997; Fish *et al.* 2015). *Sorghum halepense* has an open inflorescence with slender branches which are often pendulous (Fish *et al.* 2015) (Table 5.1). The spikelets are lanceolate without awns (Fish *et al.* 2015). Its pedicelled spikelets are 5–7 mm long (Fish *et al.* 2015) and they are longer than the sessile spikelets (Retief and Herman 1997) which are up to 5.7 mm long (Fish *et al.* 2015). Sessile spikelets are ovate with various colours when mature, and the lower glumes are indurate, shiny and adpressed pubescent (Fish *et al.* 2015). The upper lemma is minutely bilobed with awns of 10–16 mm long when present, and are then twisted and geniculate. The anthers are 2.1–4.5 mm long (Fish *et al.* 2015). Sugarcane is a large tall grass like *S. halepense* and these grasses are both perennials with nodes and rhizomes present (Retief and Herman 1997; Van Oudtshoorn 1999; James 2004; Dangol 2005; Fish *et al.* 2015; Da Silva 2017) (Table 5.1). They both further have whitish midribs on their leaves (Van Oudtshoorn 1999; Pandey *et al.* 2015). Their inflorescences are open with pedicelled spikelets which are awnless (Amalraj and Balasundarum 2006; Pandey *et al.* 2015; Fish *et al.* 2015) (Table 5.1). Like other *Sorghum*

species studied in this research, *S. halepense* was thus also found to have many similar morphological characteristics to sugarcane.

5.3 Distribution of *Saccharum* wild relatives in eastern South Africa

5.3.1. *Cleistachne sorghoides* Benth.

Cleistachne sorghoides is known to be restricted to Mpumalanga province (Germishuizen and Meyer 2003; Victor 2004; Germishuizen *et al.* 2006; Fish *et al.* 2015). *Cleistachne sorghoides* has previously only been recorded from Mariepskop in Limpopo Figure (5.3 A), but this could be a wrong identification (Soberón and Peterson 2004). Barberton, Carolina, Machadodorp, Nelspruit and Sabie were areas where *C. sorghoides* were found in Mpumalanga. Barberton and Nelspruit were the only two sugarcane quarter-degree squares (QDS) with records of this grass species in the whole study area (Table 5.2). *Cleistachne sorghoides* were not found to be occurring in KwaZulu-Natal (Figure 5.3 A). No field observations of *C. sorghoides* were made during field surveys and the data used for distribution of this grass were from sourced specimens only (Table 5.2). Our findings showed that there is co-occurrence of *C. sorghoides* QDS distributions with those of sugarcane cultivation areas in Mpumalanga, despite a limited distribution across the study area (Figure 5.3 A). A total of seven QDS of the study contained *C. sorghoides*, where two of these records were within sugarcane QDS and three localities were bordering sugarcane QDS (Table 5.2). This grass species is not threatened (Hilton-Taylor 1996) and it is therefore classified as a species of least concern (Victor 2004; Raimondo *et al.* 2009).

Table 5.2. Locality records and total QDS covered by each target species.

Species	No. of sourced herbarium specimens	No. of fieldwork collections	No. of total QDS	No. of sugarcane QDS	No. of QDS bordering sugarcane QDS
<i>Cleistachne sorghoides</i>	7	0	7	2	3
<i>Imperata cylindrica</i>	172	8	180	36	20
<i>Microstegium nudum</i>	31	0	31	5	5
<i>Miscanthidium capense</i>	87	7	94	20	6
<i>Miscanthidium junceum</i>	86	2	88	10	8
<i>Sarga versicolor</i>	56	0	56	10	10
<i>Sorghastrum nudipes</i>	17	0	17	1	1
<i>Sorghastrum stipoides</i>	34	0	34	13	5
<i>Sorghum arundinaceum</i>	82	20	102	33	10
<i>Sorghum ×drummondii</i>	1	4	5	3	1
<i>Sorghum halepense</i>	29	0	29	15	2

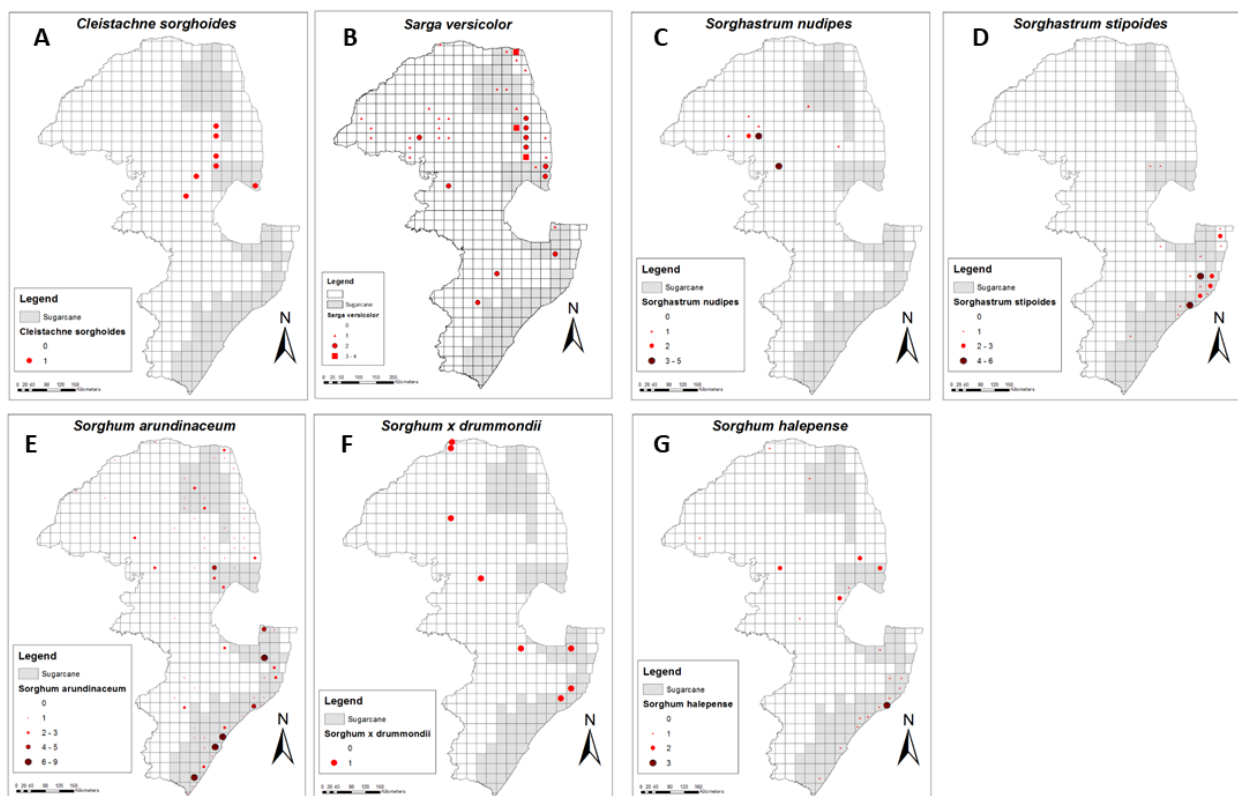


Figure 5.3. Distribution maps of Sorghinae species in eastern South Africa: *Cleistachne sorghoides* (A), *Sarga versicolor* (B), *Sorghastrum nudipes* (C), *Sorghastrum stipoides* (D), *Sorghum arundinaceum* (E), *Sorghum x drummondii* (F) and *Sorghum halepense* (G).

5.3.2. *Imperata cylindrica* (L.) Raeusch.

Imperata cylindrica was the most collected species. A total of 172 sourced localities were supplemented with eight field collections from the study sites (Table 5.2). Figure 5.4 (A) shows that *I. cylindrica* occurs in most regions of the study area including having high abundance and wider distribution within sugarcane QDS. Coastal sugarcane production regions of KwaZulu-Natal are widely depicted to be prevalent with *I. cylindrica* as shown by figure 5.4 (A). *Imperata cylindrica* is a cosmopolitan grass species and its records are well documented in the study provinces (Van Oudtshoorn 1999; Germishuizen and Meyer 2003; Fish and Victor 2006; Germishuizen *et al.* 2006; Fish *et al.* 2015). Its conservation status in the country has been classified as least concern (Raimondo *et al.* 2009).

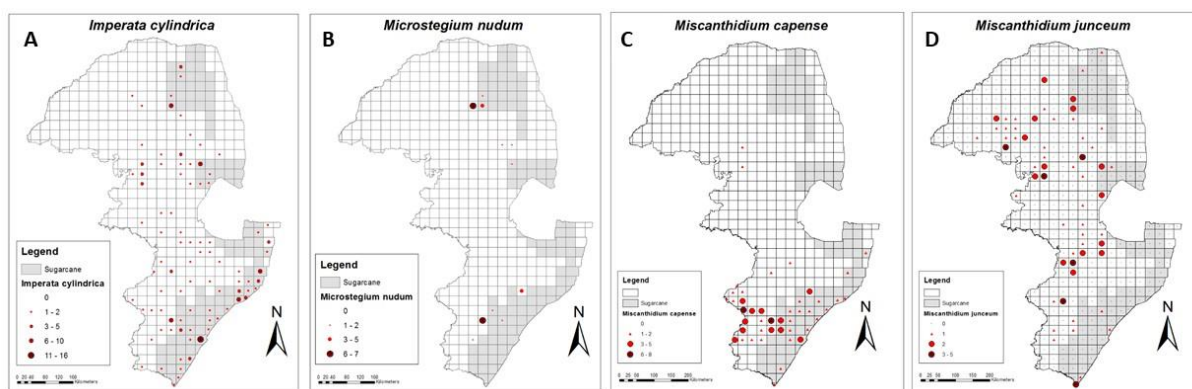


Figure 5.4. Distribution maps of Saccharinae species in eastern South Africa: *Imperata cylindrica* (A), *Microstegium nudum* (B), *Miscanthidium capense* (C) and *Miscanthidium junceum* (D).

5.3.3. *Microstegium nudum* (Trin.) A.Camus

Microstegium nudum was amongst the least collected species, with only 31 specimens sourced from six herbaria. It is distributed in three provinces (Figure 5.4 B). Polokwane and Tzaneen in Limpopo were the only two areas where this species was collected in Limpopo. For Mpumalanga, it was sampled in the Graskop, Pilgrim's Rest and Sabie areas, whereas the rest of collections showed its presence in various regions of KwaZulu-Natal. This grass species was recorded in sugarcane QDS of all the study provinces despite its scattered distribution patterns in eastern South Africa (Figure 5.4 B). Germishuizen and Meyer (2003), Germishuizen *et al.* (2006) and Fish *et al.* (2015) documented the distribution of *M. nudum* in the study area. *Microstegium nudum* was not found during field surveys of the current study (Table 5.2).

5.3.4. *Miscanthidium capense* (Nees) Andersson

Marble Hall was the only town with one record in Limpopo where *Miscanthidium capense* was sampled. Only two records were made in Mpumalanga, both of them in the Witbank area. All records from Mpumalanga provinces were not sampled in QDS containing sugarcane fields (Figure 5.4 C). Ninety-one samples were made from KwaZulu-Natal, with most of them being distributed in and around sugarcane QDS (Figure 5.4 C). All seven field observations were recorded within three QDS's of KwaZulu-Natal (Durban, Hammarsdale and New Hanover; Table 5.2), where there are extensive commercial sugarcane fields. According to the literature *M. capense* only occurs in KwaZulu-Natal within the study provinces (Germishuizen and Meyer 2003; Fish and Victor 2005a; Germishuizen *et al.* 2006; Fish *et al.* 2015). The Red List of South African Plants has grouped *M. capense* amongst species of least concern in the country (Raimondo *et al.* 2009).

5.3.5. *Miscanthidium junceum* (Stapf) Pilg.

Miscanthidium junceum is more evenly distributed (Figure 5.4 D) across study provinces when compared to the previously discussed species under section 5.3.4, even though it was less collected than *M. capense*. Most of the sourced data were localities from Limpopo followed by KwaZulu-Natal and lastly Mpumalanga. Two records were sampled from Mount Edgecombe and Paulpietersburg (KwaZulu-Natal) during field visits (Table 5.2; Appendix A). *Miscanthidium junceum* was found to be distributed in sugarcane grids of all study provinces. Although widely distributed, it was not associated with the sugarcane cultivation area (Figure 5.4 D). Occurrences of *M. junceum* across study provinces is well supported in South African literature (Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015).

5.3.6. *Sarga versicolor* (Andersson) Spangler

The occurrence of *Sarga versicolor* was mostly in Limpopo. There were more clustered occurrences of this species in the western part of Limpopo where there were no commercialized sugarcane (Figure 5.1 B). *Sarga versicolor* was associated with sugarcane QDS in Mpumalanga. The occurrences of *S. versicolor* in KwaZulu-Natal was found to be low and scattered across the province (Figure 5.1 B). *Sarga versicolor* was amongst target sugarcane wild relatives that were not found during field surveys of this study (Table 5.2). Distribution accounts in Van Oudtshoorn (1999); Bromilow (2001); Germishuizen and Meyer (2003); Germishuizen *et al.* (2006) and Fish *et al.* (2015) supports the results presented for *S. versicolor*.

5.3.7. *Sorghastrum nudipes* Nash

Only 17 collection localities were sourced for *Sorghastrum nudipes*, making it the second least collected species. Its distribution was mostly in Limpopo. Only one sugarcane QDS (from Limpopo) represented *S. nudipes* in sugarcane fields (Figure 5.1 C). All records in Mpumalanga were from the Witbank area and this grass species was not found in KwaZulu-Natal. *Sorghastrum nudipes* was also not encountered during surveys, and all the reported findings are based on historic data (Table 5.2). Distribution patterns indicate that this grass species is not commonly associated with areas where sugarcane is cultivated in the study area. Findings reported on the distribution of *S. nudipes* are in agreement with South African publications (Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015).

5.3.8. *Sorghastrum stipoides* (Kunth) Nash

All the localities of *Sorghastrum stipoides* were found to be in and around sugarcane QDS (Figure 5.1 D), mostly in sugar cultivation regions of KwaZulu-Natal. This was unlike the closely related *S. nudipes* (see sub-section 5.3.7) that was mostly found far from sugarcane QDS. Of all sugarcane wild relative species assessed in this study, *S. stipoides* was the species with highest co-occurrence with sugarcane QDS based on its total records. Based on herbarium records,

Sorghastrum stipoides does not occur in Limpopo. *Sorghastrum stipoides* was not found during surveys, and all the reported findings are based on historic data (Table 5.2). Studies support the distribution pattern of *S. stipoides*, namely that it is found in Mpumalanga and KwaZulu-Natal (Germishuizen and Meyer 2003; Germishuizen *et al.* 2006), but Fish *et al.* (2015) also presents an extended distribution into the Limpopo province.

5.3.9. *Sorghum arundinaceum* (Desv.) Stapf

Sorghum arundinaceum was the second most collected species with 102 specimen records. It was the most encountered target species during fieldwork with 20 records (Table 5.2). These records were also found co-occurring with sugarcane QDS of the following areas: Empangeni, Felixton, Hammarsdale, Kwa-Mbonambi, Mount Edgecombe, Port Shepstone, Ubombo and Umhlali from KwaZulu-Natal. Malelane was the only area from Mpumalanga where *S. arundinaceum* was collected within sugarcane QDS, and Ohrighstad the only area from Limpopo. The distribution of this species covers most of the study area (Figure 5.1 E), indicating that it is adapted to the varying conditions across eastern South Africa. Literature agrees with results obtained since this grass is a cosmopolitan species found commonly in eastern South Africa (Van Oudtshoorn 1999; Bromilow 2001; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015).

5.3.10. *Sorghum ×drummondii* (Nees ex Steud.) Millsp. & Chase

Sorghum ×drummondii is not that widely distributed and was the least sourced species with five records. These records showed that it occurs in all the study provinces, although it was poorly collected. Four collections were made from KwaZulu-Natal during fieldwork (Table 5.2; Appendix A). Figure 5.1 (F) indicates that the distribution of this species is associated with both sugarcane QDS and bordering grids. Based on literature, *S. ×drummondii* is known to occur only in two of the study provinces except for KwaZulu-Natal (Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015), which is in contrast with herbarium data and collections sampled for this study. These new localities extend the distribution of *S. ×drummondii* to KwaZulu-Natal. It is classified as least concern (Fish and Victor 2005b). In general, it is known to have a rare occurrence in South Africa (Van Oudtshoorn 1999).

5.3.11. *Sorghum halepense* (L.) Pers.

All distributions records of *Sorghum halepense* from KwaZulu-Natal were found within sugarcane QDS, with high abundances in the coastal regions (Figure 5.1 G). Limpopo was the only province where *S. halepense* was not found in or around QDS with sugarcane cultivations. In general, the distribution of *S. halepense* coincided with that of sugarcane cultivation areas of the study area. *Sorghum halepense* is documented as a common species of eastern South Africa (Van Oudtshoorn 1999; Bromilow 2001; Henderson 2001; Germishuizen and Meyer 2003; Germishuizen *et al.* 2006; Fish *et al.* 2015), which was confirmed by our findings. Bromilow (2001) reported *S.*

halepense as a weed which is more commonly distributed in Mpumalanga than other South African provinces. *Sorghum halepense* was not encountered during field surveys, and all the reported findings are based on historic data (Table 5.2).

5.4 Habitat types of *Saccharum* wild relatives in eastern South Africa

5.4.1. *Cleistachne sorghoides* Benth.

Cleistachne sorghoides is poorly collected in eastern South Africa with only seven records from three (of 11) herbaria visited during this study. Specimen data indicated that this grass species is associated with waterlogged areas such as rivers, vleis and swamps. Riverbanks and vleis were documented as common habitats of *C. sorghoides* and in accordance with specimen data (Retief and Herman 1997; Fish *et al.* 2015). These habitats are often associated with the margins of sugarcane fields in the study area. However, *C. sorghoides* was previously collected from pine plantations of Mariepskop and foothills of the Drakensberg at Pilgrim's Rest. Both these habitats are high altitude areas and not suitable for commercial sugarcane fields. *Cleistachne sorghoides* was not collected during field surveys and field observational information regarding habitat preferences was therefore based on sourced data and literature (Table 5.3). Riverbanks and vleis were documented as common habitats of *C. sorghoides* and in accordance with specimen data (Retief and Herman 1997; Fish *et al.* 2015).

Table 5.3. Habitats of *Saccharum* wild relatives studied in the eastern South Africa. Habitat types of species were provided using the following codes for source of observations: Fieldwork (F), Herbarium specimens (H) and Literature (L). Listed habitat type was classified based on species occurrence as either aquatic (A) and or terrestrial (T) systems. The following literature were used to generate the table: Retief and Herman 1997; Van Oudtshoorn 1999; Bromilow 2001; Henderson 2001; Meter *et al.* 2002; Firehun and Tamado 2006; Malan *et al.* 2007; Ahlawat 2008; Gulaati 2011; Kumar *et al.* 2011; Takim *et al.* 2014; Fish *et al.* 2015; Olabode and Sangodele 2015; Maroyi 2017 and Visser *et al.* 2017.

