Determination of the Rate and Distance of Pollen-mediated Gene Flow in Sorghum Using Cytoplasmic Male Sterile Varieties

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management has not previously been submitted by me for a degree at this or any other University; that it is my work in design and execution, and that all the material contained has been duly acknowledged.

Candidate: N.M. Mailula Signature Date----------

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DEDICATION

I dedicated this work to my wife, Mokgadi, who at all times stood by me and supported me financially and morally to accomplish this goal.

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Conducting this research has not been an easy task especially that I had no financial support. Prof. H.A. Shimelis and Prof. P.W. Mashela are sincerely thanked for their encouragement and assistance throughout the study. I also extend my gratitude to Prof. E. Nesamvuni and Dr. K. Nephawe who shared their valuable time with me and visited the research trials, where they provided feedback and encouragements. My friends Biza, Itu and Big Tom, who regularly accompanied me to the trial sites, gave me encouragement to go on with the study. The University of Limpopo provided experimental site at Syferkuil Experimental Farm, ICRISAT for supplying the cytoplasmic sterile lines while Mr. Mojapelo provided another site on his farm. Finally, I am indebted to my wife, Mokgadi and my two sons, Moeletsi and Motlatsi for creating a conducive family atmosphere which enhanced the completion of this work.

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ABSTRACT

Introducing genetically modified varieties in a new environment could potentially affect existing varieties or landraces due to gene flow as a result of cross pollination. Before the release of transgenic sorghum cultivars, detailed information is required on the rate and distance of pollen-mediated gene flow at various locations using non transgenic baseline environments. The objective of this study was to determine seed set in sorghum as affected by the direction of field establishment and distance of pollen travelled at Syferkuil and Roedtan in Limpopo Province. The study was conducted using three cytoplasmic male sterile sorghum varieties (MSVs) at the University of Limpopo in Capricorn District (Syferkuil) and Waterberg District (Roedtan) during the 2009/2010 growing season. Three MSVs (Ps213A, Ps256A and Ps278A) were planted in 100 m long rows with eight coordinates (N, NE, E, SE, S, SW, W and NW) branched from a central pollen source. Each row was marked at 5 m intervals namely at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 m away from the established pollen source. Total fertile and sterile panicles, panicle length, number of seeds per panicle, seed yield and hundred seed mass were collected. Significant ($P \leq 0.05$) interactions occurred among variety, distance and direction on the number of fertile panicles, sterile panicles and seed yield, both at Syferkuil and Roedtan. Significant ($P \leq 0.05$) differences on panicle length and hundred seed mass at both localities were observed. The number of seeds per panicle had significant ($P \leq 0.05$) interaction between variety and direction at Syferkuil and Roedtan. At Syferkuil the number of fertile panicles ranged between 1- 11 and sterile panicles between 1-11. The maximum panicle length was 30 cm. The maximum numbers of seed per panicle and seed yield were 1700 and 49 g/plot respectively. The minimum 100-seed mass

was 5 g/100 seed. At Roedtan, the number of fertile seed per panicle ranged from 14 to1723 and the lowest seed yield was 0.04g/plot. Results demonstrated the presence of clear differences among the MSVs on performance of agronomic traits. Variety Ps213A had the highest seed set and overall high performance, followed by Ps256A and Ps278A. Further, there was a considerable reduction in seed set among all varieties with continued distance from the pollen source. There was a greater seed set among the MSVs in the north easterly direction at Syferkuil and easterly direction at Roedtan. Inconsistent responses were noted for other agronomic traits with respect to direction from the pollen source. These results also illustrated the presence of considerable amount of seed set extending to 100 m away from the pollen source. In conclusion the distances used in this study were not sufficient to avoid genetic contamination. Further studies with relatively longer distances from pollen sources are therefore, required to allow for sound conclusions on the least isolation distance to preclude seed set due to cross pollination. The second order interaction was highly significant ($P \leq 0.05$) for the number of fertile panicle at Syferkuil while at Roedtan it was not. At Syferkuil, variety, direction, distance × direction and variety × distance × direction contributed 42%, 29%, 18% and 8%, respectively, to the total treatment variation (TTV) in the number of fertile panicle. At Roedtan, distance, direction and distance × direction contributed 64%, 19% and 11%, respectively, to the TTV in the number of fertile panicle. At Syferkuil, distance, direction, distance × direction, and variety × distance × direction contributed 13%, 44%, 27% and 12%, respectively, to the TTV in the number of sterile panicle while at Roedtan, direction, distance × direction and variety × distance × direction contributed 14%, 48% and 24%, respectively, to the TTV in the number of sterile panicle. At Syferkuil, variety × direction and distance × direction contributed 14% and 11%, respectively, to the TTV in the number of seeds

per panicle. At Roedtan only variety contributed 67% to the TTV in terms of number of seeds per panicle. At Syferkuil, distance direction, distance × direction contributed 40%, 28% and 17%, respectively, to the TTV in seed yield. At Roedtan, distance × direction and variety × distance ×direction contributed 29% and 50%, respectively, to the TTV in seed yield.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Sorghum (*Sorghum bicolor L*.) has the centre of biodiversity in Africa and is a critical component of food security for more than 100 million people on the continent (De Wet and Harlan, 1971). Anthropological evidence suggests that hunter-gatherers consumed sorghum as early as 8 000 BC (Smith and Frederiksen, 2000). The domestication of sorghum has its origins in Ethiopia and surrounding countries, commencing around 4000-3000 BC (De Wet and Harlan, 1971). Numerous varieties of sorghum were created through the practice of disruptive selection, whereby selection for more than one level of a particular character a population occurred (Doggett, 1970). A balance of farmer selection for cultivated traits and natural selection for wild characteristics generated improved sorghum types, wild types and intermediate sorghum types (Doggett, 1970). Improved sorghum types were incidentally spread via the movement of people and trade routes into other regions of Africa, India and Middle East, eventually into the Far East. By the time sorghum was transported to America during the late 1800s to early 1900s, the diversity of new sorghum types, varieties and races created through the movement of people, disruptive selection, geographic isolation and recombination of new types in different environments exacerbated would have been large (Doggett, 1970; Wright, 1931). Several wild relatives of cultivated sorghum are found in Africa, both in natural habitats and as weeds in farmer's fields.

Sorghum is grown predominantly in low-rainfall, arid to semi-arid environments (Rosenow and Clark, 1995). Globally, the occurrence of drought stress is a major

constraint to sorghum production (Ellis *et al.,* 1997). Two critical drought stress periods had been identified in sorghum production, which occur during panicle differentiation prior to flowering and grain filling stage (Rosenow and Clark, 1995). Identification of varieties and lines with naturally high levels of pre-anthesis drought tolerance and their selection for higher yields resulted in sorghum varieties with stable and higher yields (Ellis *et al.,* 1997). Post-anthesis drought stress can result in significant yield loss due to small grain size, premature plant death and susceptibility to diseases (Ellis *et al.,* 1997). Post-flowering drought tolerance is referred to as stay-green, with plants maintaining green leaf area and photosynthetic capability under severe moisture stress, which results in higher grain yields compared with senescent varieties (Borrell and Douglas, 1997; Borrell *et al.*, 1999). Physiological components of stay-green include green leaf area at flowering, time of onset of senescence and rate of senescence (Borrell *et al.*, 1999). These features are independently inherited and easily combined through breeding, resulting in new sorghum varieties that exhibit high levels of stay-green with stable high yields and good levels of insect resistance (Borrell *et al.,* 2000).

Sorghum production is affected by various pests and diseases. Some of the major pests are midge (*Stenodiplosis sorghicola* Coquillett), green bug (*Schizaphis graminum* Rondani), various aphids, shoot fly (*Atherigona soccata* Rondani) and stem borer (*Chilo partellus* Swinhoe) (Sharma, 1993). Major diseases include downy mildew, anthracnose, sorghum rust, leaf blight, ergot and head and kernel smut (House, 1985). Successes in breeding for resistance in sorghum varieties have been varied. Resistance to some pests is quantitatively inherited and therefore, difficult to transfer into high-yielding cultivars (Tao *et al.*, 2003). The exception to this is midge resistance, where high levels of midge immunity have been incorporated from Indian

American and Australian breeding lines into elite, high-yielding sorghum varieties in Australia, with greater than 80% of the planted area using these resistant varieties (Jordan *et al.*, 1998; Tao *et al.*, 2003).