Species	Habitat	Habitat type	Source of observation
<i>Cleistachne sorghoides</i>	Mountain foothills, pine plantations, rivers, river banks, swamps and vleis	A and T	H and L
<i>Imperata cylindrica</i>	Burnt sites, coastal forests, coastal grasslands, cultivated lands, damp soil, dams, disturbed areas, dunes, lakes, marshes, old mines, pans,	A and T	F, H and L

	poorly drained areas, railway, rivers, riverbanks, roadsides, seasonally wet places, seepages areas, streams, sugarcane fields, valleys, vleis and waterlogged areas		
<i>Microstegium nudum</i>	Cultivated areas, rivers, forests, grasslands, mountains, plantations, roadsides and waterlogged areas	A and T	H and L
<i>Miscanthidium capense</i>	Bog clumps, bushlands, dams, dunes, forests, grasslands, hills, kloofs, marshes, mountain slopes, plantations, ridges, rivers (river banks, river beds and streams), roadsides, sugarcane fields, swamps, valleys, vleis and waterlogged areas	A and T	F, H and L
<i>M. junceum</i>	Catchment areas, coarse sandy soil, marshes, pan, rivers, vleis waterlogged areas and wetland	A	F, H and L
<i>Sarga versicolor</i>	Black turf soil, bushveld, cultivated land, damp black clay soil, disturbed places, floodplain, grassland, Mopaneveld, mountain, old cultivated lands, open veld, railway, roadsides, rivers, shrubveld, strip mine, savanna, vleis and woodlands	A and T	H and L
<i>Sorghastrum nudipes</i>	Cultivated land, flooded area, forest, grassland, roadside, sand, streams, swamps, veld, vleis, wet places and waterlogged areas	A and T	H and L
<i>S. stipoides</i>	Bushveld, estuaries, forest margins, grasslands, lakes, marshes, pans, roadsides, shores, streams, swamps, tidal lagoon, vleis and wet areas	A and T	H and L
<i>Sorghum arundinaceum</i>	Beaches, clay soils, coastal woodlands, damp areas, disturbed places, grasslands, floodplains, irrigated areas, moist areas, open fields, pans, patches, railways, rivers, roadsides, savanna,	A and T	F, H and L

	sugarcane fields, valleys, vleis, and water courses		
<i>S. ×drummondii</i>	Agricultural areas, disturbed places, old lands, railways, rivers, roadsides and sugarcane fields	T	F, H and L
<i>S. halepense</i>	Cultivated land, damp clay soil, disturbed places, dongas, gardens, grasslands, maize fields, pans, railways, rivers, roadsides, sandy soil sugarcane fields and waterlogged areas	A and T	H and L

5.4.2. *Imperata cylindrica* (L.) Raeusch.

Imperata cylindrica was found growing along roadsides, rivers, and it was also frequently observed and recorded as a weed of sugarcane fields during field surveys (Table 5.3). *Imperata cylindrica* occurs in wide range of habitats, In the sourced data it is reported to frequently occur in waterlogged situations such as dams, dunes, valleys, vleis, rivers, lakes, marshes, pans, streams, seepages areas (Table 5.3). This weedy grass was further sampled as a common species in disturbed areas including burnt sites, old mines, roads, railway, and cultivated lands including sugarcane fields (Table 5.3). It was also found inhabiting coastal forests and coastal grasslands of KwaZulu-Natal based on herbarium data.

Our results from sourced data and records from field surveys confirm that *I. cylindrica* is a weedy relative of sugarcane that shares common habitats with commercially cultivated sugarcane in the study areas. South African literature support results presented from sourced data and field surveys conducted for this study and Retief and Herman (1997) and Van Oudtshoorn (1999) reported *I. cylindrica* as a water loving grass species which is associated with regions of high rainfall, riverbanks, vleis and seasonally waterlogged areas (Table 5.3). It can also grow in poorly drained areas and damp soil (Van Oudtshoorn 1999). It is known as a noxious weed in many countries (Bromilow 2001). It is found growing in drained soils along riverbank sand in vleis and seasonally wet places (Fish *et al.* 2015) (Table 5.3). In Nigeria, *I. cylindrica* is known as an aggressive weed of cultivated areas such as maize and sugarcane fields (Takim *et al.* 2014; Olabode and Sangodele 2015).

5.4.3. *Microstegium nudum* (Trin.) A.Camus

Specimens of *M. nudum* collected in Limpopo were made along rivers, except for one in the forests of Magoebaskloof. This was supported by most of KwaZulu-Natal collections which were also made in forests (Table 5.3). Meter *et al.* (2002) mentioned that *M. nudum* is a species that were associated with high altitudes areas such as mountains of KwaZulu-Natal. Another record from

forests came from a collection in the Drakensberg around Pilgrim's Rest in the Mpumalanga province. Waterlogged areas and roadsides were put forward on specimen labels as common habitats of *M. nudum* (Table 5.3). This grass species was further sampled in grasslands and plantations. It is normally found on moist-shady sites of the forests (Retief and Herman 1997; Fish *et al.* 2015). In India, it was also recorded in the Himalayan Mountains between 1800–3600 m above sea level (Gulaati 2011). It was also found in cultivated areas in India (Kumar *et al.* 2011). *Microstegium nudum* was not collected during field surveys and field observational information regarding habitat preferences was based on sourced data and literature (Table 5.3).

5.4.4. *Miscanthidium capense* (Nees) Andersson

Rivers (including river banks, river beds and streams), marshes, dams, swamps and vleis are waterlogged habitats that *Miscanthidium capense* was highly associated with (based on herbarium specimens) (Table 5.3). Grasslands were the second most preferred habitat of this species. Roadsides, forests, dunes, hills, kloofs, mountain slopes, bog clumps, plantations, ridges and valleys were other habitats where *M. capense* was found based on sourced herbarium data (Table 5.3). Only one observation was made along a river during field visits of this study, and most of the observations were made from in sugarcane fields and roadsides. These results indicate that this species is also associated with disturbed habitats. Both sourced data and field observations (Table 5.3) supported that most habitats of *M. capense*, including the common habitats such as waterlogged areas and grasslands, are marginal to habitats where commercial sugarcane is cultivated, and suggests that this species become weedy in such areas. Van Oudtshoorn (1999) and Maroyi (2017) reported *M. capense* to be growing in damp places such as along rivers and forests and support the findings of this study (Table 5.3). This grass species also inhabits forest margins and watercourses (including river banks) (Fish *et al.* 2015). Malan *et al.* (2007) recorded *M. capense* in bushlands and grasslands of KwaZulu-Natal (Table 5.3).

5.4.5. *Miscanthidium junceum* (Stapf) Pilg.

Miscanthidium junceum was mostly collected along rivers, vleis and marshes, but only recorded once in wetlands according to sourced data (Table 5.3). Herbarium data showed that *M. junceum* is a common weed growing along roadsides (Table 5.3). It was further sampled in grassland, bushveld and kloofs. The above-mentioned results were extracted from herbarium data (Table 5.3). This grass species was only found inhabiting pan sites of KwaZulu-Natal, during field surveys conducted in the current study (Table 5.3). Riverbanks and vleis are mostly prominent habitats of *M. junceum*, and growing in standing water is usually its most common habitat (Retief and Herman 1997; Van Oudtshoorn 1999; Fish *et al.* 2015) (Table 5.3). Coarse sandy soil is its preferred soil type (Van Oudtshoorn 1999). *Miscanthidium junceum* was also recorded in wet areas of KwaZulu-Natal midlands (Malan *et al.* 2007) (Table 5.3). Maroyi (2017) reported this grass in the catchment areas of Eastern Cape province.

5.4.6. *Sarga versicolor* (Andersson) Spangler

Specimen data showed that roadsides, cultivated land and damp black soils are preferred habitats of *Sarga versicolor*, which were also supported by literature (Retief and Herman 1997; Van Oudtshoorn 1999; Fish *et al.* 2015). Rivers and vleis were other common habitats of *S. versicolor*. Locality records were also in savanna, namely bushveld, Mopaneveld and woodlands (Table 5.3). *Sarga versicolor* was infrequently recorded from mountains, grassland, floodplains, along railways, shrubveld, and strip mine habitats. Common other habitats of *S. versicolor* are areas similar to where commercial sugarcane is cultivated in the study area (Van Oudtshoorn 1999; Fish *et al.* 2015). *Sarga versicolor* was not collected during field surveys and field observational information regarding habitat preferences was therefore be based on sourced data and literature (Table 5.3).

5.4.7. *Sorghastrum nudipes* Nash

Sorghastrum nudipes is mostly associated with vleis based from data on herbarium specimens. It was collected once in each of the following habitats: cultivated land, streams, flooded area, forest, grassland, roadside and sand veld (Table 5.3). *Sorghastrum nudipes* is mostly associated with wet places (Retief and Herman 1997) including stream banks, swamps and road drains (Fish *et al.* 2015). South African literature support the localities provided by herbarium specimens and reports this grass as a common species of waterlogged areas. *Sorghastrum nudipes* was not collected during field surveys and field observational information regarding habitat preferences was therefore based on sourced data and literature (Table 5.3).

5.4.8. *Sorghastrum stipoides* (Kunth) Nash

Occurrences of *Sorghastrum stipoides* was not found to be associated with disturbed habitats, based on its single recording from a roadside. It was found to commonly in grasslands. Other habitat types with noticeably higher records using herbarium data were swamps, streams and marshes, but similar wet areas such as vleis, estuaries, pans, lakes, shores and tidal lagoon where only found with single instances in *S. stipoides* (Table 5.3). Bushveld and forest margins where other habitats were this grass species was sampled from. It favours wet places (Retief and Herman 1997; Fish *et al.* 2015). *Sorghastrum stipoides* was not collected during field surveys and field observational information regarding habitat preferences was therefore based on sourced data and literature (Table 5.3).

5.4.9. *Sorghum arundinaceum* (Desv.) Stapf

Rivers, roadsides and sugarcane fields were habitats where *Sorghum arundinaceum* was mostly collected. Sourced specimen data showed that this species is a weed of sugarcane in the study area. Many specimen records came from Grassland, floodplains and savanna. Valleys, pans, railways, patches, vleis, beaches, open fields and coastal woodlands were other habitats where *S. arundinaceum* were sampled from based on sourced data (Table 5.3). All records sampled during

the field surveys were made in sugarcane field margins, except one record which was sampled along a roadside outside of sugarcane fields (Table 5.3). *Sorghum arundinaceum* normally favours moist and disturbed places (Retief and Herman 1997; Fish *et al.* 2015), this is also supported by the results obtained in this study. Riverbanks and vleis were undisturbed habitats that were preferred by this grass species (Van Oudtshoorn 1999). It is a common weed of water courses and damp areas such as irrigated areas of sugarcane fields (Van Oudtshoorn 1999). This grass species usually grows in clay soils (Van Oudtshoorn 1999). *Sorghum arundinaceum* is a noxious weed of agricultural areas in South Africa (Bromilow 2001), including sugarcane fields (Van Oudtshoorn 1999). Firehun and Tamado (2006) also reported *S. arundinaceum* as an aggressive weedy grass of sugarcane fields in Ethiopia.

5.4.10. *Sorghum xdrummondii* (Nees ex Steud.) Millsp. & Chase

From sourced specimens *Sorghum xdrummondii* was only found along railways, rivers and in old lands (Table 5.3), however two specimens did not provide information of where this grass was collected. For the field survey conducted for this study it was recorded along roadsides and in sugarcane fields (Table 5.3). Besides the collections that were sampled in sugarcane fields, other localities showed that the species can share similar habitats with commercial sugarcane in South Africa. *Sorghum xdrummondii* is an alien weedy grass species which was introduced to South Africa for agricultural purposes (Bromilow 2001; Visser *et al.* 2017). Bromilow (2001) reported this grass amongst aggressive weeds of agricultural regions in South Africa. It is generally found in cultivated lands and disturbed areas (Fish *et al.* 2015). *Sorghum xdrummondii* was recorded as a major weedy grass of sugarcane in Ethiopia (Firehun and Tamado 2006).

5.4.11. *Sorghum halepense* (L.) Pers.

Sorghum halepense was mostly associated with rivers, roadsides and sugarcane fields. Cultivated land, dongas, gardens, pans and railways were other sites where *S. halepense* was recorded using sourced data (Table 5.3). *Sorghum halepense* was not found to be a common weed of sugarcane like *S. arundinaceum*. The results reported were based on sourced data, since this *Sorghum* species was not found during the study (Table 5.3). *Sorghum halepense* is weedy grass inhabiting disturbed places such as roadsides and cultivated areas (Retief and Herman 1997; Henderson 2001; Fish *et al.* 2015). It also grows on damp clay or sandy soil (Van Oudtshoorn 1999). It is reported to be found in grasslands and waterlogged areas such as riverbanks and riverbeds (Henderson 2001). It is an alien invasive grass species in South Africa being introduced from Europe (Bromilow 2001; Visser *et al.* 2017) and it is also categorised as an Invader 2 (Henderson 2001). This grass is known to be a competitive weed of agricultural areas such as sugarcane and maize fields in South Africa (Bromilow 2001). *Sorghum halepense* is also known as a cosmopolitan weed of sugarcane fields in India (Ahlawat 2008).

5.5 References

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CHAPTER 6: SPATIAL AND GENE FLOW ASSESSMENTS

6.1 Relatedness of *Saccharum* wild relatives to *Saccharum* hybrids and one another

6.1.1. *Cleistachne sorghoides* Benth.

Sarga versicolor shares its most recent common ancestor with *Cleistachne sorghoides*, which makes this grass the most related species to *C. sorghoides* within the target grass species in this study (Figures 6.1 and 6.2). A grouping of *Sorghum* species from the *Sorghum sensu stricto* clade (*S. arundinaceum*, *S. bicolor* BTx623, *S. halepense*, *S. halepense* 2, *S. laxiflorum*, *S. propinquum*, *S. ×drummondii*) are amongst the closest sister species to *C. sorghoides* and *Sarga versicolor* (though they are in a divergent clade, diverging less than 3.4 million years ago). *Miscanthidium capense*, *M. junceum* and *Saccharum* species are also close relatives of *C. sorghoides* with less than 7.4 million years of divergence (Figure 6.2 and Table 6.1). *Microstegium nudum* and the *Sorghastrum* species (*S. nudipes* and *S. stipoides*) are not closely related to *C. sorghoides* and, *Imperata cylindrica* (at 12.1 million years divergence) should not be considered as a close relative to *C. sorghoides* (Figure 6.1).

Chloroplast-based analyses indicated that *C. sorghoides* emerged amongst the immediate relatives of *Sorghum* spp. (Dillon *et al.* 2001; 2007). In both the latter studies, *C. sorghoides* and *Saccharum officinarum* were the only two species apart from *Sorghum* sp., which were not included as outgroups of the phylogeny. In these studies, *C. sorghoides* was not found to be a close relative of *S. officinarum*. In fact *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* were closer to *S. officinarum* (Dillon *et al.* 2001). In the review by Spangler *et al.* (1999) *C. sorghoides* was the only species from any other genus that was nested within the *Sorghum* clade which also included *Sorghum arundinaceum*, *S. bicolor* and *S. halepense* (using *ndhF* sequences and the neighbor-joining (NJ) methodology).

The above-mentioned chloroplast-based study illustrated the close relationship of the two genera in association with other genera that were included such as: *Andropogon*, *Apluda*, *Arundinella*, *Bothriochloa*, *Capillipedium*, *Chionachne*, *Chrysopogon*, *Coix*, *Coelorachis*, *Cymbopogon*, *Danthoniopsis*, *Dichanthium*, *Elionorus*, *Heteropogon*, *Hyparrhenia*, *Ischaemium*, *Microstegium*, *Miscanthus*, *Panicum*, *Phacelurus*, *Rattryaya*, *Schizachyrium*, *Setaria*, *Sorghastrum*, *Tripsacum*, *Thysanolaena* and *Zea*. Their results were confirmed by the current study whereby *C. sorghoides* was placed as a sister to *Sarga versicolor* and the *Sorghum sensu stricto* spp. as sister to the core Andropogoneae, with this super-clade being sister to the clade containing *Cleistachne* and *Saccharum*.

Chloroplast phylogenies are known to be based on uniparentally inherited chloroplast DNA and due to the ease of sequencing have been a primary source of molecular variations used for phylogenetic analyses in photosynthetic eukaryotes (Chu *et al.* 2004). However, chloroplast-based phylogenies may often differ from gene or genomic region based phylogenies due to reticulation (hybridisation), differential evolutionary pressures and organellar exchange. In contrast, genomic phylogenies are based on relationships among genomes (Beiko *et al.* 2008).

Genomic phylogenies are currently believed to yield the more accurate tree topology and therefore the tree presented in this study (Figure 6.1) is based on genomic DNA. Previous studies based on chloroplast DNA may need to be re-evaluated. It should also be noted that gene-based studies, as presented here and in Estep *et al.* (2014) and Welker *et al.* (2015), cast doubt on previous chloroplast-based phylogenies. Monophyly of *Sorghum* is not supported by genomic phylogenies, with *Sorghum sensu stricto* being more closely allied to the core Andropogoneae and *Sarga* spp. (*Sarga versicolor* and *Cleistachne*) being more closely related to the Saccharinae. These studies also support the clear separation of *Miscanthus sensu stricto* and *Miscanthidium*.

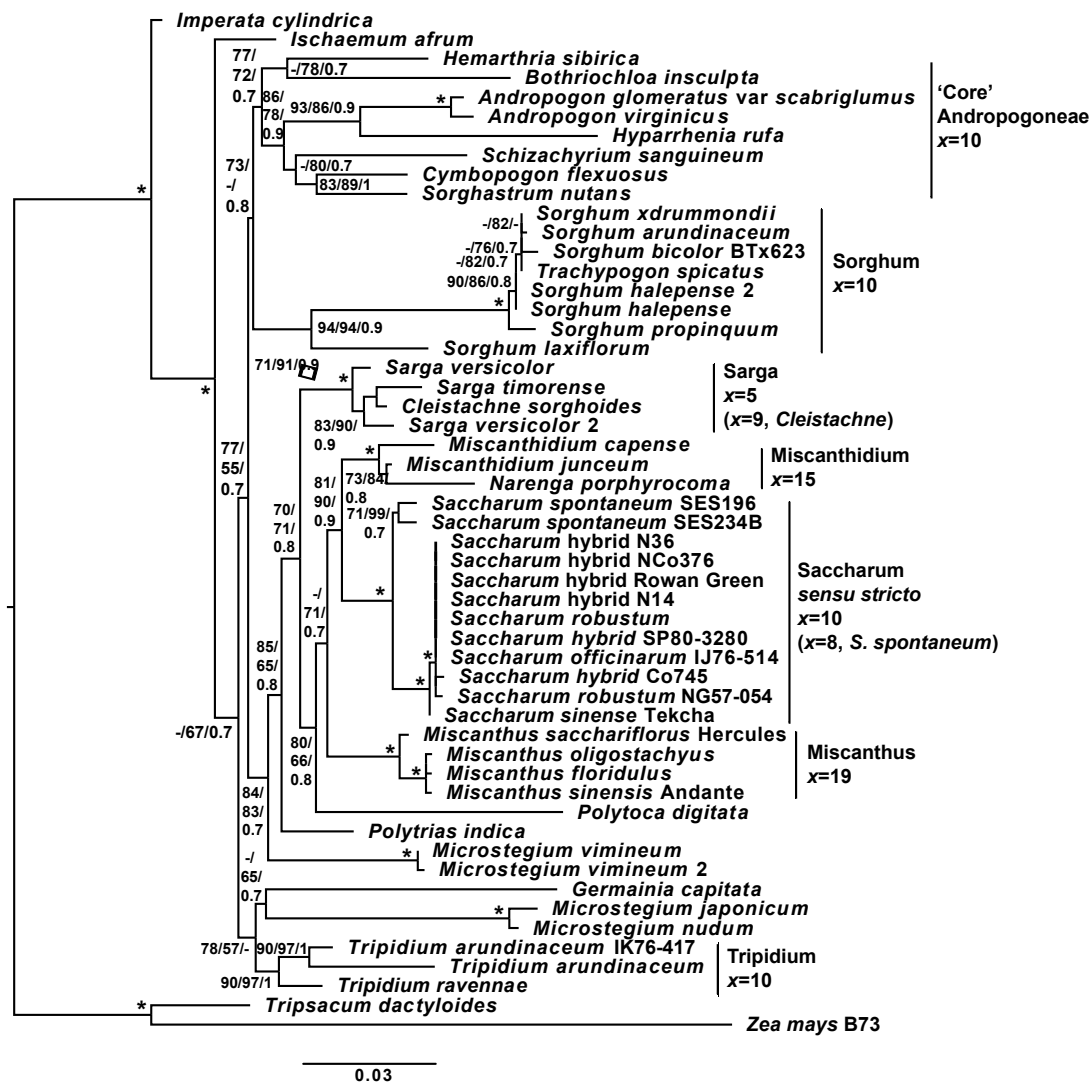


Figure 6.1. Phylogeny of sugarcane and related genera, based on the ITS cassette. A phylogeny of *Saccharum*, *Sorghum* and related genera based on the ITS (18s rRNA partial, ITS1 complete, 5.8s rRNA complete, ITS2 complete and 28s rRNA partial) genomic cassette. Tree terminals are the species name and cultivar or accession, where appropriate. Numbers at nodes represent SH-aLRT/non-parametric bootstrap/Bayesian inference support values. Bars to the right of the tree represent major clades, with associated base or monoploid (x) chromosome numbers. Branch lengths (scale on the bottom) correspond to the expected numbers of substitutions per sides. Monoploid chromosome numbers are derived from: *Sorghum* and *Sarga* (Gu *et al.* 1984); *Miscanthus* (Adati 1958); *Miscanthidium* (Strydom *et al.* 2000); *Saccharum spontaneum* (Ha *et al.* 1999); *Saccharum officinarum* (Li *et al.* 1959); *Tripidium* (Jagathesan and Devi 1969) and *Cleistachne* (Celarier 1958). The code “*” represents complete support for a node (100% SH-aLRT, 100% non-parametric bootstrap and Bayesian inference of 1), whilst “-” represents support that is below the threshold (65% for SH-aLRT, 50% for non-parametric bootstrap and 0.7 for Bayesian inference). Within *Saccharum sensu stricto*, between the sister relationship of *Saccharum robustum* NG57-054, *Saccharum hybrid* cv Co745 and *Saccharum officinarum* IJ76-514 with the remaining species there was insufficient sequence divergence within the ITS cassette to yield any

meaningful branch supports between the species. The Tripsacinae (*Tripsacum dactyoides* and *Zea mays*) were employed as an outgroup (Snyman *et al.* 2018). Reproduced with kind permission of D Lloyd Evans.

Table 6.1. Relatedness of sugarcane relatives with sugarcane. Sugarcane relatives were scored based on their divergent age in million years from sugarcane using a phylogeny (Figure 6.1) and chronogram (Figure 6.2). Sugarcane relatives were ranked from highest to lowest, with recent divergence scoring 11 and distant related species scoring 1.

Species	Divergent age from sugarcane in million years	Score
<i>Cleistachne sorghoides</i>	7.4	9
<i>Imperata cylindrica</i>	12	1
<i>Microstegium nudum</i>	11.4	3
<i>Miscanthidium capense</i>	3	11
<i>Miscanthidium junceum</i>	3.8	11
<i>Sarga versicolor</i>	7.4	9
<i>Sorghastrum nudipes</i>	11	3
<i>Sorghastrum stipoides</i>	11	3
<i>Sorghum arundinaceum</i>	10.4	6
<i>Sorghum ×drummondii</i>	10.4	6
<i>Sorghum halepense</i>	10.4	6

6.1.2. *Imperata cylindrica* (L.) Raeusch.

Figure 6.1 demonstrates that *Imperata cylindrica* is found near the root of the phylogenetic tree with 12 million years divergence from sugarcane, as shown by Figure 6.2 and Table 6.1. In this study, *Sorghastrum* (represented by *S. nutans*) was the most closely related grass species to *I. cylindrica* with 7.7 million years of divergence (Figure 6.2). *Imperata cylindrica* is the species closest to the Tripsacinae (treated as outgroup in this study) (Figure 6.1). The genus *Sarga* (including *Cleistachne sorghoides*), diverged from *I. cylindrica* 11 million years ago (Figure 6.2) and represents one of the most distant relatives of *I. cylindrica* from the studied species. Target species from *Sorghum* (*S. arundinaceum*, *S. ×drummondii*, *S. halepense* and *S. halepense* 2 along with *M. nudum* and *Cleistachne sorghoides* are distant relatives of *I. cylindrica* (7 and 11 million years, respectively) (Figure 6.2). The chronogram in Figure 6.2 further illustrates that *Miscanthidium* species and *Saccharum* hybrids are more than eight million years divergent from *I. cylindrica* and this corresponds with findings discussed under *Saccharum* hybrids in section 6.1.1, whereby *I. cylindrica* was reported as the most basal relative of *Saccharum* hybrids in this study.

The study of Welker *et al.* (2015) also showed *I. cylindrica* to be a distant relative of *Saccharum* and *Miscanthus* species. ITS data revealed *Sorghum arundinaceum* and *S. halepense* are more recently diverged from *I. cylindrica* than *Sorghastrum nutans*, whereas opposing results were obtained when a *trnL-F* sequence were used as described by Skendzic *et al.* (2007). Contradicting results, again, were presented by Hodkinson *et al.* (2002), who indicated that *I. cylindrica* was sister to the species *S. arundinaceum* and *S. ravennae* (now re-classified as *Tripsidium*). Although this study also used ITS, their smaller number of characters (266 as opposed to 950 in study) and incomplete taxon sampling may have biased their phylogeny (as demonstrated by relatively low branch support values).

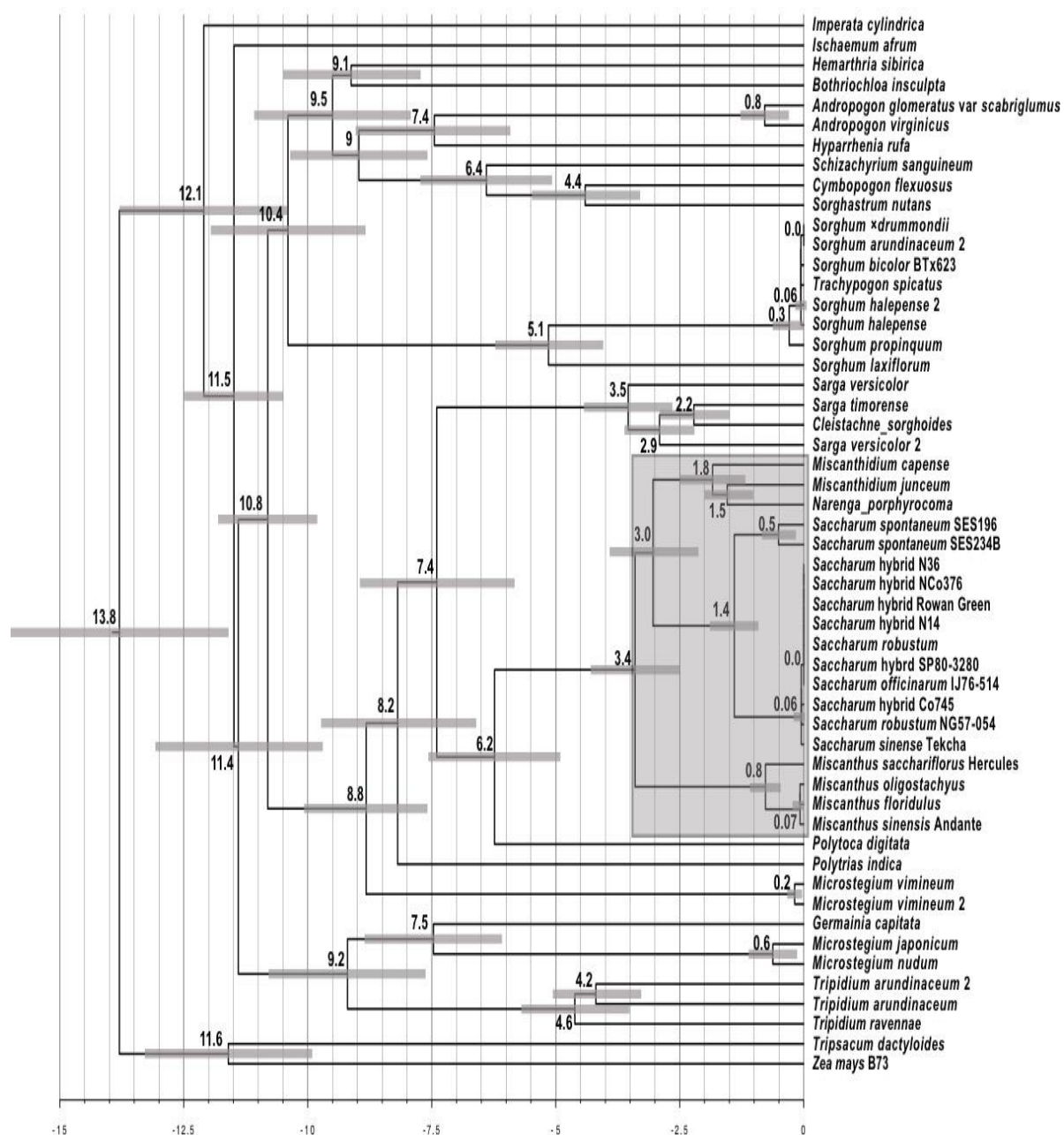


Figure 6.2. Chronogram derived from the alignment of Andropogoneae ITS cassette sequences. The chronogram was generated with r8s from the Maximum Likelihood ITS phylogeny from Figure 6.1. The scale at the bottom represents millions of years before present. Numbers at nodes represent the age of that node as millions of years before present. Scale bars at nodes represent the central 95% of the age distribution (i.e. 95% confidence interval) as determined by bootstrap resampling. The shaded region centred on *Saccharum* represents the 3.4 million year window in which wild hybridisations between *Saccharum* and other genera is possible (Lloyd Evans and Joshi 2016). Reproduced with kind permission of D Lloyd Evans.

6.1.3. *Microstegium nudum* (Trin.) A.Camus

Figure 6.1 demonstrates that *Microstegium* is not a monophyletic genus, with *Microstegium vimineum* an outgroup of the Saccharinae, and *M. vimineum* and *M. nudum* basal in the Germaininae clade. The Germaininae are directly ancestral to both the Saccharinae and Sorghinae + 'core' Andropogoneae clades. Thus, *M. nudum* is part of a clade that is directly ancestral to many of the species studied in this research namely: *Cleistachne sorghoides*, *Miscanthidium capense*, *M. junceum*, *Saccharum* hybrids, *Sarga versicolor*, *Sorghum arundinaceum*, *S. halepense* and *S. ×drummondii* (Figure 6.2). *Microstegium nudum* diverged from *Saccharum* hybrids 11.4 million years ago (Table 6.1). Lying within the core Andropogoneae, *Sorghastrum* spp. are more distantly related to *M. nudum* as compared with *Sorghum* (1 million years divergent) and *Sarga* (4 million years divergent) species (Figure 6.2). *Imperata cylindrica* is not regarded as a species closely related to *M. nudum* (Figure 6.1) with 11.5 million years of divergence (Figure 6.2).

Microstegium nudum was identified as sister to *Miscanthus japonicus* (a homonym for *Miscanthus floridulus*) and placed in the same clade as the *Sorghum* spp., whereby *Sorghastrum nutans* was placed more distantly from *M. nudum* in the review by Spangler *et al.* (1999), a finding that was confirmed in this study. Estep *et al.* (2014) and Welker *et al.* (2015) found *Microstegium vimineum* to be more closely related to *Sorghastrum nutans* than to *Miscanthus* and *Saccharum* spp., as they share recent ancestor, although they were placed in disparate branches of their phylogenies. Their findings differ from our results (but they had fewer *Microstegium* spp. and their study might have suffered from incomplete taxon sampling), although they placed *Microstegium* as a close relative to *Saccharum* hybrids, which compliments the current study. *Sorghum* spp. were grouped as more distal relatives to *Microstegium* spp. (Estep *et al.* 2014), which is supported by our findings.

6.1.4. *Miscanthidium capense* (Nees) Andersson and *M. junceum* (Stapf) Pilg.

Amongst all the species selected for this study, *Miscanthidium capense* and *M. junceum* are each other's closest relatives. These species were found in a distinct clade (*Miscanthidium*) as shown in Figure 6.1, and Figure 6.2 shows that they diverged less than 2 million years ago. All *Saccharum sensu stricto* species and cultivars sampled viz.: *Saccharum* hybrids: N14, N36, NCo376, NCo745, Rowan Green, SP80-3280 along with *S. officinarum* IJ76-514, *S. robustum*, *S. sinense* cv. Tekcha *S. spontaneum* cv. SES196 and *S. spontaneum* cv. SES234B are form a monophyletic clade that is sister to *Miscanthidium*. This places *Saccharum* species as the closest relatives of *Miscanthidium* species amongst all the species of interest in this study (Figure 6.1 and Table 6.1). *Sarga* species, *Sarga versicolor* and *Cleistachne sorghoides* were also revealed as close relatives of *Miscanthidium* species, being a basal clade that diverged from *Saccharum* within 3.9 million

years ago (Figures 6.1 and 6.2 and Table 6.1). Figure 6.1 clearly show that *Microstegium nudum* and *Sorghum* species are much more distantly related to *Miscanthidium* species in the phylogeny (last sharing common ancestors 8.8 and 10.8 million years ago, respectively). *Imperata cylindrica* and *Sorghastrum* species were found to be the most distant relatives of *Miscanthidium* species of all the species assessed in this study (Figure 6.1).

Miscanthus species (which are distinct from *Miscanthidium* in this study) are the next closest relatives to *Saccharum* hybrids (2.8 million years divergent), a finding that is consistent with the combined gene locus datasets presented in the work of Welker *et al.* (2015). The findings of Welker *et al.* (2015) placed *Sorghastrum* (represented by *S. elliotii* and *S. nutans*) and *Microstegium* (represented by *M. vimineum*) as a monophyletic and ancestral to *Saccharum* based on a Bayesian phylogeny using a combined gene locus data set (*apo1*, *d8*, *ep2-ex7*, *ep2-ex8*, and *rep1*), but with relatively poor branch support and insufficient sampling of *Microstegium* species. Other species analysed by Welker *et al.* (2015) that were of interest in the current study were *Sorghum* (represented by *S. bicolor*) and *Imperata* (represented by *I. cylindrica*), whereby the latter was also revealed as a distant relative to *Saccharum* species (being grouped in a more distant clade, similar to our results). Hodkinson *et al.* (2002) analysed Saccharinae species using both ITS and *trnL-F* sequences, and they also demonstrated that *Miscanthidium* species were the closest relatives of *Saccharum* hybrids among *Saccharum* complex species in both analyses. In another analysis, *Miscanthus* species were the only wild relatives close to the main *Saccharum* clade. *Sorghum* species were however distal from both *Miscanthus* and *Saccharum* hybrids (Cordeiro *et al.* 2003). *Miscanthus ecklonii* (syn. *Miscanthus capensis*, *Miscanthidium capense*) was also found as the immediate sister of *Saccharum* species (Estep *et al.* 2014).

6.1.5. *Saccharum* hybrids

Miscanthidium capense and *M. junceum* are the most closely related species to *Saccharum* hybrids based on the ITS cassette phylogeny as indicated in figure 6.1. Figure 6.2 indicates that *Miscanthidium* species diverged from *Saccharum* less than 3.4 million years ago, which is within the wild hybridisation window of the Saccharinae. Phylogenetic trees group closely related species together, and many studies have been performed for *Saccharum* species and their wild relatives (Dillon *et al.* 2001; Virtudazo *et al.* 2001; Cordeiro *et al.* 2003; Hawkins *et al.* 2015; Soreng *et al.* 2015; Arthan *et al.* 2017; Soreng *et al.* 2017; Saarela *et al.* 2018). Early studies suggested that Saccharinae and Sorghinae species are typically clustered closer to each other than to other Andropogoneae subtribes (Hodkinson *et al.* 2002). However, recent molecular data (Estep *et al.* 2014; Welker *et al.* 2015) and ITS based phylogeny (Figure 6.1) strongly indicate that these subtribes need to be re-circumscribed as follows: Saccharinae: *Erianthus* (American species only), *Miscanthidium*, *Narenga*, *Pseudosorghum*, *Miscanthus*, *Saccharum* and *Sarga* and that the Sorghinae be limited to *Sorghum sensu stricto*. However, for this study the selection of the target

Saccharum wild relatives were classified into two subtribes as follows based on older literature: Saccharinae (*Imperata cylindrica*, *Microstegium nudum*, *Miscanthidium capense* and *M. junceum*) and Sorghinae (*Cleistachne sorghoides*, *Sarga versicolor*, *Sorghastrum nudipes*, *S. stipoides*, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* (Skendzic et al. 2007; Fish et al. 2015; Lloyd Evans and Joshi 2016; Soreng et al. 2017). This classification of the target species for this research will therefore be used for discussions in this study.

As expected, the target Saccharinae wild relatives (*M. capense* and *M. junceum*) were found to be most closely related to *Saccharum* hybrids. *Imperata cylindrica* and *Microstegium nudum* (previously included in the Saccharinae subtribe) are species that were found to be less closely related to sugarcane than the following former Sorghinae species: *Cleistachne sorghoides* and *Sarga versicolor* (as shown in Figure 6.1). Wild species that are closely related to cultivated crops are of interest due to their gene flow (introgression) potential from transgenic cultivars. Overall, *Sorghum* species did not score as being closely related to *Saccharum* species (they are closer to the 'core' Andropogoneae) as they were ranked 6 (Table 6.1), except for *S. versicolor* (now reclassified as a *Sarga* species) which was previously classified amongst the closest relatives to sugarcane (Figures 6.1 and 6.2 and Table 6.1). *Imperata cylindrica*, *Sorghastrum* spp. and *Sorghum* spp. do not share a recent common ancestor with *Saccharum* hybrids.

Sorghastrum (represented by *S. nutans*) is the only genus that falls within the core Andropogoneae amongst the genera that has been selected for this study. From eleven target grass species that were chosen for *Saccharum* risk assessment in the eastern South African context, *I. cylindrica* was found to be the most distantly related species to *Saccharum* hybrids (Figure 6.1 and Table 6.1).