Development of disease-resistant sorghum varieties has relied on identifying sorghum varieties/landraces with natural genetic resistance to the particular disease. Commercial sorghum varieties are being developed with resistance to grain moulds and anthracnose (Reddy *et al.*, 2006). Although domestication and the resulting super-domestication of sorghum have relied on principally *S. bicolor* subsp. *bicolor* varieties/landraces/lines for significant gains in agricultural production, the undomesticated *Sorghum* species offer an untapped wealth of novel traits for both biotic and abiotic stress resistance and yield (*Jordan et al.*, 1998; *Tao et al.,* 2003).

Recently, sorghum breeding efforts have utilized a range of biotechnological tools to enhance (or speed up) cultivar development. Advances in biotechnology culminated in genetic engineering of most crops with economic importance (Conner, 1997). Undoubtedly, the technology had profound potential in improving the ever increasing demand for food, more so in developing countries. Nonetheless, critical concerns have been raised about the potential risks posed by genetically modified (GM) crops on the environment (Ellstrand, 2003a). Foremost, among these concerns is the potential escape of trans-genes from cultivated crops to their wild and weedy relatives through gene-flow. The possible harmful consequences of such escape are the evolution of more aggressive weeds in agricultural systems, the generation of more invasive species in natural habitats, the gradual replacement of wild gene pools by cultivated ones and in some extreme cases, the extinction of crop wild relative populations (Conner, 1997; Ellstrand, 2003a). Scientific assessment of these

potential environmental risks is an integral part of biosafety regulations and therefore precedes any decision to release a GM crop (Ellstrand, 2003a).

1.2 Research problem

GM sorghum, being developed to solve agronomic or nutritional problems in developing countries, may be grown near centres of origin and biodiversity of sorghum, where the plants were first domesticated and remain a major crop (Baker, 1972). Often genetically modified sorghums (GMs) are being developed by publicly funded, non-profit institutions. Spontaneous, morphologically-intermediate plants between cultivated sorghum varieties and their wild relatives have been reported in and near sorghum fields in Africa (Baker, 1972; De Wet 1978; Dogget and Majisu, 1968; Dogget and Prasada-Rao, 1995; Mutegi *et al*., 2009; Tesso *et al*., 2008). Moreover, crop-to-wild plant hybridisation in sorghum had been implicated in the origin of *S. almum Parodi* a noxious weed, and on enhanced weediness and invasiveness of Johnson grass (*S. halfpence* (L.) Pers.) (Ellstrand *et al*.,1999). Prediction of the extent and direction of introgression between sorghum varieties and wild weedy relatives is, thus, an important part of environmental risk assessment of transgenic sorghum. Although, Africa is the centre of origin and biodiversity for sorghum, unfortunately environmental risk assessment studies have been reported with limited information. This study would provide the much needed information regarding the male sterile varieties (MSVs) when planted at eight coordinates N, NE, E, SE, S, SW, W and NW, branched from a central pollen source. The MSVs would be assessed for seed and number of productive panicles set at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 m away from the established pollen source. This study would show if there were any significant

differences among varieties, as well as distances and directions from the pollen source for seed set and number of productive panicles per plot. Seed set (g/plot) variation among varieties, arm directions, as well as arm distances would be determined. In general, the study would inform concerned groups about the distance and magnitude of seed set and number of productive panicles away from the pollen source.

1.3 Motivation of the study

Before release of GM cultivars, detailed research is needed on the distance and direction of pollen-mediated gene-flow in sorghum at various locations using non-transgenic baseline environments. Thus, results from this study would help in risk assessment and to provide baseline information regarding safe introduction and deployment of GM cultivars. Also, baseline information would be valuable towards protecting existing germplasm from genetic contamination in farmers' fields.

1.4 Aim and objectives of the study

1.4.1 Aim

The aim of this study was to generate baseline information with regard to genetic contamination of non-GM sorghum varieties with GM sorghum varieties.

1.4.2 Objective

To determine seed set and agronomic responses in sorghum through pollen-mediated gene-flow at two localities using three cytoplasmic male sterile varieties planted at eight coordinates and 20 distance intervals from a fertile pollen parent.