The co-occurrence of *Saccharum* hybrids and all related grass species within the sugarcane production areas of this study was confirmed (Table 6.2). Sugarcane's closest relative (*Miscanthidium capense*) also ranked amongst the species that overlapped significantly with sugarcane in its cultivation areas (Table 6.3). *Miscanthidium* (syn. *Miscanthus*) species are known to rank amongst the closest wild relatives of *Saccharum*, and these two genera are often presented as recently divergent from a common ancestor in numerous phylogenetic studies (Hodkinson et al. 2002; Skendzic et al. 2007; Estep et al. 2014; Kim et al. 2014; Hawkins et al. 2015; Welker et al. 2015; Lloyd Evans and Joshi 2016). This close relationship was also confirmed by the current study.

All *Saccharum* species were grouped as a monophyletic clade, with *Narenga* and *Miscanthidium* species as outgroups (i.e. representing their most immediate wild relatives) (Figure 6.1). However, *Narenga* was not amongst the target species for this study since there was no wild *Narenga* species growing naturally in the study area. These findings therefore suggest that *Miscanthidium* species (*M. capense* and *M. junceum*) is the most closely related species to *Saccharum* hybrids which should be thoroughly investigated for this assessment.

6.1.6. *Sarga versicolor* (Andersson) Spangler

All *Sorghum* species selected for this study were grouped in a sister clade to the *Sarga* clade (Figure 6.1) with the chronogram (Figure 6.2) showing that they are 3.5 million years divergent. However, the analyses presented here place *Cleistachne sorghoides* closer to *Sarga versicolor* (syn. *Sorghum versicolor*) than *Sorghum sensu stricto* spp. *Miscanthidium* species (*M. capense* and *M. junceum*) and *Saccharum* hybrids are close relatives to *Sarga versicolor* with less than 7.4 million years of divergence between them (Figure 6.2 and Table 6.1). Indeed, these species grouped in the same clade (Figure 6.1). *Microstegium nudum* and *Sorghastrum* species are more distal clades to *S. versicolor* and they are therefore less evolutionarily related to *S. versicolor*. *Imperata cylindrica* is the most distally related species amongst target species, being the out group to the phylogeny (Figure 6.1).

A study conducted by Hodkinson *et al.* (2002) included *S. versicolor* with the following target species: *I. cylindrica*, *Miscanthus ecklonii* (syn. *Miscanthidium capense*), *Miscanthus junceus* (syn. *Miscanthidium junceum*), *Saccharum* hybrids and *Sorghum halepense*. However, though using ITS sequences, Hodkinson *et al.* (2002) employed a low number of characters (266) and as a result, many of their species collapsed to a polytomy at the root of the tree. Dillon *et al.* (2001) found *C. sorghoides* closer to *S. versicolor* than *S. arundinaceum*, *S. bicolor*, *S. ×drummondii* and *S. halepense* on the basis of ITS sequence, with *Saccharum officinarum* being placed further due to numerous *Sorghum* species sampled. When *Adh1*, ITS1 and *ndhF* sequences were combined by Dillon *et al.* (2001), different findings from the above-mentioned study were obtained because *C. sorghoides* was not found to be the closest relative of *S. versicolor*. Other target *Sorghum* species for the current study was closer to *S. versicolor*. The combining of genomic and chloroplast sequence data, which present different evolutionary signals, undoubtedly skewed the results presented by Dillon *et al.* (2001). *Sorghastrum* is therefore the only genus from the current definition of the Sorghinae that is the most distant relatives to *S. versicolor* (Figure 6.1). Indeed, our analyses suggest that the Sorghinae as a subtribe needs to be re-circumscribed to only include *Sorghum sensu stricto* with the exclusion of *Sorghastrum* and *Sarga*.

6.1.7. *Sorghastrum nudipes* Nash and *S. stipoides* (Kunth) Nash

Sorghum species (*S. arundinaceum*, *S. halepense* and *S. ×drummondii*) are the closest relatives of *Sorghastrum* (*S. nudipes* and *S. stipoides*) within the target species assessed in this study as shown by Figure 6.1. *Sorghastrum nudipes* and *S. stipoides* are represented by *Sorghastrum nutans* in Figure 6.1. *Cleistachne sorghoides*, *Miscanthidium capense*, *M. junceum*, *Saccharum* hybrids and *Sarga versicolor* cannot be considered close sister groupings to *Sorghum*, since they are almost 11 million years divergent (Figure 6.2 and Table 6.1). All other species of interests are not close relatives of *Sorghastrum* species.

Welker *et al.* (2015) used a combined nuclear gene locus data set (*apo1*, *d8*, *ep2-ex7*, *ep2-ex8*, and *rep1*) and reported different results to our findings, whereby *Microstegium vimineum* was placed as sister to *Sorghastrum nutans*, whilst *Miscanthus* species and *Saccharum* species are closer relatives of *Sorghastrum nutans*. This need not be a contradiction however, as ITS regions may be presenting a different evolutionary signal to nuclear genes. In addition, their grouping of *Microstegium* + *Sorghastrum* had relatively poor branch support and possibly under sampling of *Microstegium* spp. Indeed, our study shows that *Microstegium* is not monophyletic and *Microstegium vimineum* represents a separate and novel genus to other *Microstegium* species.

Moreover, Spangler *et al.* (1999) reported similar findings to our study where *Sorghastrum nutans* was grouped within the core Andropogoneae which lies in a group where there are no other target species for the current study. In their study *Miscanthus japonicus* (syn. *Miscanthus floridulus*), the only species outside *Saccharum sensu stricto* proven to have hybridized with *Saccharum officinarum* in the wild. *Microstegium nudum* and *Cleistachne sorghoides* were placed closer to *Sorghastrum nutans* than *Sorghum arundinaceum* and *S. halepense* with *ndhF* (Spangler *et al.* 1999), which is in contradiction to our findings (but we know that chloroplast data can be anomalous). *Imperata*, *Saccharum* and *Sarga* were not investigated by Spangler *et al.* (1999) and this study found *Sorghastrum nutans* to be closer to Saccharinae grasses than its related Sorghinae species. *Sorghastrum* species are not close relatives of *Saccharum* species (Skendzic *et al.* 2007).

6.1.8. *Sorghum arundinaceum* (Desv.) Stapf and *S. ×drummondii* (Nees ex Steud.) Millsp. & Chase

Sorghum arundinaceum and *Sorghum ×drummondii* are each other's closest wild relatives amongst the target species for the current study and they are both placed as sisters in the phylogenetic tree (Figure 6.1). *Sorghum halepense* was the second closest relative to these previously mentioned species from our target species with less than half a million years divergence from *S. arundinaceum* and *S. ×drummondii* (Figure 6.2). Target members of the *Sarga* clade (*Cleistachne sorghoides* and *Sarga versicolor*), though evolutionarily distant still share an immediate ancestor with *Sorghum* as they represent a direct sister clade of *Sorghum sensu stricto* clade which consists of *S. arundinaceum* and *S. ×drummondii* (Figure 6.1). However, the chronogram (Figure 6.2) places these two groups as evolutionarily distinct, having diverged less than 3.9 million years ago. *Imperata cylindrica*, *Microstegium nudum*, *Miscanthidium* spp, *Saccharum* hybrids and *Sorghastrum* spp. are not close relatives of *Sorghum* spp. (*S. arundinaceum* and *S. ×drummondii*) (Figure 6.1). *Sorghum arundinaceum* diverged from sugarcane within 10.4 million years ago (Table 6.1). *Cleistachne sorghoides* and *Sarga versicolor* were considered close relatives of *Sorghum* (Dillon *et al.* 2001; 2007; Ng'uni *et al.* 2010). Both *Microstegium nudum* and *Miscanthus japonicus* were put as sisters to *Sorghum* species whereas

Sorghastrum nutans were furthest from *Sorghum* spp. (Spangler *et al.* 1999), which is in accordance with our findings. *Sorghastrum* spp. were grouped as closer relatives of *Sorghum* spp. by Skendzic *et al.* (2007), whereas *Imperata* and *Miscanthus* taxa were amongst more distant relatives. Findings reported by Skendzic *et al.* (2007) on *Imperata* and *Miscanthus* are well supported by the current assessment, except for *Sorghastrum* spp. However, genomic studies demonstrated that *Saccharum* spp. and *Sorghum* should not be considered as close relatives (Skendzic *et al.* 2007; Estep *et al.* 2014; Welker *et al.* 2015).

6.1.9. *Sorghum halepense* (L.) Pers.

Both *Sorghum arundinaceum* and *S. ×drummondii* are the closest relatives of *Sorghum halepense* within target species for this study (Figure 6.1). Other species studied in this project are similarly related to *S. halepense* (as discussed under *Sorghum arundinaceum* and *S. ×drummondii* in subsection 6.1.7) since these *Sorghum* spp. were in the same clade with little divergence, indicating recent radiation from a common ancestor (Figures 6.1 and 6.2). Similarly to *Sorghum arundinaceum*, *S. halepense* also diverged from sugarcane within 10.4 million years ago as shown in Table 6.1. Skendzic *et al.* (2007) reported *Sorghum arundinaceum* and *S. bicolor* as sisters of *S. halepense* on the basis of ITS data. Ng'uni *et al.* (2010) used similar analyses and reported similar results for the placement of *S. ×drummondii*, as supported by the outcomes from the current study. Reports by Dillon *et al.* (2001) revealed a similar pattern of relatedness between *S. halepense*, *S. arundinaceum* and *S. ×drummondii*. However, their analyses placed *Sorghum* as more closely related to *Saccharum officinarum*. However, in genomic studies (including our analyses) it was unexpected to find it to be closer to *Sorghum* spp., except for *S. versicolor* (now re-defined as being within a separate genus, *Sarga*). *Sorghum halepense* is considered a closer relative of *Cleistachne sorghoides*. The study by Dillon *et al.* (2001), where there were more *Sorghum* spp. included, placed *C. sorghoides* in a more distal branch. Other studies placed *Sarga versicolor* as a close relative of *Sorghum halepense* (Dillon *et al.* 2001; 2007), however, this was not supported by the current study as they are 10.8 million years divergent. Based on the ITS tree, this places *Imperata cylindrica* and *Sorghastrum* species as more closely related to *S. halepense*, than what *S. halepense* is to *Miscanthus* and *Saccharum* spp. as also reported by (Skendzic *et al.* 2007).

6.2 Prevalence of *Saccharum* wild relatives in *Saccharum* cultivation areas

A total of 815 herbarium specimens of 11 *Saccharum* wild relative species were sourced from 11 herbaria. These records were supplemented by 34 observations of *Saccharum* wild relatives during field visits to sugarcane cultivation areas in South Africa. All 11 wild relatives of the Andropogoneae have been recorded from sugarcane cultivation areas. Six species occurred

throughout the sugar cultivation region, but *Miscanthidium capense* (previously *Miscanthus capensis*), *Sorghum ×drummondii* and *Sorghastrum stipoides* were restricted to the southern parts, and *Cleistachne sorghoides* and *Sorghastrum nudipes* to the northern parts of the cultivation area. *Imperata cylindrica*, *Sorghum arundinaceum* and *Miscanthidium capense* showed the highest prevalence within sugarcane cultivation areas (Table 6.2). Three species from the Sorghinae, namely *Cleistachne sorghoides*, *Sorghastrum nudipes* and *Sorghum ×drummondii* showed low prevalence within sugarcane quarter-degree squares (QDS) (Table 6.2). Agro-ecosystems have been found to have more biodiversity including grasses, when compared to non-agricultural areas (Oberhauser *et al.* 2001; Botha *et al.* 2017). The presence of crop wild relatives in cultivated areas has been an interesting topic for studying their potential gene flow from their related crops, since prevalence is amongst the factors considered in the assessment of hybridisation potential between transgenic crops and their non-transgenic relatives (Ellstrand *et al.* 1999; FitzJohn *et al.* 2007; Barnaud *et al.* 2008; Morales and Traveset 2008; Tesso *et al.* 2008; Rabbi *et al.* 2011; Nieh *et al.* 2014).

Sourced data from herbarium records was used as a link to study and collect target species in their growing regions (Jaca and Mkhize 2018). In addition to this, observations and recording of presence of relevant plant species in cultivated fields enhances our understanding of their distribution in such areas. This information was also needed for generating assessment maps for cultivated regions (Rew and Cousens 2001; McGeoch *et al.* 2009; Jaca and Mkhize 2018), as used in this current study. The data generated for prevalence (Table 6.2) and other factors assessed in this study will therefore improve our understanding of the likelihood of gene flow from *Saccharum* hybrids to their wild relatives in South Africa, and will inform future risk assessments of GM sugarcane. Prevalence of sugarcane wild relatives was confirmed in sugar productions regions of the study area. Consequently, prevalence (presence) of wild relatives in the areas where transgenic sugarcane is cultivated does not necessarily give assurance that there will be gene flow because other factors should be taken into account for introgression to occur (Légère 2005; Bonnett *et al.* 2008).

Table 6.2. Prevalence or commonness of individuals (based on herbarium specimens) of *Saccharum* wild relatives in sugarcane cultivation areas. Calculation of scores was based on ranking the commonness of species from highest to lowest, with most common species scoring 11 and least common receiving a score of 1.

Species	No. of individuals within sugarcane QDS	Proportion of individuals within sugarcane QDS	No. of individuals bordering sugarcane QDS	Proportion of individuals bordering sugarcane QDS	Total proportion of sugarcane QDS + bordering sugarcane QDS	Score
<i>Cleistachne sorghoides</i>	2	1	3	2	3	3
<i>Imperata cylindrica</i>	99	33	31	25	58	11
<i>Microstegium nudum</i>	10	3	14	11	14	5
<i>Miscanthidium capense</i>	35	12	14	11	23	9
<i>Miscanthidium junceum</i>	15	5	13	11	16	7
<i>Sorghastrum nudipes</i>	1	0.3	1	1	1.3	1
<i>Sorghastrum stipoides</i>	26	9	8	7	16	7
<i>Sorghum arundinaceum</i>	85	28	14	11	39	10
<i>Sorghum ×drummondii</i>	3	1	1	1	2	2
<i>Sorghum halepense</i>	16	5	4	3	8	4
<i>Sarga versicolor</i>	10	3	20	16	19	8

6.3 Spatial overlap of *Saccharum* wild relatives with *Saccharum* cultivation areas

Sugarcane related grass species with highest spatial overlap percentages in sugar productions areas were *Imperata cylindrica*, *Sorghum arundinaceum* and *Miscanthidium capense* (Table 6.3). Figure 6.3 indicates the presence of *Miscanthidium capense* weedy populations encountered during field visits in sugarcane fields in New Hanover, KwaZulu-Natal, South Africa. Three species

from the Sorghinae, namely *Cleistachne sorghoides*, *Sorghastrum nudipes* and *Sorghum ×drummondii* showed low prevalence within sugarcane QDS (Table 6.2). The highest spatial overlap of wild relatives with sugarcane grids revealed a similar outcome to the prevalence rankings (see Sub-heading 6.2). In both cases (i.e. prevalence and spatial overlap), the highest and lowest score values differed substantially. *Imperata cylindrica* showed the highest likelihood for spatial congruence with sugarcane and *Sorghastrum nudipes* the least.

Scoring systems have been used for assessing potential risks associated with invasive species that are not native to South African ecosystems to detect environmental threats that can be posed by these organisms, for example adverse effects on biodiversity and agricultural productivity (Blackburn *et al.* 2011; Keller and Kumschick 2017; Visser *et al.* 2017). The latest review of invasive grasses of South Africa (Visser *et al.* 2017) reported *Sorghum ×drummondii* and *S. halepense* amongst 256 weedy grasses that were introduced to agricultural systems. Weedy relatives may be considered as high risk for gene flow when they are geographically associated with GM crops (Bonnett *et al.* 2008; OECD 2013). In general, most problematic weeds of sugarcane are in the Andropogoneae (Cheavegatti-Gianotto *et al.* 2011; OECD 2013).

Imperata cylindrica and members of *Sorghum* have been documented as aggressive weeds in agricultural systems including sugarcane fields in many countries (Van Oudtshoorn 1999; Firehun and Tamado 2006; Bonnett *et al.* 2008; OECD 2013; Takim *et al.* 2014), as well as being noxious weeds in other habitats (Skendzic *et al.* 2007). *Sorghum arundinaceum* and *S. ×drummondii* are considered weeds of sugarcane in South Africa (Van Oudtshoorn 1999). Studies from Nigeria reported *Imperata cylindrica* amongst problem weeds of sugarcane (Takim *et al.* 2014), and both *Sorghum arundinaceum* and *S. ×drummondii* are regarded as major weeds of sugarcane in Ethiopia (Firehun and Tamado 2006). In the South African context, as assessed in this study, habitats of the weeds are usually common in sugarcane cultivation areas and field margins (Van Oudtshoorn 1999; Milton 2004; Fish *et al.* 2015).



Figure 6.3. *Miscanthidium capense* growing within sugarcane fields in New Hanover, KwaZulu-Natal Province, South Africa [Photo: DM Komape].

Table 6.3. Spatial overlap (shared occurrence) of *Saccharum* wild relatives (based on herbarium specimens) with sugarcane cultivation areas (113 QDS). Calculation of scores was based on ranking species occurrences from highest to lowest, with highest ranked species that scored 11 and lowest scoring 1.

Species	Sugarcane QDS	Overlapping %	Score
<i>Cleistachne sorghoides</i>	2	2	2
<i>Imperata cylindrica</i>	38	34	11
<i>Microstegium nudum</i>	5	4	4
<i>Miscanthidium capense</i>	19	17	9
<i>Miscanthidium junceum</i>	10	9	5
<i>Sarga versicolor</i>	15	13	7
<i>Sorghastrum nudipes</i>	1	1	1
<i>Sorghastrum stipoides</i>	14	12	6
<i>Sorghum arundinaceum</i>	35	31	10
<i>Sorghum ×drummondii</i>	3	3	3
<i>Sorghum halepense</i>	16	14	8

6.4 Proximity of *Saccharum* wild relatives to *Saccharum* hybrids in cultivation areas

Cleistachne sorghoides, *Microstegium nudum*, *Sarga versicolor*, *Sorghastrum nudipes* and *S. stipoides* are 5 of the 11 target grass species that were not found within 700 m of sugarcane fields during the spatial assessment done in this study (Table 6.4). The above-mentioned species are

therefore not perceived as habitual weeds of South African sugarcane fields besides their prevalence and spatial overlap with some sugarcane QDS. Species of importance in assessments done in this study are those that occur in sugarcane fields, namely *Imperata cylindrica*, *Miscanthidium capense*, *M. junceum*, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* (Table 6.4). *Sorghum arundinaceum* scored the highest for proximity (Table 6.4) and was also depicted in Figure 6.4 to grow in close proximity to sugarcane in the study area. *Imperata cylindrica* recorded the second highest score as shown in Table 6.4. In general, members of *Sorghum* scored higher for proximity to sugarcane fields, except for *S. versicolor* (syn. *Sarga versicolor*) (Table 6.4). This is ascribed to preferences for habitat associated with sugarcane fields. *Miscanthidium* species were moderately associated with sugarcane fields (Table 6.4). Both *I. cylindrica* and *M. capense* were found to be weeds in sugarcane fields during field surveys although these species were not documented in South African literature as such. In our study, *Imperata cylindrica*, *Miscanthidium capense*, *M. junceum*, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* were found in relatively close proximity to sugarcane fields (Table 6.4). The information of distribution patterns of reproductively compatible species that are related to the crop species in their growing regions is essential for assessing their potential gene flow (Warwick *et al.* 1999).