1.5 Hypothesis

There is no difference in seed set and agronomic responses across locations among the cytoplasmic male sterile sorghum varieties when established at eight coordinates and 20 distance intervals from a fertile pollen parent.

1.6 Significance of the study

In this study, seed set in sorghum as affected by the distance and direction of gene-flow would be quantified. Outputs from the work would contribute to development of biosafety regulations and guidelines for the introduction of transgenic sorghum in Limpopo Province, South Africa. The significance of the study was to contribute to the understanding of the gene-flow related environmental risks of releasing GM sorghum into Limpopo Province agro-ecosystem and contribute to biosafety, conservation and utilization decisions regarding sorghum in the Province. Understanding the distance and direction of movement of genes between domesticated and wild and/or weedy relative populations of sorghum is the first step in characterizing the potential environmental risks of escaped transgenes. Such information is currently lacking, but is critically needed for science-based decision making by biosafety regulators.

1.7 Format of dissertation

Following this General Introduction, Literature on the research problem would be reviewed (Chapter 2), with Chapter 3 addressing the listed objective. Finally, the summary, conclusion and recommendations for future research would wrap up the study (Chapter 4).

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Worldwide, Sorghum [*Sorghum bicolor (*L.) Moench] is the fifth most important grain crop, after maize (*Zea mays*), wheat (*Triticum species*), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) in terms of production and area planted (Taylor and Emmambux, 2008). In Africa, sorghum is the second highly produced cereal after maize (Markus and Gurling, 2006). Sorghum is able to grow with less water than other cereals such as maize and wheat, and is well-adapted to growing in many arid and semi-arid regions of the world (Markus and Gurling, 2006; Taylor and Emmambux, 2008). Africa is the centre of diversity for sorghum species, (Ellstrand *et al*., 1999). Thus, with increased interest in introducing GM sorghum cultivars, there is an inherent possibility of having trusted local sorghum varieties cross-pollinated from the introduced or tested GM varieties. This cross-pollination would constitute a resource for risk, since the produced plants may end-up out-competing the trusted local varieties. The aim of this chapter was to review the work already done on the previously outlined research problem.

2.2 Work done on the research problem

2.2.1 Interspecific cross pollination in sorghum

Sorghum belongs to the family Poaceae, and the genus *sorghum* is subdivided into five sections. The section sorghum includes three species *S. halepense* (weed, Johnson grass), *S. propinquum* (Kunth) Hitchc. (perennial, fully fertile with *S. bicolor*), and *S. bicolor* with three subspecies (De Wet, 1978; Doggett, 1988;

Frederiksen, 2000; Smith and Raemakers, 2001). Three *S. bicolor* subspecies are *S. bicolor* subsp. *bicolor* (cultivated species, five main races), *S. bicolor* subsp. *Arundinaceum* (Desv.) synonym to subsp. *Verticilliflorum* (Steud). Stapf (the wild progenitor of cultivated sorghum, four main races and *S. bicolor* subsp. *Drummondii* (Steud). (Sudan grass, weed) (De Wet, 1978). No reproduction barrier exists between cultivated *S. bicolor* subsp. *bicolor* and its wild progenitor *S. bicolor* subsp. *arundinaceum* (hybrids form shatter cane-type weeds) and the weed *S. bicolor* subsp. *drummondii* (De Wet, 1978; Doggett, 1988). *Sorghum bicolor*-almum Parodi is a rhizomatous hybrid between *S. bicolor* and *S. halepense* and is occasionally cultivated as a fodder grass (De Wet, 1978), when backcrossed to *S. halepense*, *S. bicolor alumum* can give rise to aggressive weeds, including Johnson grass, which was classified as one of the most noxious weeds in the world (Holm *et al*., 1977).

Sorghum is largely self-pollinated, but wind pollination between plants does occur, (De Wet, 1978). Subspecies or varieties of sorghum with open, grass-like panicles, such as Sudan grass have a higher rate of out-crossing than sorghum, with compact heads typical of commercial hybrids. Out-crossing also varies by location on the panicle, with much higher rates at the top of the panicle, where flowering initiates (Maunder and Sharp, 1963).