Gene transfer from crop species is highest to their wild relatives that grow in close proximity and introgression can occur when other risk factors for gene flow are crossed (Schmidt and Bothma 2006; FitzJohn *et al.* 2007; McGeoch *et al.* 2009; Bonnett *et al.* 2008; Nieh *et al.* 2014). *Sorghum* and *Imperata* are two genera with highest scores for proximity to *Saccharum* hybrids in sugar production regions (Table 6.4) and species from these genera has previously been reported as problem grasses in agricultural systems (Schmidt and Bothma 2006; OECD 2013). Tesso *et al.* (2008) reported gene flow from cultivated *Sorghum* to their wild and weedy relatives where these species were found growing in close proximity on farms in Ethiopia and Niger. Barnaud *et al.* (2008) also reported similar results to the study by Tesso *et al.* (2008).

Warwick *et al.* (1999) stated that the formation of "Super-weeds" can be as a result from such introgression which will be harmful to the natural environment. *Imperata cylindrica* is known to be a grass species with invasive potential in various biomes across the world (King and Grace 2000; Ling *et al.* 2009). This grass species is difficult to control in agricultural areas and its presence and competition with crop species results in economic losses through decreased crop yield (Avav 2000; Olabode and Sangodele 2015), for example in sugarcane fields in Nigeria (Takim *et al.* 2014). These examples of both *Sorghum* and *Imperata*, which were also recorded in close proximity to sugarcane in the current study, are therefore known to be problem plants in agro-ecosystems.



Figure 6.4. *Sorghum arundinaceum* growing in close proximity to *Saccharum* hybrids within a sugarcane field in Greytown, KwaZulu-Natal Province, South Africa [Photo: DM Komape].

Table 6.4. Proximity or closeness of *Saccharum* wild relatives (based on herbarium specimens, field observations and literature) to sugarcane fields in the study area. Calculation of scores was based on ranking species proximity to fields from highest to lowest, with highest ranked species that scored 11. A score of 0 was given when no records could be found and therefore proximity data is not currently known (absence equates to no ranking).

Species	Recorded from field	Literature	fm + li	Score
	and margins (fm)	confirmations (li)		
<i>Cleistachne sorghoides</i>	-	-	Absent	0
<i>Imperata cylindrica</i>	7	1	8	10
<i>Microstegium nudum</i>	-	-	Absent	0
<i>Miscanthidium capense</i>	3	-	3	7
<i>Miscanthidium junceum</i>	1	-	1	6
<i>Sorghastrum nudipes</i>	-	-	Absent	0
<i>Sorghastrum stipoides</i>	-	-	Absent	0
<i>Sorghum arundinaceum</i>	25	2	27	11
<i>Sorghum ×drummondii</i>	3	1	4	9
<i>Sorghum halepense</i>	3	1	4	9
<i>Sarga versicolor</i>	-	-	Absent	0

6.5 Potential hybridisation of *Saccharum* hybrids with their wild relatives

A literature review of hybridisation events between cultivated sugarcane and its relatives reported 39 hybridisation incidents in 23 different studies dating from 1935 to 2014 (Bourne 1935; Gao *et al.* 2014). From these, there were only three claims of spontaneous hybridisation (Parthasarathy 1948; Ellstrand *et al.* 1999), with the remaining crosses requiring human intervention in artificially controlled conditions using experimental procedures that maximised flowering, pollination and seedling survival. Crosses were performed to integrate the beneficial traits of one species to another to enhance agronomic traits such as growth, ratoonability and biomass accumulation (Brett 1950; Piperidis *et al.* 2000; Aitken *et al.* 2007; Gao *et al.* 2014). The genus previously known as *Erianthus* (now divided into *Tripidium* and *Saccharum*) was utilised in 18 of the artificial man-made crosses, predominantly with *Saccharum arundinaceum* (syn. *Erianthus arundinaceus*, *Tripidium arundinaceum*). Similarly, the number of crosses made with cultivated sugarcane was mainly with the *Saccharum* genus (10 crosses) and with *Saccharum arundinaceum* (4 crosses).

Other genera which have been crossed with sugarcane include *Bambusa*, *Imperata*, *Miscanthidium*, *Sorghum* and *Zea*. Of the 18 species that have been involved in hybridisation with sugarcane, seven occur in South Africa and comprise 30.77% of the total hybridisation events. The highest number of seedling survival in cultivation resulting from *Saccharum* hybrids x *Sorghum bicolor* (L.) Moench, was 9.7% (starting with 14 141 seedlings produced by crosses) (Hodnett *et al.* 2010). The lowest seedling survival was from a cross involving *Zea mays*, where only one from more than 1 000 seedlings survived (Bonnett *et al.* 2008). One of the reported crosses involving *Sorghum bicolor* failed with no true seedlings obtained (Bourne 1935). With the exclusion of the former attempt, 48.72% of these studies used molecular markers to verify the presence of the maternal and paternal alleles from putative hybrids, whereas the remaining crosses (51.38%) relied on visual inspection of inherited morphological characteristics against those of parent lines as well as chromosome counts (Khanyi 2018).

Imperata cylindrica, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* were the only species that were found to be reproductively compatible with *Saccharum* species based on literature (Table 6.5). *Miscanthidium capense* and *M. junceum* were not part of any species-specific hybridisation studies, but were scored as compatible reproductive species based on the literature reporting on other species of the genus hybridising with *Saccharum* species (Table 6.5). Six successful *Miscanthidium* hybridisation cases have been documented in the literature (17 publications).

Hybridisation potential between *Miscanthidium* and *Saccharum* ranked highest. *Imperata cylindrica* was reported in five publications with one success and *Sorghum halepense* was recorded in two publications with one success (Table 6.5). There were considerably more publications on other *Sorghum* species hybridising with *Saccharum* species, which was not included in the analyses since they were not *Sorghum* spp. that were assessed for this study. There were relatively higher numbers of published papers on intra generic hybridisation of *Saccharum* species, compared to the Sorghinae grass species that were targeted for this assessment. In general, there were insufficient literature reports for South African *Saccharum* relative species considering that species with highest number of reports (*Miscanthidium* species) were assessed at genus level and the species with the second highest number of reports (*Imperata cylindrica*) were from a single experimental study.

Pollen gene flow from transgenic crops to their wild relatives, GM crops becoming invasive weeds, insect resistance, herbicide resistance, disease resistance, drought-tolerance traits, creation of super-weeds escaping from agricultural activities to natural areas, and gene flow effects from GM to non-target species are ecological uncertainties that have been associated with growing GM crops (Altieri 1999; Altieri 2000; Barton and Dracup 2000; Crawley *et al.* 2001; Senior and Dale 2002; Ellstrand 2003, Légère 2005; Andow and Zwahlen 2006; Peterson *et al.* 2006; FitzJohn *et al.* 2007; Barnauid *et al.* 2008; Warwick *et al.* 2009). It is therefore essential to assess the potential hybridisation between crop species and their closely related wild species for potential gene flow when assessing GM studies (Ellstrand *et al.* 1999; FitzJohn *et al.* 2007; McGeoch *et al.* 2009) including sugarcane relative species (Janaki-Ammal 1942; Chapman and Burke 2006; Schmidt and Bothma 2006; Bonnett *et al.* 2008; OECD 2013; Chae *et al.* 2014; Welker *et al.* 2015; Lloyd and Joshi 2016). In a South African study, gene flow has been reported from cultivated sorghum to a wild relative (Schmidt and Bothma 2006). *Saccharum* hybrids have not been reported to spontaneously hybridise with their related wild grasses (Bonnett *et al.* 2008; Cheavegatti-Gianotto *et al.* 2011). Natural hybridisation in sugarcane have not been reported before besides it being listed by Ellstrand *et al.* (1999) amongst the world's important crop species which could hybridise with their wild relatives.

Table 6.5. Summary of hybridisation reports between *Saccharum* hybrids and wild relatives from the literature for genera present in sugarcane cultivation areas in South Africa. Rankings were based on the number of successful hybridisation events, with the highest ranking scoring 11. A score of 0 was given when no instances of hybridisation were reported in the literature and therefore no gene flow risk is currently known (no evidence equates to no ranking). *Miscanthidium* was treated at species level as hybridisation was not conducted with species found in South Africa.

Species	No. publications reporting hybridisation	No. reports of successful hybridisation	Success %	Score
<i>Cleistachne sorghoides</i>	-	-	0	0
<i>Imperata cylindrica</i>	5	1	20	7
<i>Microstegium nudum</i>	-	-	0	0
<i>Miscanthidium</i> spp.	9	3	33	8
<i>Sarga versicolor</i>	-	-	0	0
<i>Sorghastrum nudipes</i>	-	-	0	0
<i>Sorghastrum stipoides</i>	-	-	0	0
<i>Sorghum arundinaceum</i>	1	1	100	11
<i>Sorghum ×drummondii</i>	1	1	100	11
<i>Sorghum halepense</i>	2	1	50	9

6.6 Dispersal potential of *Saccharum* wild relatives across the study area

Imperata cylindrica, *Miscanthidium junceum* and *Sorghum arundinaceum* were ranked highest in terms of having road and railway networks associated with their QDS of occurrence (Table 6.6). These networks present a higher likelihood for these species to spread into and within sugarcane cultivation areas compared with species that have fewer dispersal networks. Species that are in isolated QDS and that are normally restricted to certain locations will also lack these dispersal networks. Dispersal networks of sugarcane relatives overlap spatially with those of sugarcane areas and their analyses per species revealed that they are all widely distributed across grids with commercial sugarcane, including weedy relatives such as *Imperata cylindrica*, *Sorghum arundinaceum* and *S. halepense*.

Consequently, competitive plants have better chances to adapt well in various environments, which makes them prone to occur even at undesignated areas in future through their survival mechanisms, especially with more dispersal channels which adds to their advantages (Rew and

Cousens 2001; Meier *et al.* 2011; Chauhan *et al.* 2012). Vehicles were reported to be amongst the main factors associated with the spread of weedy grasses in South Africa (Milton 2004). Railways are additional sources of transport to roads, whereby railways has been mentioned as the main source of transport of sugarcane to South African mills (Giles 2009). The transport network therefore provides an indication of the potential for weedy relatives of sugarcane to spread, with denser networks implying higher chances for migrations.

Furthermore, sugarcane relatives are often associated with roadsides as a preferred habitat (Retief and Herman 1997; Van Oudtshoorn 1999; Fish *et al.* 2015). Potential dispersal networks of related species in our study show that most would be able to spread from the areas in which they are found. For example, *Miscanthidium capense* is associated with vast road and rail networks (Table 6.6), which suggests that anthropogenic activities may enhance seed dispersal and potentially increase gene flow potential (Andow and Zwahlen 2006).

Table 6.6. Dispersal potential of *Saccharum* wild relatives (based on road and railway networks) in sugarcane cultivation areas. Calculation of scores was based on ranking species from highest to lowest using the number of roads and railways present in the grids of wild relatives, and scoring the largest network as 11 and the smallest as 1.

Sugarcane wild relative	QDS with railway line (rl ₁)	QDS with railway line bordering (rl ₂)	QDS with roads (rd ₁)	QDS with roads bordering (rd ₂)	rl ₁ + rl ₂ + rd ₁ + rd ₂	Rank
<i>Cleistachne sorghoides</i>	6	14	7	28	55	1
<i>Imperata cylindrica</i>	49	65	85	165	364	11
<i>Microstegium nudum</i>	7	29	11	55	102	5
<i>Miscanthidium capense</i>	25	38	41	77	181	7
<i>Miscanthidium junceum</i>	36	63	59	161	319	10
<i>Sorghastrum nudipes</i>	5	8	8	44	65	2
<i>Sorghastrum stipoides</i>	12	26	18	37	93	4
<i>Sorghum arundinaceum</i>	28	57	60	164	309	9
<i>Sorghum ×drummondii</i>	4	20	6	42	72	3
<i>Sorghum halepense</i>	16	36	21	85	158	6
<i>Sarga versicolor</i>	18	29	37	101	185	8

6.7 Flowering times of *Saccharum* hybrids and their wild relatives

Information sourced from herbarium labels and field surveys highlighted that *Imperata cylindrica* (Figure 6.5) and *Sorghum arundinaceum* flower throughout the year, suggesting a 100% flowering synchrony with *Saccharum* hybrids (Table 6.7). *Miscanthidium capense* has an 83% overlap in flowering time with *Saccharum* hybrids. More than 66% of flowering synchrony was further depicted for *Microstegium nudum*, *Miscanthidium junceum*, *Sorghum ×drummondii* and *S.*

halepense (Table 6.7). Specimen data with inflorescence and collection dates were used to fill time lag of flowering period that could have been missed during specified field surveys (Greve *et al.* 2016; Jaca and Mkhize 2018). A South African study by McGeoch *et al.* (2009) mentioned the importance of herbarium specimens for providing valuable flowering data in addition to field surveys even for rapid assessment studies. Field surveys conducted in sugarcane cultivation areas and literature used for assessing flowering times also provide essential data in this regard.

The likelihood of gene flow to and hybridisation of recipient plant species that flowers at the same time as donor crop species is higher when they are in closer geographical areas than those that are distant from donor plants (Ellstrand *et al.* 1999; Chapman and Burke 2006; Schmidt and Bothma 2006; FitzJohn *et al.* 2007; Bonnett *et al.* 2008; Tesso *et al.* 2008; Nieh *et al.* 2014). A review by Lu and Snow (2005) indicated that the rate of gene flow from crops and their relatives is low during asymmetrical flowering. Crop-to-crop and crop-to-wild gene flow potential with flowering synchrony has been reported for *Sorghum* species and their wild relatives (Schmidt and Bothma 2006; Barnaud *et al.* 2008; Tesso *et al.* 2008; Rabbi *et al.* 2011), which is known to be related to *Saccharum* species (Soreng *et al.* 2015; Welker *et al.* 2015). Similar results have been found in other important crops such as rice (Song *et al.* 2004) and maize (Nieh *et al.* 2014).

The likelihood of flowering weedy relatives of transgenic crops have been considered to be at higher risk for receiving pollen and this is an environmental concern since hybrids may be invasive and have an adverse effect in agro-ecosystems (Heard *et al.* 2003; Roy *et al.* 2003; Warwick *et al.* 2009). In the current study, sugarcane relative species with high flowering overlapping with sugarcane which were not present in close proximity to sugarcane fields may presented less likelihood for gene flow compared with species that are found within sugarcane fields, especially if other gene flow barriers are not crossed.



Figure 6.5. *Imperata cylindrica* flowering in sugarcane fields in Mtubatuba, KwaZulu-Natal Province, South Africa [Photo: DM Komape].

Table 6.7. Flowering times of *Saccharum* wild relatives (based on literature, herbarium specimens and field observations) in sugarcane cultivation areas. Calculation of scores was based on ranking the percentage flowering synchrony with *Saccharum* hybrids (flowering from March to August in South Africa). *Saccharum* wild relative species were ranked from highest to lowest, with highest overlap scoring 11 and lowest 1.

Species	Flowering period (literature)	Flowering period (herbarium records and field observations)	Com- bined flowering period	No. months with flowering synchrony	Over- lapping months (%)	Score
<i>Cleistachne sorghoides</i>	Feb-Apr	Mar-Apr	Feb-Apr	2	33	2
<i>Imperata cylindrica</i>	Aug-Jun	Jan-Dec	Jan-Dec	6	100	11
<i>Microstegium nudum</i>	Jan-May	Jan-Jun	Jan-Jun	4	67	7
<i>Miscanthidium capense</i>	Nov-Apr	Dec-Jul, Sep	Sep-Jul	5	83	9
<i>Miscanthidium junceum</i>	Nov-Jun	Nov-Jun, Sep	Sep-Jun	4	67	7
<i>Sarga versicolor</i>	Dec-May	Jan-May	Dec-May	3	50	3
<i>Sorghastrum nudipes</i>	Jan-Apr	Jan-Feb, Apr	Jan-Apr	2	33	2
<i>Sorghastrum stipoides</i>	Dec-Apr	Nov-May, Aug	Aug-May	4	67	7
<i>Sorghum arundinaceum</i>	Jan-Jun	Jan-Dec	Jan-Dec	6	100	11
<i>Sorghum ×drummondii</i>	Jan-Jun	Jan-Mar, Jun-Jul, Nov	Nov-Jul	5	83	9
<i>Sorghum halepense</i>	Dec-May	Nov-Mar, May, Jul- Sep	Nov-Sep	4	67	7

6.8 Likelihood scores

Imperata cylindrica scored the highest during the spatial and temporal assessment, followed by *Sorghum arundinaceum* and *Miscanthidium capense* (Table 6.8). *Miscanthidium junceum*, *Sorghum ×drummondii* and *S. halepense* also had high scores. However, based on relatedness assessment, *I. cylindrica* and the above *Sorghum* species are not closely related to commercial sugarcane (Figure 6.1) and are therefore not candidates to consider for gene flow. Although *Sorghum arundinaceum* had the highest overall score, its distance from *Saccharum* in the phylogeny generated in our study makes it low risk for out crossing. Species with low scores are not considered to present any likelihood for gene flow, especially if these species have diverged from *Saccharum* at more than 7.3 million years ago (e.g. *Sorghum*).

Miscanthidium capense is a *Saccharum* wild relative species with the highest likelihood for potential gene transfer from sugarcane for South African case study according to the risk factors assessed in this study (Table 6.8). *M. capense* is the closest relative to *Saccharum* species (Figure 6.1 and Tables 6.1 and 6.8) with highest gene flow potential (Tables 6.5 and 6.8). This species also scored higher than 6 for the following risk factors: prevalence (Tables 6.2 and 6.8), spatial overlap (Figure 6.3 and Tables 6.3 and 6.8), proximity (Figure 6.4 and Tables 6.4 and 6.8), flowering (Figure 5 and Tables 6.7 and 6.8) and distribution potential (Tables 6.6 and 6.8).

Table 6.8. Score per species calculated by equal weighting of factors obtained per each of the spatial (prevalence, spatial overlap, proximity and distribution potential), temporal (flowering time) and relatedness (hybridisation and phylogenetics (Figure 6.1)) assessments. Gene flow likelihood score was calculated by weighting the spatial, temporal and relatedness assessments at 1:1:2.

Species	Prevalence	Spatial overlap	Proximity	Dispersal potential	Spatial assessment	Temporal assessment	Hybridisation	Phylogenetics	Relatedness assessment	Likelihood score S:T:R (1:1:2)
<i>Sorghum arundinaceum</i>	10	10	11	9	10	11	11	6	8.5	38
<i>Miscanthidium capense</i>	9	9	7	7	8	9	8	11	9.5	36
<i>Miscanthidium junceum</i>	7	5	6	10	7	7	8	11	9.5	33
<i>Sorghum ×drummondii</i>	2	3	9	3	4	9	11	6	8.5	30
<i>Imperata cylindrica</i>	11	11	10	11	11	11	7	1	4	30
<i>Sorghum halepense</i>	4	8	9	6	7	7	9	6	7.5	29
<i>Microstegium nudum</i>	5	4	0	5	4	7	0	7	3.5	18
<i>Sarga versicolor</i>	8	7	0	8	6	3	0	9	4.5	18
<i>Sorghastrum stipoides</i>	7	6	0	4	4	7	0	3	1.5	14
<i>Cleistachne sorghoides</i>	3	2	0	1	2	2	0	9	4.5	13
<i>Sorghastrum nudipes</i>	1	1	0	2	1	2	0	3	1.5	6

Closely related species with high spatial congruity pose the highest likelihood for gene flow and certain areas can be flagged where this is the case. The spatial assessment showed that the highest likelihood for gene flow to occur should be species that are regarded as reproductively compatible with sugarcane. No sugarcane QDS with very high likelihood for gene flow was found in Limpopo but there were two of high likelihood in Modjadjiskloof and Tzaneen (Figure 6.6). There was one QDS with very high likelihood in Nelspruit in addition to one QDS with high likelihood in Mpumalanga province. Thirteen QDS with high and 7 with very high likelihood were identified for KwaZulu-Natal. These were Durban, Felixton, Gingindlovu, Port Edward, Port Shepstone, Richards Bay and Verulam. Overall it appears as if coastal and southern-inland KwaZulu-Natal has the highest likelihood for gene flow to occur based on relatedness, temporal and spatial congruity (Figure 6.6).

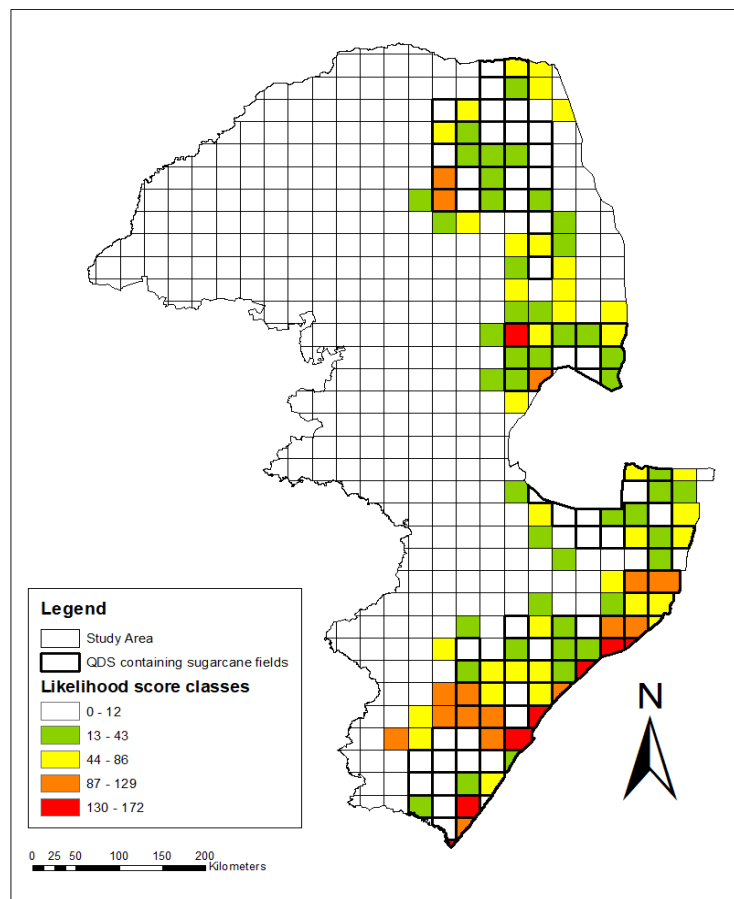


Figure 6.6. Spatial, temporal and relatedness assessment indicating the levels of likelihood for gene flow to occur between sugarcane and wild relatives in the sugar production region of South Africa. Grid values were calculated by summing the likelihood scores allocated per species (from Table 6.8) for all the species recorded per grid. QDS with sugarcane fields are indicated with bold lines, whereas other QDS of the study area without sugarcane fields are not in bold. Likelihood for gene flow: *Sorghastrum nudipes* scored 6 and there was no sugarcane QDS containing only this wild relative species. QDS with sugarcane fields without wild relatives (0–12); sugarcane QDS fields with wild relatives: very low (13–43); low (44–86); high (87–129); very high (130–172).

The herbarium voucher specimens that were sourced during this study (Figure 6.7) in addition to the field collections, were important in developing spatial maps to assess potential gene flow (Figure 6.6; Appendix B). Specimen data are botanical resources which are used as ecological surrogates for information that is essential for environmental assessments (Simon and Proença 2000; Aikio *et al.* 2010; Soltis 2017), although some areas are often better documented with plant collections than other areas (Simon and Proença 2000). In South Africa, for example, most botanical projects are guided by specific biodiversity interests which results in such areas having more herbarium collections of certain species than regions of lower biological interest (Smith and Klopper 2002; McGeoch *et al.* 2009; Victor *et al.* 2016; Visser *et al.* 2017). Herbarium records have been used as baseline data for plant species of conservation concern (Greve *et al.* 2016), ethnobotanical research (Souza and Hawkins 2017), endemic plant species (Simon and Proença

2000), invasive plant species (Aikio *et al.* 2010) and discovery of new plant species (Bebber *et al.* 2010).



Figure 6.7. Voucher specimen of *Sorghum arundinaceum* (syn. *S. verticilliflorum*) sourced from Pretoria National Herbarium (PRE). Locality: KwaZulu-Natal Province, Umzimkulu River, Port Shepstone, Roadside alongside sugarcane fields. QDS: 3030CB. Collector: Nicholson, H.B. no 1379. Date: 1974/2/5.

6.9 Implications of the research outcomes

Imperata cylindrica, *Miscanthidium capense*, *M. junceum*, *Sarga versicolor*, *Sorghastrum nudipes*, *Sorghum arundinaceum*, *S. xdrummondii* and *S. halepense* were presented as target species in Chapter 5 under the ‘Morphology’ sub-heading (5.2), due to their morphological similarities with sugarcane. Of these, only *Miscanthidium capense* and *M. junceum* are considered to have likelihood for gene flow with *Saccharum* hybrids. Subsequently, this study reports that morphological similarities of crop species with their wild relatives are not always indicative of gene flow potential. Mapped distribution patterns of all target sugarcane relatives were found to co-occur with sugarcane cultivations, which enhance the chance of gene flow to occur unlike if they didn’t overlap in space. Habitat data also supported the co-existence of the relative species to share similar habitats with sugarcane. Sexually compatible species (*Miscanthidium capense* and *M. junceum*) with *Saccharum* hybrids were found to share similar habitats with sugarcane and they

were also recorded as weeds of sugarcane fields in the study area. These findings, therefore, imply that both *Miscanthidium* are species that could cross gene flow barriers and therefore further studies on pollen compatibility and viability are required as part of a risk assessment (Chapman and Burke 2006; Bonnett *et al.* 2008).

Areas of lower gene flow likelihood, as shown by the likelihood map (Figure 6.6), suggested that there was an overall limited possibility for gene flow to occur from *Saccharum* hybrids to their wild and weedy relatives in eastern South Africa. This low likelihood of gene flow is expected as most wild relatives considered in this study were only distantly related to sugarcane hybrids. Sugarcane production regions with high to very high gene flow likelihood was characterised by a spatial overlap and prevalence of *Miscanthidium* species which means several spatial and temporal gene flow barriers were crossed. These areas should probably be avoided for cultivation of genetically modified sugarcane until risk assessments have been conducted. Sexually compatible indigenous sugarcane relatives growing in sugarcane fields might receive genes from GM sugarcane which may introgress undesired traits into wild relatives. This may in turn impact the environment negatively, such as weedy sugarcane relatives becoming herbicide resistant (Hokanson *et al.* 2010; Tepfer *et al.* 2013; DiTommaso *et al.* 2016).

Outcomes obtained in this study will therefore inform target wild relative species for risk assessments which is part of the process of risk analysis to make informed decisions for regulating GM crops (Johnson *et al.* 2006; Chandler and Dunwell 2008; Jansen van Rijssen *et al.* 2015; Raybould and Macdonald 2018). The following four stages of risk analysis for GM crops were described by Johnson *et al.* (2006) and guide the process from here on:

1. key issue identification (of which this study makes a major contribution), which entails management goals then carrying out assessment endpoints,
2. risk assessment with testing endpoints, conducting risk assessment (assessing hazards and exposure) and scientific risk evaluation,
3. risk decisions dealing with threshold values, risk management and risk decisions,
4. risk communication, which deals with communicating the risk decision and how the decision was reached.

6.10 References

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CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 Taxonomy and distribution of target species

This study has shown that when wild relatives are considered in biosafety studies, it is important to use both accepted names and synonyms when literature and herbarium surveys are conducted. A very good understanding is required of the nomenclature to collect and present data accurately. Description of the morphological characteristics of target species is essential for accurate identification and collection of *Saccharum* relatives during surveys. For example, although *Cleistachne sorghoides*, *Microstegium nudum*, *Sarga versicolor*, *Sorghastrum nudipes*, *S. stipoides* and *Sorghum halepense* were not found during field visits despite having access to accurate collection records, these were indicated in literature to occur at these specific sampling sites. *Miscanthidium capense* and *M. junceum* were reported to share more morphological characters with sugarcane than other target species. Furthermore, it was revealed in this study that one cannot rely on morphological similarities of crop species with their wild relatives to support their likelihood for gene flow as relatedness assessments showed that this was not the case.

Mapping sugarcane cultivation regions of eastern South Africa was achieved in this study. The distribution records of all target species were found to overlap with sugar production areas. The mere presence of wild relatives in cultivation areas does not mean there will be gene flow from crops to their relatives, but additionally other gene flow factors should be assessed. Distribution patterns of reproductively compatible species of sugarcane (*Miscanthidium capense* and *M. junceum*) in sugarcane growing regions were essential for assessing their potential gene flow. Reproductive wild species that are present in sugar production areas, which ranked high for the assessed factors in this study have the highest likelihood to receive gene flow from cultivated crops. Habitats of the studied wild and weedy sugarcane relatives commonly occurred within sugarcane production areas within the study area.

7.2 Spatial and gene flow assessments

Cleistachne sorghoides, *Miscanthidium capense*, *M. junceum* and *Sarga versicolor* were the only target species that were considered by this study as close relatives of *Saccharum* hybrids. *Miscanthidium capense* and *M. junceum* diverged within 3.9 million years from sugarcane, whereas both *Cleistachne sorghoides* and *Sarga versicolor* was reported to have diverged from sugarcane at 7.4 million years ago. Therefore, Saccharinae wild relatives (*Miscanthidium capense* and *M. junceum*) are most closely related to *Saccharum* hybrids even though *Imperata cylindrica* and *Microstegium nudum* (both Saccharinae species) were found to be distantly related to sugarcane compared to the following Sorghinae species: *Cleistachne sorghoides* and *Sarga versicolor*. This study shows that all local close relatives of sugarcane (*Cleistachne sorghoides*,

Miscanthidium capense, *M. junceum* and *Sarga versicolor*) were found to occur in sugarcane cultivation areas of eastern South Africa. Close wild relatives of crops are more likely to receive gene flow from crops than relative species which are more diverged from their relative crops. These findings were imperative in achieving the objective to determine which close relatives of *Saccharum* occur in the sugarcane cultivation areas of eastern South Africa.

The current research showed that grids with dispersal networks of all sugarcane relatives overlapped spatially with those of sugarcane cultivation areas. The relevant grass species recorded with QDS containing most roads and railways networks were *Imperata cylindrica*, *Miscanthidium junceum* and *Sorghum arundinaceum*. The distribution channels assessed in this study were also found to be common habitats of sugarcane wild relatives. Consequently, closely related species of *Saccharum* hybrids (*Miscanthidium capense* and *M. junceum*) were found with high dispersal potential networks including regions of sugarcane QDS. These are likely to have more chances to spread into other QDS of the study area in future and such instances will then be subjected to increase gene flow potential.

All 11 selected *Saccharum* relatives were found to be prevalent (presence) within QDS of sugarcane areas. This finding were achieved after studying and using the information of 815 sourced herbarium specimens and 34 field observation records of *Saccharum* wild relatives in sugarcane cultivation areas of the study area. This research confirms the presence of target *Saccharum* relatives in the commercially cultivated sugarcane areas of eastern South Africa. Similar findings of spatial overlap with prevalence were attained since records of all target species also overlapped spatially with sugarcane QDS. The same species which were found to have the highest prevalence (*Imperata cylindrica*, *Sorghum arundinaceum* and *Miscanthidium capense*) were also reported with the highest overlapping percentages with sugarcane QDS in the same order. The lowest prevalent species (*Cleistachne sorghoides*, *Sorghastrum nudipes* and *Sorghum ×drummondii*) were found to show less overlapping percentage with sugarcane QDS. Therefore, it can be concluded that *Imperata cylindrica* was the sugarcane relative with the highest likelihood for spatial congruence with sugarcane and that *Sorghastrum nudipes* is the least congruent target species.

Imperata cylindrica, *Miscanthidium capense*, *M. junceum*, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* were assessed and found to be growing in close proximity (<700 m) from sugarcane fields and were therefore used for assessing the likelihood of gene flow from sugarcane to these target species in eastern South Africa. Gene flow is higher from crops to their related wild species when they grow in closer proximity to the crops and introgression can therefore occur if other barriers to gene flow can be crossed. From the above-listed species, *Imperata cylindrica* and *Sorghum arundinaceum* scored the highest for proximity and both these species are known to be problem weeds of sugarcane fields in the study area. *Cleistachne*

sorghoides, *Microstegium nudum*, *Sarga versicolor*, *Sorghastrum nudipes* and *S. stipoides* were not found within 700 m of sugarcane fields in this study and as a result, these species were not classified as habitual weeds, even though they were found to be prevalent and overlapped spatially with some sugarcane cultivation areas.

The literature review conducted in this study recorded 39 hybridisation events from 23 assessed studies between cultivated sugarcane and its relatives. From the assessed studies it was found that only three claimed spontaneous hybridisation for sugarcane and related species. Based on the assessed literature in the current research, it is shown that *Imperata cylindrica*, *Sorghum arundinaceum*, *S. ×drummondii* and *S. halepense* are the only species that are reproductively compatible with *Saccharum* species. The incidence of reported hybridisation events was the highest for *Miscanthidium* (17 publications) with six of these reporting successful hybridisation events with sugarcane. Hybridisation potential between *Miscanthidium* and *Saccharum* ranked the highest for wild relatives.

This study reported the potential hybridisation between *Saccharum* hybrids and their closely related species in eastern South Africa. It was identified that there is a lack of literature for South African *Saccharum* relative species considering that species with highest number of reports (*Miscanthidium* species) were assessed only at generic level while there was only one report regarding the species with the second highest incidence of reporting (*Imperata cylindrica*).

This study showed that *Imperata cylindrica* and *Sorghum arundinaceum* in the study area flowers throughout the year, indicating a 100% flowering synchrony with *Saccharum* hybrids. Another target species which was found with high flowering overlap with *Saccharum* hybrids was *Miscanthidium capense*, with an 83% overlap in flowering time. Despite these results, it should be noted that sugarcane relatives with high flowering overlap with sugarcane which are not present in close proximity to sugarcane fields may present less transgene risks compared to species that are found within sugarcane fields, especially if other gene flow barriers are not crossed.

Imperata cylindrica was the target species which scored the highest for both spatial and temporal overlap. *Sorghum arundinaceum* had the overall highest score. However, findings of a recent phylogenetic analysis showed that *I. cylindrica* and *Sorghum* species are not closely related to commercial sugarcane and are therefore not candidates to consider for gene flow. Subsequently, this research project flagged *Miscanthidium capense* as the *Saccharum* wild relative species with high risk for potential gene transfer from GM sugarcane in eastern South Africa. The findings listed above were based on the assessments that *M. capense* was found to be the closest relative to *Saccharum* hybrids with highest gene flow potential and this grass was ranked with higher scores for the following risk factors: prevalence, spatial overlap, proximity, flowering and distribution potential.

Likelihood scores that were presented in this study were based on all the assessed risk factors and were indicated to be essential for highlighting areas with high spatial congruity which pose the highest likelihood for gene flow from *Saccharum* hybrids to their closest related species. This study showed that KwaZulu-Natal was the province with most areas of high and very high likelihood for gene flow to occur from *Saccharum* hybrids to wild relatives because a total of 13 sugarcane QDS with high and seven with very high likelihood occurred in this province. In conclusion, this study showed that coastal and southern-inland KwaZulu-Natal has the highest likelihood for gene flow to occur based on relatedness, temporal and spatial congruity.

7.3 Recommendations and future studies

Gene flow studies (as obtained in the current study) are important to guide risk assessments and to determine whether GM crops threatens biological diversity. If gene flow occurs between transgenic crops and their wild relatives it may affect related species in an agro-ecosystem in a negative way (Bonnett *et al.* 2008; OECD 2013; Akinbo *et al.* 2015; Bøhn *et al.* 2016; Raybould and Macdonald 2018). Risk assessments regulate transgenic crops to protect the biodiversity in the proposed areas where these crops will be cultivated (Johnson *et al.* 2006; Chandler and Dunwell 2008; Tepfer *et al.* 2013; Raybould and Macdonald 2018).

Despite the rare occurrence of wild hybridisation of crop species with their relatives, genes of GM crops may cause unintended effects on biodiversity if released in the natural environment (Chapman and Burke 2006). For example, in Ethiopia, a study by Adugna *et al.* (2013) showed that gene flow from GM crops and to their wild relatives is very high when there are no hybridisation barriers and that this can impact on gene pools of wild species.

Gene confinement strategies could be evaluated by means of field trials which can be used to test if introgression from crops to their reproductively compatible species do occur (Chapman and Burke 2006; Chandler and Dunwell 2008; Hokanson *et al.* 2010). Since gene flow barriers such as proximity and flowering time was shown to be crossed in the current study, further studies should be conducted to assess pollen compatibility and viability of sugarcane and related species (*Miscanthidium capense* and *M. junceum*).

7.4 References

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Appendix A: Wild and weedy grasses flowering with *Saccharum* hybrids (flowering synchrony of *Saccharum* hybrids and other grasses) which were observed during field surveys in commercial sugarcane regions of eastern South Africa. Target grass species selected for this study are highlighted in bold.

Species name	Region								
	Empangeni	Jozini	Hammarisdale	Kwa-Mbonambi	Malelane	Mount Edgecombe	Mkuze	Mtubatuba	Mtunzini
<i>Avena fatua</i> L.	1								
<i>Bothriochloa insculpta</i> (A.Rich.) A.Camus					1				
<i>Chloris gayana</i> Kunth	1								
<i>Chloris pycnothrix</i> Trin.	1								
<i>Chloris virgata</i> Sw.	2								
<i>Chrysopogon zizanioides</i> (L.) Roberty						1			
<i>Cynodon dactylon</i> (L.) Pers.		1							
<i>Dactyloctenium australe</i> Steud.	1								
<i>Digitaria ternata</i> (A.Rich.) Stapf	1								
<i>Eleusine corocana</i> (L.) Gaertn. subsp. <i>africana</i> (Kenn.-O'Byrne) Hilu & de Wet	1	1						1	
<i>Eragrostis curvula</i> (Schrad.) Nees	1								
<i>Eragrostis superba</i> Peyr.		1							
<i>Hyparrhenia schimperi</i> (Hoch. ex A.Rich.) Andersson ex Stapf					1				
<i>Imperata cylindrica</i> (L.) Raeusch.			1	3		1		1	1
<i>Melinis minutiflora</i> P.Beauv						1			
<i>Melinis repens</i> (Willd.) Zizka					1				
<i>Miscanthidium capense</i> (Nees) Stapf			1			1			
<i>Miscanthidium junceum</i> (Stapf) Pilg.								1	
<i>Panicum maximum</i> Jacq.	2	3			1			1	

<i>Pennisetum purpureum</i> Schumach.	1	1							
<i>Phragmites mauritanus</i> Kunth	1								
<i>Sorghum arundinaceum</i> (Desv.) Stapf	6	1	1		1		8		
<i>Sorghum ×drummondii</i> (Nees ex Steud.) Millsp. & Chase	2	1							
<i>Sporobolus africanus</i> (Poir.) Robyns & Tournay	2	1			1				
<i>Tragus berteronianus</i> Schlult.	1								
<i>Urochloa mosambicensis</i> (Hack.) Dandy	1								
<i>Zea mays</i> L.		1							

Appendix A: Continues...