Existing potential in wild sorghum gene pool for providing new sources of resistance and adaptation in breeding has been documented (Dillon *et al.,* 2007; Gurney *et al.,* 2002; Hajjar and Hodgkin, 2007; Kamala *et al.,* 2002; Kameswara *et al.,* 2003; Komolong *et al.,* 2002; Sharma and Franzmann, 2001). The relationship between cultivated sorghum and its wild relatives for being inter-fertile and growing in

sympatric patterns in most agro-ecosystems of sub-Saharan Africa had long been documented (Dogget, 1988; Dogget and Majisu, 1968; Dogget and Prasada-Rao, 1995). For example, Dogget and Majisu (1968) studied the relationship between cultivated sorghum and its wild relatives in East African countries and provided morphological evidence of cultivated wild complexes. The rate and direction of gene-flow between cultivated varieties and their ecotypes in Africa have not been investigated. In most parts of Africa a wide assortment of intermixed cultivars of sorghum are being grown in scattered cultivated plots often in close proximity to its wild forms (Barnaud *et al*. 2007; Dogget, 1988; Tesso *et al*., 2008), with eminent outcrossing risks.

2.2.2 Advances in developing GM sorghum

Optimised protocols for genetical transformation of sorghum based on either agrobacterium or particle bombardment techniques have been in place for some time (Casas *et al.,* 1993; Casas *et al.,* 1997; Gao *et al.,* 2005; Howe, 2000; Zhao *et al.,* 2000). Successful genetic engineering of sorghum had been reported for chitinase genes against fungal diseases (Ayoo, 2008; Krishnaveni *et al.,* 2000; Zhu *et al.,* 1998), *Bacillus thuringiensis* (bt) genes against stalkborer (Girijashankar *et al*., 2005) and alpha-hordothionin protein gene originating in barley (*H. vulgare*) for high lysine content (Zhao *et al*., 2000). Ayoo (2008) used the Kenyan sorghum landraces. Elsewhere, similar efforts to transform sorghum have been underway, prominent among them being the initiative by the Bill Gates African Bio-fortified Sorghum Project, with the primary aim of deploying a nutritionally improved and highly digestible transgenic sorghum to subsistence farmers in Africa (Zhao, 2000). Currently there is an urgent need to generate science-based gene-flow data in

cultivated-wild complexes of sorghum for use in African biosafety regulators with regard to testing and commercially releasing transgenic sorghum. In Kenya for example, a biosafety law had been enacted (Zhao, 2000), paving a way for on-farm deployment of transgenic varieties.

2.2.3 Inter-specific gene-flow in sorghums

Gene-flow involves the movement and incorporation of alleles from the gene pool of one population into the gene pool of another distinct population (Slatkin, 1987). Gene-flow in its broadest sense is the dispersal of genes through pollen grains, seeds or vegetative reproduction units and could not said to have occurred if the migrant alleles do not persist in the progeny of the recipient population (Slatkin, 1987). Natural out-crossing rate is variable among *S. bicolor* strains and varieties, while preferential self-pollinated and out-crossing rates among Sorghum species can reach 26% for grain-type sorghum with compact panicle typical in commercial hybrids and 61% for open grass-like panicle such as *S. sudanensis* (Ellstrand and Schierenbeck, 2000).

Gene-flow naturally exists between individuals that belong to different Sorghum species and within or between gene pools. The most widely recognised interspecific sorghum weed, shatter cane arose from the hybridisation of subspecies S. *bicolor* and its wild relatives (Harlan, 1992). Though not widely adopted, shatter canes are also described as hybrids between sorghum crop and *S. halepense* (Harlan, 1992). Outcrossing can also involve individuals with different ploidy levels (Dogget; 1988). For instance, *almum* is a tetraploid grass that arose from a natural cross between the tetraploid *S. halepense* and the diploid *S. bicolor* (Doggett, 1988). Sorghum

halepense weed was also described as an allopolyploid perennial weed resulting from the cross between *S. propinquum* and *S. bicolor* (Ellstrand and Schierenbeck, 2000). Hybrids between grain sorghum (2n = 20) and *S. halepense* (2n = 40) had been shown to include highly sterile 30-chromosome and relatively fertile 40-chromosome types (Ellstrand and Schierenbeck, 2000). In contrast, the process that includes mechanisms and circumstances that promote spontaneous transfer of pollen and successful fertilisation is not gene-flow; but introgressive hybridisation (Doggett, 1988). Incidentally, from an evolutionary, ecological and risk assessment viewpoint it is important to make the distinction between gene-flow and hybridisation. Hybridisation alone is not gene-flow, but successful hybridisation is essential if introgression or gene-flow is ultimately to occur (Ellstrand and Schierenbeck, 2000).

2.2.4 Hybridisation

Plant hybridisation has been actively studied for decades, with the significance of this phenomenon as an evolutionary force been a subject of intense debate, was it through hybridisation that naturally growing crops changed into modern evolved crops? Anderson (1949) outlined conditions for successful fertilisation and the potential consequences of such events, particularly as they relate to speciation. Others have explored the subject from a modern evolutionary perspective (Ellstrand *et al*., 1999). According to Ellstrand (2003a), if hybridisation is to be successful, a simple set of conditions must be met: ranges of the population in question must overlap. If the populations are not reasonably close to each other geographically, then high levels of hybridisation are unlikely to occur, regardless of any other condition, centres on populations sharing a common pollination vector should allow for spontaneous pollen transfer under field and/or natural conditions. Such pollen

transfer requires that the plant populations in question have overlapping flowering times in the area of sympatric patterns (Ellistrand *et al*., 1999). In addition, some degree of sexual compatibility must exist between the donor and recipient plants (Ellstrand, 2003a). Even modest levels of compatibility that produce only a few viable offspring per reproductive cycle, would be sufficient for establishing a population of hybrid plants with different lineages (Ellistrand *et al*., 1999). The presence of a population of hybrid plants with different lineages, referred to as a hybrid swarm, may also facilitate gene-flow (Ellstrand, 2003a).

Conditions for hybridisation are often met among plant groups (Doggett, 1988; Ellstrand, 2003a). Raybould and Gray (1993) proposed that most populations of *S. halepense* in North America represented a hybrid swarm that resulted from repeated mating events between *S. bicolor* and the weedy Johnson grass introduced to USA in the mid1800s. Johnson grass and sorghum are fully sympatric and they are wind-pollinated and sexually compatible, producing roughly 5% viable hybrid offspring when allowed to cross under field conditions (Arriola and Ellstrand, 1996). Successful hybridisation between the two species is fairly common, with different cases tending to have idiosyncratic features (Ellistrand, 2003a).

Many variables such as pollen load from the donor or competition on the stigmatic surface can affect successful fertilisation in the field. However, available data suggest that, in most cases, spontaneous hybridisation occurs at levels that are experimentally detectable and are most likely to be evolutionarily significant (Ellstrand, 2003a). Studies conducted on model systems, using domesticated species and compatible wild relatives have continuously demonstrated that pollen

exchange and hybrid formation can occur at great distances and pollen movement cannot be easily controlled or managed (Gressel and Rotteveel, 2000).

2.2.5 Introgression

Introgression is the permanent incorporation of genes from one set of differentiated populations to another (Rieseberg and Wendel, 1993). Hybridisation is only the first part of the gene-flow process. Introgression of introduced alleles is also required for gene-flow to occur. Whether the introduced alleles persist in the long-term ultimately depends on factors such as the nature of the introduced trait, the genetic and environmental background in which it is expressed and levels of local selection (Rissler and Mellon, 1996). Introduced alleles have the inherent capacity to persist (Linder *et al.,* 1998; Whitton *et al*., 1997). Although most studies used crop-wild complexes (Whitton *et al*., 1997), the data remain important and could be viewed to infer that persistence of introduced alleles is a more general situation. Although the presence of F1 plants in the short term does not generally guarantee introgression (Linder, 1998), continued exchanges of pollen in hybrid swarms can increase the likelihood for introgression. However the swarm's successive generations of introgressed plants are most likely to be generated as a result of repeated backcrossing between F1 and parental types (Whitton *et al*., 1997).