Species name	Region								Number of records per species
	Empangeni	Jozini	Hammarisdale	New Hanover	Paulpietersburg	Port Shepstone	Richards Bay	Umhlali	
<i>Avena fatua</i> L.	1								1
<i>Bothriochloa insculpta</i> (A.Rich.) A.Camus									1
<i>Chloris gayana</i> Kunth	1								1
<i>Chloris pycnothrix</i> Trin.	1								1
<i>Chloris virgata</i> Sw.	2								2
<i>Chrysopogon zizanioides</i> (L.) Roberty									1
<i>Cynodon dactylon</i> (L.) Pers.		1				1			2
<i>Dactyloctenium australe</i> Steud.	1								1
<i>Digitaria ternata</i> (A.Rich.) Stapf	1								1
<i>Eleusine corocana</i> (L.) Gaertn. subsp. <i>africana</i> (Kenn.-	1	1				1		1	5

O'Byrne) Hilu & de Wet									
<i>Eragrostis curvula</i> (Schrud.) Nees	1								1
<i>Eragrostis superba</i> Peyr.		1							1
<i>Hyparrhenia schimperi</i> (Hoch. ex A.Rich.) Andersson ex Stapf									1
<i>Imperata cylindrica</i> (L.) Raeusch.			1		1				8
<i>Melinis minutiflora</i> P.Beauv									1
<i>Melinis repens</i> (Willd.) Zizka									1
<i>Miscanthidium capense</i> (Nees)Stapf			1	5					7
<i>Miscanthidium junceum</i> (Stapf) Pilg.					1				2
<i>Panicum maximum</i> Jacq	2	3				1		1	9
<i>Pennisetum purpureum</i> Schumach.	1	1							2
<i>Phragmites mauritianus</i> Kunth	1								1
<i>Sorghum arundinaceum</i> (Desv.) Stapf	6	1	1		1	4	1	1	20
<i>Sorghum xdrummondii</i> (Nees ex Steud.) Millsp. & Chase	2	1			1				4
<i>Sporobolus africanus</i> (Poir.) Robyns & Tournay	2	1				1		1	6
<i>Tragus berteronianus</i> Schlult.	1								1
<i>Urochloa mosambicensis</i> (Hack.) Dandy	1								1
<i>Zea mays</i> L.		1							1

Appendix B: Likelihood scores per target species recorded in ArcGIS sheet and QDS of the eastern South Africa obtained from Figure 6.6. Only totals of sugarcane QDS are presented here, since they were used to generate the likelihood map with various likelihood classes in Figure 6.6.

Sheet number	<i>Cleistachne sorghoides</i>	<i>Imperata cylindrica</i>	<i>Microstegium nudum</i>	<i>Miscanthidium capense</i>	<i>Miscanthidium junceum</i>	<i>Sarga versicolor</i>	<i>Sorghastrum nudipes</i>	<i>Sorghastrum stipoides</i>	<i>Sorghum arundinaceum</i>	<i>Sorghum halepense</i>	<i>Sorghum xdrummondii</i>	Sugarcane QDS
2228BD	0	0	0	0	0	0	0	0	0	0	0	0
2228CA	0	0	0	0	0	0	0	0	0	0	0	0
2228CB	0	0	0	0	0	0	0	0	38	0	0	0
2228CC	0	0	0	0	0	0	0	0	0	0	0	0
2228CD	0	0	0	0	0	0	0	0	0	0	0	0
2228DA	0	0	0	0	0	0	0	0	0	0	0	0
2228DB	0	0	0	0	0	0	0	0	0	0	0	0
2228DC	0	0	0	0	0	0	0	0	0	0	0	0
2228DD	0	0	0	0	0	0	0	0	0	0	0	0
2229AA	0	0	0	0	0	18	0	0	0	0	30	0
2229AB	0	0	0	0	0	0	0	0	38	0	0	0
2229AC	0	0	0	0	0	0	0	0	0	29	30	0
2229AD	0	0	0	0	0	0	0	0	0	0	0	0
2229BA	0	0	0	0	0	0	0	0	0	0	0	0
2229BB	0	0	0	0	0	0	0	0	0	0	0	0
2229BC	0	0	0	0	0	0	0	0	0	0	0	0
2229BD	0	0	0	0	0	0	0	0	0	0	0	0
2229CA	0	0	0	0	0	0	0	0	0	0	0	0
2229CB	0	0	0	0	0	0	0	0	0	0	0	0
2229CC	0	0	0	0	0	0	0	0	0	0	0	0
2229CD	0	0	0	0	0	0	0	0	0	0	0	0
2229DA	0	0	0	0	0	0	0	0	0	0	0	0
2229DB	0	0	0	0	0	0	0	0	0	0	0	5
2229DC	0	0	0	0	0	0	0	0	0	0	0	0
2229DD	0	0	0	0	0	0	0	0	0	0	0	0
2230AC	0	0	0	0	0	0	0	0	0	0	0	0
2230AD	0	0	0	0	0	0	0	0	0	0	0	0
2230BC	0	0	0	0	0	0	0	0	0	0	0	0
2230BD	0	0	0	0	33	18	0	0	0	0	0	51
2230CA	0	0	0	0	0	0	0	0	0	0	0	0
2230CB	0	0	0	0	0	0	0	0	0	0	0	0
2230CC	0	0	0	0	0	0	0	0	0	0	0	0
2230CD	0	30	0	0	33	0	0	0	0	0	0	63
2230DA	0	0	0	0	0	0	0	0	0	0	0	0
2230DB	0	0	0	0	0	0	0	0	38	0	0	38
2230DC	0	0	0	0	0	0	0	0	0	0	0	0
2230DD	0	0	0	0	0	0	0	0	0	0	0	0
2231AC	0	0	0	0	0	18	0	0	38	0	0	56
2231AD	0	0	0	0	0	0	0	0	38	0	0	0
2231CA	0	0	0	0	0	18	0	0	38	0	0	56
2231CB	0	0	0	0	0	0	0	0	0	0	0	0
2231CC	0	0	0	0	0	0	0	0	0	0	0	0
2231CD	0	0	0	0	0	18	0	0	38	0	0	56
2326DB	0	0	0	0	0	0	0	0	0	0	0	0
2326DD	0	0	0	0	0	0	0	0	0	0	0	0
2327AC	0	0	0	0	0	0	0	0	0	0	0	0
2327AD	0	0	0	0	0	0	0	0	38	0	0	0
2327BA	0	0	0	0	0	0	0	0	0	0	0	0

2327BB	0	0	0	0	0	0	0	0	0	0	0	0
2327BC	0	0	0	0	0	0	0	0	0	0	0	0
2327BD	0	0	0	0	0	0	0	0	0	0	0	0
2327CA	0	0	0	0	0	0	0	0	0	0	0	0
2327CB	0	0	0	0	0	0	0	0	0	0	0	0
2327CC	0	0	0	0	0	0	0	0	0	0	0	0
2327CD	0	0	0	0	0	0	0	0	0	0	0	0
2327DA	0	0	0	0	0	0	0	0	0	0	0	0
2327DB	0	0	0	0	0	0	0	0	0	0	0	0
2327DC	0	0	0	0	0	0	0	0	0	0	0	0
2327DD	0	0	0	0	0	0	0	0	0	0	0	0
2328AA	0	0	0	0	0	0	0	0	0	0	0	0
2328AB	0	0	0	0	0	0	0	0	0	0	0	0
2328AC	0	0	0	0	0	0	0	0	0	0	0	0
2328AD	0	0	0	0	0	0	0	0	0	0	0	0
2328BA	0	0	0	0	0	0	0	0	0	0	0	0
2328BB	0	0	0	0	0	0	0	0	0	0	0	0
2328BC	0	0	0	0	0	0	0	0	0	0	0	0
2328BD	0	0	0	0	0	0	0	0	0	0	0	0
2328CA	0	0	0	0	0	0	0	0	38	0	0	0
2328CB	0	0	0	0	0	0	0	0	0	0	0	0
2328CC	0	0	0	0	0	0	0	0	0	0	0	0
2328CD	0	0	0	0	0	0	0	0	0	0	0	0
2328DA	0	0	0	0	0	0	0	0	0	0	0	0
2328DB	0	0	0	0	0	0	0	0	0	0	0	0
2328DC	0	0	0	0	0	0	0	0	0	0	0	0
2328DD	0	0	0	0	0	18	0	0	0	0	0	0
2329AA	0	0	0	0	0	0	0	0	0	0	0	0
2329AB	0	0	0	0	33	0	0	0	0	0	0	0
2329AC	0	0	0	0	0	0	0	0	0	0	0	0
2329AD	0	0	0	0	0	0	0	0	0	0	0	0
2329BA	0	0	0	0	0	0	0	0	0	0	0	0
2329BB	0	0	0	0	0	0	0	0	0	0	0	0
2329BC	0	0	0	0	0	0	0	0	0	0	0	0
2329BD	0	0	0	0	0	0	0	0	0	0	0	0
2329CA	0	30	0	0	0	0	0	0	0	0	0	0
2329CB	0	0	0	0	0	0	0	0	0	0	0	0
2329CC	0	0	0	0	0	0	0	0	0	0	0	0
2329CD	0	30	0	0	33	0	0	0	0	0	0	0
2329DA	0	0	0	0	0	0	0	0	0	0	0	0
2329DB	0	0	0	0	0	0	0	0	0	0	0	0
2329DC	0	0	0	0	0	0	0	0	0	0	0	0
2329DD	0	0	18	0	0	0	0	0	0	0	0	18
2330AA	0	0	0	0	0	0	0	0	38	29	0	67
2330AB	0	30	0	0	0	0	0	0	0	0	0	30
2330AC	0	0	0	0	0	0	0	0	0	0	0	0
2330AD	0	0	0	0	0	0	0	0	38	0	0	38
2330BA	0	0	0	0	0	0	0	0	0	0	0	0
2330BB	0	0	0	0	0	0	0	0	0	0	0	0
2330BC	0	0	0	0	0	18	0	0	0	0	0	18
2330BD	0	0	0	0	0	18	0	0	0	0	0	18
2330CA	0	30	18	0	33	0	0	0	38	0	0	119
2330CB	0	0	0	0	0	0	0	0	0	0	0	0
2330CC	0	30	18	0	33	0	6	0	38	0	0	125

2330CD	0	0	0	0	0	0	0	0	0	0	0	0
2330DA	0	0	0	0	0	0	0	0	38	0	0	38
2330DB	0	0	0	0	0	0	0	0	0	0	0	0
2330DC	0	0	0	0	0	0	0	0	38	0	0	38
2330DD	0	0	0	0	0	0	0	0	0	0	0	0
2331AA	0	0	0	0	0	0	0	0	0	0	0	0
2331AB	0	0	0	0	0	0	0	0	0	0	0	0
2331AC	0	0	0	0	0	0	0	0	0	0	0	0
2331AD	0	0	0	0	0	0	0	0	0	0	0	0
2331BA	0	0	0	0	0	0	0	0	0	0	0	0
2331BC	0	0	0	0	0	0	0	0	0	0	0	0
2331CA	0	0	0	0	0	0	0	0	0	0	0	0
2331CB	0	0	0	0	0	0	0	0	0	0	0	0
2331CC	0	0	0	0	0	18	0	0	0	0	0	18
2331CD	0	0	0	0	0	0	0	0	0	0	0	0
2331DA	0	0	0	0	0	0	0	0	0	0	0	0
2331DC	0	0	0	0	0	0	0	0	38	0	0	0
2331DD	0	0	0	0	0	0	0	0	0	0	0	0
2426BB	0	0	0	0	0	0	0	0	0	0	0	0
2426BC	0	0	0	0	0	0	0	0	0	0	0	0
2426BD	0	0	0	0	0	0	0	0	0	0	0	0
2426CB	0	0	0	0	0	0	0	0	0	0	0	0
2426CD	0	0	0	0	0	0	0	0	0	0	0	0
2426DA	0	0	0	0	0	0	0	0	0	0	0	0
2426DB	0	0	0	0	0	0	0	0	0	0	0	0
2426DC	0	0	0	0	0	0	0	0	0	0	0	0
2426DD	0	0	0	0	0	0	0	0	0	0	0	0
2427AA	0	0	0	0	0	18	0	0	0	0	0	0
2427AB	0	0	0	0	0	0	0	0	0	0	0	0
2427AC	0	0	0	0	0	0	0	0	0	0	0	0
2427AD	0	0	0	0	0	18	0	0	0	0	0	0
2427BA	0	0	0	0	0	0	0	0	0	0	0	0
2427BB	0	0	0	0	0	0	0	0	0	0	0	0
2427BC	0	0	0	0	0	0	0	0	0	0	0	0
2427BD	0	0	0	0	0	0	0	0	0	0	0	0
2427CA	0	0	0	0	0	0	0	0	0	0	0	0
2427CB	0	0	0	0	0	18	0	0	0	29	0	0
2427CC	0	0	0	0	0	0	0	0	0	0	0	0
2427CD	0	0	0	0	0	0	0	0	0	0	0	0
2427DA	0	0	0	0	33	0	0	0	0	0	0	0
2427DB	0	0	0	0	0	0	0	0	0	0	0	0
2427DC	0	0	0	0	0	0	0	0	0	0	0	0
2427DD	0	0	0	0	0	0	0	0	0	0	0	0
2428AA	0	0	0	0	33	0	0	0	0	0	0	0
2428AB	0	0	0	0	33	0	0	0	0	0	0	0
2428AC	0	0	0	0	33	0	0	0	0	0	0	0
2428AD	0	0	0	0	33	0	0	0	0	0	0	0
2428BA	0	0	0	0	33	0	6	0	0	0	0	0
2428BB	0	0	0	0	0	0	0	0	0	0	0	0
2428BC	0	0	0	0	33	0	0	0	0	0	0	0
2428BD	0	0	0	0	0	0	6	0	0	0	0	0
2428CA	0	0	0	0	0	0	6	0	0	0	0	0
2428CB	0	0	0	0	33	18	0	0	0	0	0	0
2428CC	0	0	0	0	0	0	0	0	0	0	0	0

2428CD	0	0	0	0	33	18	0	0	0	0	0	0
2428DA	0	0	0	0	33	18	6	0	0	0	0	0
2428DB	0	0	0	0	33	0	6	0	38	0	0	0
2428DC	0	0	0	0	0	0	0	0	0	0	0	0
2428DD	0	0	0	0	0	0	0	0	0	0	0	0
2429AA	0	0	0	0	33	18	0	0	0	0	30	0
2429AB	0	0	0	0	0	18	0	0	0	0	0	0
2429AC	0	0	0	0	0	18	0	0	0	0	0	0
2429AD	0	0	0	0	0	0	0	0	0	0	0	0
2429BA	0	0	0	0	33	0	0	0	0	0	0	0
2429BB	0	0	0	0	0	0	0	0	0	0	0	0
2429BC	0	0	0	0	0	0	0	0	0	0	0	0
2429BD	0	0	0	0	0	0	0	0	38	0	0	0
2429CA	0	0	0	0	0	18	0	0	0	0	0	0
2429CB	0	0	0	0	0	18	0	0	0	0	0	0
2429CC	0	0	0	0	0	0	0	0	0	0	0	0
2429CD	0	30	0	36	33	0	0	0	0	0	0	0
2429DA	0	0	0	0	0	0	0	0	0	0	0	0
2429DB	0	0	0	0	0	0	0	0	0	0	0	0
2429DC	0	0	0	0	0	0	0	0	0	0	0	0
2429DD	0	0	0	0	0	0	0	0	0	0	0	0
2430AA	0	0	0	0	33	0	0	0	0	0	0	33
2430AB	0	30	0	0	0	0	0	0	38	0	0	68
2430AC	0	0	0	0	0	0	0	0	0	0	0	0
2430AD	0	0	0	0	0	0	0	0	0	0	0	0
2430BA	0	0	0	0	0	0	0	0	0	0	0	0
2430BB	0	0	0	0	0	0	0	0	0	0	0	0
2430BC	0	0	0	0	0	0	0	0	0	0	0	0
2430BD	13	0	0	0	33	0	0	0	0	0	0	46
2430CA	0	0	0	0	0	0	0	0	0	0	0	0
2430CB	0	0	0	0	33	0	0	0	0	0	0	0
2430CC	0	0	0	0	0	0	0	0	0	0	0	0
2430CD	0	0	0	0	0	0	0	0	0	0	0	0
2430DA	0	30	0	0	0	0	0	0	38	0	0	0
2430DB	13	0	0	0	0	0	0	0	0	0	0	13
2430DC	0	0	18	0	33	0	0	0	38	0	0	0
2430DD	0	30	18	0	0	0	6	0	0	0	0	54
2431AA	0	0	0	0	0	0	0	0	0	0	0	0
2431AB	0	0	0	0	0	18	0	0	0	0	0	18
2431AC	0	0	0	0	0	18	0	0	38	0	0	56
2431AD	0	0	0	0	0	18	0	0	0	0	0	18
2431BA	0	0	0	0	0	0	0	0	38	0	0	0
2431BB	0	0	0	0	0	0	0	0	0	0	0	0
2431BC	0	0	0	0	0	0	0	0	0	0	0	0
2431BD	0	0	0	0	0	0	0	0	0	0	0	0
2431CA	0	0	0	0	0	0	0	0	0	0	0	0
2431CB	0	0	0	0	0	18	0	0	38	0	0	56
2431CC	0	0	0	0	0	0	0	0	0	0	0	0
2431CD	0	0	0	0	0	18	0	0	38	0	0	56
2431DA	0	0	0	0	0	0	0	0	38	0	0	0
2431DB	0	0	0	0	0	18	0	0	0	0	0	0
2431DC	0	0	0	0	0	0	0	0	38	0	0	0
2431DD	0	0	0	0	0	0	0	0	0	0	0	0
2527AA	0	0	0	0	0	0	0	0	0	0	0	0