Backcrossed generations, most likely contain most fertile hybrids that have significant potential for persistence and spread of newly introduced alleles (Oka and Chang, 1961). Hybridisation and the subsequent introgression of novel allelic variation had been of an evolutionarily significant in plant groups (Rieseberg, 1997). However the challenge had been to identify populations where introgression has

occurred. Historical levels of gene-flow could be estimated but plants that share common ancestry or experience similar selection pressures deepened the challenge as these phenomena might work to increase degrees of genetic and morphological similarities among interacting groups (Barrett, 1983; Small, 1984). The challenges of interpreting the outcome of introgression simply demonstrate the complexities of the process.

2.2.6 Consequences of gene-flow

Slatkin (1987) argued that gene-flow was a constraining force that could result in either the homogenization of distinct gene pools or the loss of allelic diversity. Earlier, Baker (1974) suggested that hybridization and introgression among crops and their wild relatives have led to increased development of weedy characteristics of most wild plants, thus improving their competitive ability under agrestic conditions. Gene-flow can be a potent evolutionary force that had since influenced the genetic structure of the gene-pools involved. In the early 1930s, theorists predicted that gene-flow occurring at a rate of one incorporated individual per generation suppress the effect of genetic drift in small populations (Wright, 1931). It was furthermore thought that gene-flow could suppress local selection pressures in small populations provided that the rate of influx of new alleles was sufficiently high. However, predicting the specific outcome of any gene-flow event was then difficult. Some theorists suggested that gene-flow could enhance genetic variation and fitness in recipient populations.

Concern had repeatedly been expressed that introgression of alleles into small or rare populations might lead to the extinction of those populations (Blancas, 2001;

Ellstrand, 1992; Rhymer and Simberloff, 1996), which was particularly true for gene-flow between crops and their wild relatives. However, few empirical studies available to date suggest that wild domesticated hybrids were usually fit and would likely persist as either their crop or wild parent (Arriola and Ellstrand, 1997; Klinger and Ellstrand, 1994; Langevin *et al*., 1990). However, conclusions on outcomes of gene-flow events had been unwisely given the idiosyncratic nature of both hybridisation and introgression. Further, attempts to control or manage gene-flow would have been difficult and ineffective which raised questions regarding the fate of transgenes in crop plants with sympatric and compatible relatives.

2.2.7 Gene pools

The ability of plants to interbreed is often described in terms of gene pool, based on the degree of sexual compatibility (Harlan and De Wet, 1971). Plants belonging to the primary gene pool easily cross with genes being readily and simply transferred (Harlan and De Wet, 1971). Plants belonging to the secondary gene pool include biological species that would cross with the crop, with the possibility of gene transfer being difficult to achieve. Tertiary gene pool plants can cross, but are normally anomalous, lethal or completely sterile (Harlan and De Wet, 1971). Gene transfer between plants in this category is either impossible or requires innovative artificial techniques.

Traditionally, plant breeders used materials from within the primary gene pool as sources of genetic diversity (Gepts and Papa, 2003). Domesticated crops generally belong to the same biological species as their wild progenitors and are fully compatible with them when hybridised. Use of genetic engineering to bypass sexual

incompatibility barriers and introduce new genes into existing cultivars had since given rise to a fourth gene pool (Gepts and Papa, 2003).

2.2.8 Proximity

Most crops in the world had been exported from their centres of diversity and therefore are not cultivated close to their wild relatives (Harlan and De Wet, 1971). For example, no wild relatives of maize or soybean are found in USA while In Europe gene-flow between crops and their wild relatives is also restricted because European crops are mostly exotic plants that lack relatives among the European flora (Harlan and De Wet, 1971). However, oilseed rape, grasses and several fruit crops have varying degrees of sexual compatibility with a number of wild relatives in Europe.

Other crops, such as maize, have no wild relatives with which they can potentially cross-pollinate in Europe. Similarly, maize, cotton and soybean have no close wild relatives in USA because maize and cotton were domesticated in Mexico or South America and soybean was domesticated in China (Harlan and De Wet, 1971). In contrast, sunflower and strawberries have wild relatives in USA, which is the centre of their origin (Harlan and De Wet, 1971). In some cases, introduced crops do not have progenitors but have wild relatives with which they could be compatible. Examples include *Gossypium* spp. in Australia and *S. halepense* in North America and Europe (Gepts and Papa, 2003).