2527AB	0	0	0	0	0	0	0	0	0	0	0	0
2527BA	0	0	0	0	0	0	0	0	0	0	0	0
2527BB	0	0	0	0	0	0	0	0	0	0	0	0
2528AA	0	0	0	0	0	0	0	0	0	0	0	0
2528AB	0	0	0	0	0	18	0	0	0	0	0	0
2528AD	0	0	0	0	0	0	0	0	0	0	0	0
2528BA	0	0	0	0	0	0	0	0	0	0	0	0
2528BB	0	0	0	0	0	0	0	0	0	0	0	0
2528BC	0	0	0	0	0	0	0	0	0	0	0	0
2528BD	0	0	0	0	0	0	0	0	0	0	0	0
2528DA	0	0	0	0	0	0	0	0	0	0	0	0
2528DB	0	0	0	0	0	0	0	0	0	0	0	0
2528DC	0	0	0	0	0	0	0	0	0	0	0	0
2528DD	0	0	0	0	0	0	0	0	0	0	0	0
2529AA	0	0	0	0	0	0	0	0	0	0	0	0
2529AB	0	0	0	0	33	0	0	0	0	0	0	0
2529AC	0	0	0	0	33	0	0	0	38	0	0	0
2529AD	0	30	0	36	33	0	6	0	38	29	0	0
2529BA	0	0	0	0	0	0	0	0	0	0	0	0
2529BB	0	30	0	0	0	0	0	0	0	0	0	0
2529BC	0	0	0	0	0	0	0	0	0	0	0	0
2529BD	0	30	0	0	0	0	0	0	0	0	0	0
2529CA	0	30	0	0	33	0	0	0	0	0	0	0
2529CB	0	30	0	0	33	0	0	0	0	0	0	0
2529CC	0	0	0	0	0	0	0	0	0	0	0	0
2529CD	0	30	0	0	0	18	0	0	0	0	0	0
2529DA	0	0	0	0	0	0	0	0	0	0	0	0
2529DB	0	0	0	0	0	0	0	0	0	0	30	0
2529DC	0	0	0	0	0	0	0	0	0	0	0	0
2529DD	0	0	0	0	0	0	0	0	0	0	0	0
2530AA	0	0	0	0	0	0	0	0	0	0	0	0
2530AB	0	30	0	0	33	0	0	0	0	0	0	0
2530AC	0	0	0	0	33	0	0	0	0	0	0	0
2530AD	0	30	0	0	0	0	0	0	38	0	0	0
2530BA	0	0	0	0	0	0	0	0	0	0	0	0
2530BB	13	0	0	0	0	0	0	0	0	0	0	13
2530BC	0	30	0	0	0	0	0	0	0	0	0	30
2530BD	13	30	18	0	33	0	0	14	38	0	0	146
2530CA	0	0	0	0	0	0	0	0	0	0	0	0
2530CB	13	30	0	0	33	0	0	0	0	0	0	0
2530CC	0	0	0	0	0	0	0	0	0	0	0	0
2530CD	0	0	0	0	0	0	0	0	0	0	0	0
2530DA	0	0	0	0	0	0	0	0	0	0	0	0
2530DB	0	0	0	0	0	0	0	0	38	0	0	38
2530DC	0	30	0	0	0	0	0	0	0	0	0	30
2530DD	0	30	0	0	0	0	0	0	0	0	0	30
2531AA	0	0	0	0	0	0	0	0	38	0	0	38
2531AB	0	30	0	0	0	18	0	0	0	29	0	77
2531AC	0	0	0	0	33	0	0	14	0	0	0	47
2531AD	0	0	0	0	0	0	0	0	38	0	0	38
2531BA	0	0	0	0	0	0	0	0	0	0	0	0
2531BB	0	0	0	0	0	18	0	0	38	0	0	56
2531BC	0	0	0	0	0	18	0	0	0	0	0	18
2531BD	0	0	0	0	0	18	0	0	0	29	0	47

2531CA	0	30	0	0	0	0	0	0	0	0	0	30
2531CB	0	0	0	0	0	0	0	0	0	0	0	0
2531CC	0	30	0	0	0	0	0	0	38	29	0	97
2531CD	0	0	0	0	0	0	0	0	0	0	0	0
2531DA	0	0	0	0	0	0	0	0	0	0	0	0
2531DB	0	0	0	0	0	18	0	0	0	0	0	18
2531DC	0	0	0	0	0	0	0	0	0	0	0	0
2531DD	13	0	0	0	0	0	0	0	0	0	0	13
2628AB	0	0	0	0	0	0	0	0	0	0	0	0
2628BA	0	0	0	0	33	0	0	0	0	0	0	0
2628BB	0	0	0	0	0	0	0	0	0	0	0	0
2628BC	0	0	0	0	0	0	0	0	0	0	0	0
2628BD	0	0	0	0	0	0	0	0	0	0	0	0
2628CB	0	0	0	0	0	0	0	0	0	0	0	0
2628CC	0	0	0	0	0	0	0	0	0	0	0	0
2628CD	0	0	0	0	0	0	0	0	0	0	0	0
2628DA	0	0	0	0	0	0	0	0	0	0	0	0
2628DB	0	0	0	0	0	0	0	0	0	0	0	0
2628DC	0	0	0	0	0	0	0	0	0	0	0	0
2628DD	0	0	0	0	0	0	0	0	0	0	0	0
2629AA	0	0	0	0	0	0	0	0	0	0	0	0
2629AB	0	0	0	0	0	0	0	0	0	0	0	0
2629AC	0	0	0	0	0	0	0	0	0	0	0	0
2629AD	0	0	0	0	0	0	0	0	0	0	0	0
2629BA	0	0	0	0	0	0	0	0	0	0	0	0
2629BB	0	0	0	0	0	0	0	0	0	0	0	0
2629BC	0	0	0	0	0	0	0	0	0	0	0	0
2629BD	0	0	0	0	0	0	0	0	0	0	0	0
2629CA	0	0	0	0	0	0	0	0	0	0	0	0
2629CB	0	0	0	0	0	0	0	0	0	0	0	0
2629CC	0	0	0	0	0	0	0	0	0	0	0	0
2629CD	0	30	0	0	0	0	0	0	0	0	0	0
2629DA	0	0	0	0	0	0	0	0	0	0	0	0
2629DB	0	30	0	0	0	0	0	0	38	29	0	0
2629DC	0	0	0	0	0	0	0	0	0	0	0	0
2629DD	0	0	0	0	33	0	0	0	0	0	0	0
2630AA	13	0	0	0	0	0	0	0	0	0	0	0
2630AB	0	0	0	0	0	0	0	0	0	0	0	0
2630AC	0	0	0	0	0	0	0	0	0	0	0	0
2630AD	0	0	0	0	33	0	0	0	0	0	0	0
2630BA	0	0	0	0	0	0	0	0	0	0	0	0
2630BB	0	0	0	0	33	0	0	0	0	29	0	62
2630BC	0	0	0	0	0	0	0	0	0	0	0	0
2630BD	0	0	0	0	0	0	0	0	0	0	0	0
2630CA	0	30	0	0	0	0	0	0	0	0	0	0
2630CB	0	0	0	0	0	0	0	0	0	0	0	0
2630CC	0	0	0	0	0	0	0	0	0	0	0	0
2630CD	0	0	0	0	33	0	0	0	0	0	0	0
2630DA	0	0	0	0	0	0	0	0	0	0	0	0
2630DB	0	0	0	0	0	0	0	0	0	0	0	0
2630DC	0	0	0	0	0	0	0	0	0	0	0	0
2630DD	0	0	0	0	0	0	0	0	0	0	0	0
2631AA	0	0	0	0	0	0	0	0	0	0	0	0
2632CC	0	0	0	0	0	18	0	0	38	0	0	56

2632CD	0	0	0	0	0	0	0	0	38	0	0	38
2632DC	0	30	0	0	0	0	0	14	0	0	0	44
2632DD	0	0	0	0	0	0	0	0	0	0	0	0
2728AB	0	0	0	0	0	0	0	0	0	0	0	0
2728BA	0	0	0	0	0	0	0	0	0	0	0	0
2728BB	0	0	0	0	0	0	0	0	0	0	0	0
2729AA	0	0	0	0	0	0	0	0	0	0	0	0
2729AB	0	0	0	0	0	0	0	0	0	0	0	0
2729AD	0	0	0	0	0	0	0	0	0	0	0	0
2729BA	0	0	0	0	0	0	0	0	0	0	0	0
2729BB	0	30	0	0	0	0	0	0	0	0	0	0
2729BC	0	0	0	0	0	0	0	0	0	0	0	0
2729BD	0	0	0	0	0	0	0	0	0	0	0	0
2729DA	0	0	0	0	0	0	0	0	0	0	0	0
2729DB	0	0	0	0	0	0	0	0	0	0	0	0
2729DC	0	0	0	0	0	0	0	0	0	0	0	0
2729DD	0	30	0	0	33	0	0	0	0	0	0	0
2730AA	0	0	0	0	0	0	0	0	0	0	0	0
2730AB	0	30	0	0	33	0	0	0	0	0	0	0
2730AC	0	0	0	0	0	0	0	0	0	0	0	0
2730AD	0	30	0	0	33	0	0	0	0	0	0	0
2730BA	0	0	0	0	0	0	0	0	0	0	0	0
2730BB	0	0	0	0	33	0	0	0	0	0	0	33
2730BC	0	30	0	0	0	0	0	0	0	0	0	0
2730BD	0	30	0	0	33	0	0	0	0	0	30	0
2730CA	0	0	0	0	33	0	0	0	0	0	0	0
2730CB	0	0	0	0	33	0	0	0	0	0	0	0
2730CC	0	0	0	0	33	0	0	0	0	0	0	0
2730CD	0	0	0	0	0	0	0	0	0	0	0	0
2730DA	0	0	0	0	0	0	0	0	0	0	0	0
2730DB	0	30	0	0	33	0	0	0	0	0	0	0
2730DC	0	30	0	36	0	0	0	0	0	0	0	0
2730DD	0	0	0	0	0	0	0	0	0	0	0	0
2731AA	0	0	0	0	0	0	0	0	0	0	0	0
2731AC	0	30	0	0	0	0	0	14	38	0	0	82
2731AD	0	0	0	0	0	0	0	0	0	0	0	0
2731BC	0	0	0	0	0	0	0	0	0	0	0	0
2731BD	0	0	0	0	0	0	0	0	0	29	0	29
2731CA	0	30	0	0	0	0	0	0	0	0	0	30
2731CB	0	0	0	0	0	0	0	0	0	0	0	0
2731CC	0	0	0	0	0	0	0	0	0	0	0	0
2731CD	0	30	0	0	0	0	0	0	0	0	0	30
2731DA	0	0	0	0	0	0	0	0	0	0	0	0
2731DB	0	0	0	0	0	0	0	0	0	0	0	0
2731DC	0	0	0	0	0	0	0	0	0	0	0	0
2731DD	0	0	0	0	0	0	0	0	0	0	0	0
2732AA	0	0	0	0	0	0	0	0	0	0	0	0
2732AB	0	30	0	0	0	0	0	0	0	0	0	30
2732AC	0	0	0	0	0	0	0	0	0	0	30	30
2732AD	0	0	0	0	0	0	0	0	0	0	0	0
2732BA	0	0	0	0	0	0	0	14	0	0	0	14
2732BC	0	30	0	0	0	0	0	14	0	0	0	44
2732CA	0	0	0	0	0	18	0	14	38	0	0	70
2732CB	0	0	0	0	0	0	0	0	0	0	0	16

2732CC	0	0	0	0	0	0	0	0	0	0	0	0
2732CD	0	0	0	0	0	0	0	0	38	0	0	38
2732DA	0	30	0	36	0	0	0	0	0	0	0	66
2732DC	0	0	0	0	0	0	0	0	0	0	0	0
2828DB	0	0	0	0	0	0	0	0	0	0	0	0
2828DD	0	0	0	36	0	0	0	0	0	0	0	0
2829AC	0	0	0	36	0	0	0	0	0	0	0	0
2829AD	0	0	0	0	0	0	0	0	0	0	0	0
2829BA	0	30	0	0	0	0	0	0	0	0	0	0
2829BB	0	0	0	0	0	0	0	0	0	0	0	0
2829BC	0	0	0	0	0	0	0	0	0	0	0	0
2829BD	0	0	0	0	0	0	0	0	0	0	0	0
2829CA	0	0	0	36	0	0	0	0	0	0	0	0
2829CB	0	0	0	36	0	0	0	0	0	0	0	0
2829CC	0	30	0	36	0	0	0	0	0	0	0	0
2829CD	0	0	0	36	0	0	0	0	0	0	0	0
2829DA	0	0	0	0	0	0	0	0	0	0	0	0
2829DB	0	30	0	0	0	0	0	0	38	0	0	0
2829DC	0	0	0	0	33	0	0	0	0	0	0	0
2829DD	0	0	0	0	33	0	0	0	0	0	0	0
2830AA	0	30	0	36	33	0	0	0	38	0	0	0
2830AB	0	0	0	0	0	0	0	0	0	0	0	0
2830AC	0	0	0	0	0	0	0	0	0	0	0	0
2830AD	0	0	0	0	0	0	0	0	38	0	0	0
2830BA	0	0	0	0	0	18	0	0	0	0	0	0
2830BB	0	0	0	0	0	0	0	0	0	0	0	0
2830BC	0	30	0	0	0	0	0	0	0	0	0	0
2830BD	0	0	0	0	0	0	0	0	0	0	0	0
2830CA	0	0	0	0	0	0	0	0	0	0	0	0
2830CB	0	30	0	0	0	0	0	0	0	0	0	30
2830CC	0	0	0	0	0	18	0	0	38	0	0	56
2830CD	0	0	0	0	0	0	0	0	0	0	0	0
2830DA	0	0	0	0	0	0	0	0	0	0	0	0
2830DB	0	0	0	0	0	0	0	0	0	0	0	0
2830DC	0	0	0	0	0	0	0	0	0	0	0	0
2830DD	0	0	0	36	0	0	0	0	0	0	0	36
2831AA	0	0	0	0	0	0	0	0	0	0	0	0
2831AB	0	0	0	0	0	0	0	0	0	0	0	0
2831AC	0	30	0	0	0	0	0	0	0	0	0	30
2831AD	0	0	0	0	0	0	0	0	0	0	0	0
2831BA	0	0	0	0	0	0	0	0	0	0	0	0
2831BB	0	30	0	0	0	0	0	14	0	0	0	44
2831BC	0	0	0	0	0	0	0	0	0	0	0	0
2831BD	0	30	0	0	0	0	0	0	0	0	0	30
2831CA	0	0	18	36	0	0	0	0	0	0	0	54
2831CB	0	30	0	0	0	0	0	0	0	0	0	30
2831CC	0	0	0	0	0	0	0	0	0	0	0	0
2831CD	0	0	0	36	0	0	0	0	0	0	0	36
2831DA	0	0	0	0	0	0	0	0	0	0	0	0
2831DB	0	30	0	0	0	0	0	0	38	0	30	98
2831DC	0	0	0	0	0	0	0	14	0	0	0	14
2831DD	0	30	0	36	0	0	0	14	38	29	0	147
2832AA	0	0	0	36	0	0	0	14	38	29	0	117
2832AB	0	30	0	0	0	0	0	14	38	29	0	111

2832AC	0	30	0	0	0	0	0	14	0	0	30	74
2832AD	0	30	0	0	0	0	0	14	0	29	0	73
2832CA	0	30	0	0	0	0	0	14	38	29	0	111
2832CB	0	30	0	0	0	0	0	14	0	0	0	44
2832CC	0	30	0	36	0	0	0	0	38	29	0	133
2929AA	0	0	0	0	0	0	0	0	0	0	0	0
2929AB	0	30	0	36	0	0	0	0	0	0	0	0
2929AD	0	0	0	36	0	0	0	0	0	0	0	0
2929BA	0	30	0	36	0	0	0	0	0	0	0	0
2929BB	0	30	0	36	0	0	0	0	0	0	0	0
2929BC	0	0	0	0	0	0	0	0	0	0	0	0
2929BD	0	0	0	36	0	0	0	0	0	0	0	0
2929CA	0	0	0	0	0	0	0	0	0	0	0	0
2929CB	0	0	0	36	0	0	0	0	0	0	0	0
2929CC	0	0	0	36	0	0	0	0	0	0	0	0
2929CD	0	0	0	36	0	0	0	0	0	0	0	0
2929DA	0	0	0	0	33	0	0	0	0	0	0	0
2929DB	0	30	0	36	0	0	0	0	0	0	0	66
2929DC	0	30	0	36	33	0	0	0	0	0	0	99
2929DD	0	0	18	36	0	0	0	0	0	0	0	54
2930AA	0	0	0	0	0	0	0	0	0	0	0	0
2930AB	0	0	18	0	0	0	0	0	0	0	0	18
2930AC	0	30	18	36	33	0	0	0	0	0	0	117
2930AD	0	30	0	36	0	0	0	0	38	0	0	104
2930BA	0	30	0	36	0	0	0	0	0	0	0	66
2930BB	0	0	0	36	33	0	0	0	0	0	0	69
2930BC	0	0	18	36	0	0	0	0	0	0	0	54
2930BD	0	0	0	0	0	0	0	0	0	0	0	0
2930CA	0	30	0	36	33	0	0	0	0	0	0	99
2930CB	0	30	0	36	0	0	0	14	38	0	0	118
2930CC	0	0	0	0	0	0	0	0	0	0	0	0
2930CD	0	0	0	0	0	0	0	0	0	0	0	0
2930DA	0	30	0	36	0	0	0	0	38	0	0	104
2930DB	0	0	0	0	0	0	0	0	0	0	0	0
2930DC	0	30	0	36	0	0	0	0	38	0	0	104
2930DD	0	30	0	36	0	0	0	0	38	29	0	133
2931AA	0	30	0	36	0	0	0	0	0	0	0	66
2931AB	0	0	0	0	0	0	0	0	0	29	0	29
2931AC	0	30	0	0	0	0	0	0	38	0	0	68
2931AD	0	30	0	36	0	0	0	0	0	29	0	95
2931BA	0	30	0	36	0	0	0	14	38	29	0	147
2931CA	0	30	0	36	33	0	0	0	38	0	0	137
3029AA	0	0	0	0	0	0	0	0	0	0	0	0
3029AB	0	0	0	0	0	0	0	0	0	0	0	0
3029AC	0	0	0	0	0	0	0	0	0	0	0	0
3029AD	0	0	0	0	0	0	0	0	0	0	0	0
3029BA	0	0	0	0	0	0	0	0	0	0	0	0
3029BB	0	0	0	0	0	0	0	0	0	0	0	0
3029BC	0	0	0	0	0	0	0	0	0	0	0	0
3029BD	0	0	0	0	0	0	0	0	0	0	0	0
3029CA	0	0	0	0	0	0	0	0	0	0	0	0
3029CB	0	30	0	0	0	0	0	0	0	0	0	0
3029DA	0	0	0	0	0	0	0	0	0	0	0	0
3029DB	0	30	0	0	0	0	0	0	0	0	0	30

3029DD	0	0	0	0	0	0	0	0	0	0	0	0
3030AA	0	0	0	0	0	0	0	0	0	0	0	0
3030AB	0	0	0	0	0	0	0	0	0	0	0	0
3030AC	0	0	0	0	0	0	0	0	0	0	0	0
3030AD	0	30	0	0	0	0	0	0	0	0	0	30
3030BA	0	0	0	0	0	0	0	0	0	0	0	0
3030BB	0	0	0	0	0	0	0	0	38	0	0	38
3030BC	0	30	0	0	0	0	0	0	38	0	0	68
3030CA	0	0	0	0	0	0	0	0	0	0	0	0
3030CB	0	30	0	36	0	0	0	0	38	29	0	133
3030CC	0	0	0	0	0	0	0	0	0	0	0	0
3030CD	0	30	0	0	33	0	0	0	38	0	0	101
3030DA	0	0	0	0	0	0	0	0	0	0	0	0
3130AA	0	30	0	36	33	0	0	0	38	0	0	137