2.2.9 Phenology

In addition to being compatible and in close proximity, introgression requires that any pre-mating and post-mating barriers be overcome. Parents need to have overlapping flowering times and common pollinators or modes of pollination (Freyre *et al.*, 1996; Langevin *et al*., 1990; Ottaviano *et al*., 1988). Flowering times between crops and wild relatives must coincide so that pollen from one can potentially effect fertilisation in the other. Weedy relatives can act as pollen donors and increase the probability of overlap between the flowering periods of wild and crop species.

2.2.10 Hybrid fitness

For gene-flow through hybridisation to occur, pollen grains must achieve fertilisation and seeds must germinate and produce sexually mature plants. Hence, first generation hybrids should be sufficiently fit to survive to sexual maturity and yield viable and fertile progeny that can backcross to the wild progenitor to maintain the newly transferred genes in the wild relative's gene pool. The descendants of the backcross also need to survive and establish a new population that will contain the novel genetic material in addition to parental genetic material. The term fitness indicates a short-term measure of expected reproductive success and includes characteristics such as age-dependent viability, age-dependent fecundity, age at maturity, seed production rate and germination success. Generally, the permanent transfer of genes from a crop to wild or weedy relatives is more likely to succeed if the trait is neutral or beneficial to the hybrid population. The fitness of crop hybrids is generally assumed to be low under field conditions (Hancock *et al.,* 1996) but successful hybrids have been found between most crops and their wild populations (Smartt and Simmonds, 1995). Hybrids are generally rare, often sterile and relatively

few populations exist, except where the parents remain in contact or where they are able to spread vegetatively (Raybould and Gray, 1993).

2.2.11 Pollen dispersal and pollination

The extent of cross-pollination between fields of crops or between plant populations is dependent largely on the scale of pollen emission and to some extent on pollen movement (Raybould and Gray, 1993). Pollen dispersal can be heavily influenced by changes in temperature, humidity, light, wind and rain. Seed and pollen flow contributed to the transfer of genes from weed populations of European sugar beet into neighbouring wild populations and that seed flow play a major role in the establishment of weed plants.

Different crop species have different pollination mechanisms and different seed dispersal patterns. As well as being dispersed by air currents and wind, pollen can be effectively distributed by insects to distances of at least 1 km (Devlin and Ellstrand, 1990; Ellstrand and Marshall, 1985). Wind-carried pollen has been found hundreds of kilometres from its point of origin (Ehrlich and Raven, 1969). Physical features in the immediate local environment such as the nature of the plant canopy, surrounding vegetation and topography can heavily influence patterns of pollen dispersal. The probability of gene transfer via pollen is much smaller when crops and weeds are self-pollinating. Self-pollinating plants such as wheat and rice use their own pollen to produce seeds. They make minimal use of pollen that might be available from nearby plants, although some exchange among plants does occur. In general, outcrossing species have a wider pattern of pollen distribution than inbreeding ones (Hancock *et al*., 1996).

Harmonic radar showed that most bees regularly fly over the range 70-631 m from the nest to forage. The results support the hypothesis that Bumble bees do not necessarily forage close to the nest, and illustrate that studies on a landscape scale are required to evaluate bee foraging ranges fully with respect to resource availability. Such evaluations are required to underpin assessments of gene-flow in bee-pollinated crops and wild flowers.

2.2.12 Crop to weed gene-flow

Natural gene-flow between cultivated and wild and weedy sorghums in areas where they are sympatric has also led to gene exchange between the cultivated crop and wild relatives. This natural crossing has enhanced co-evolution and improved adaptation of crops to changing environmental conditions, which is a major advantage of *in situ* conservation strategies for plant genetic resources over *ex situ* techniques. Several studies (Arriola and Ellstrand, 1996; Sangduen and Hanna, 1984) showed that under natural conditions, crop-to-weed gene exchange is very likely in sorghum.

Success in moving genes between crop and wild relatives depends on several factors including cross ability, spontaneous hybridisation, fertility, and fitness of the resultant hybrids. Potential hybridisation of cultivated sorghum (*S. bicolor*) with Sudan grass (*S. sudanense*) and its feral relatives (*S. almum* and *S. halepense*) was assessed for three congeners commonly growing in natural habitats near sorghum fields. However, although the potential for gene-flow among this group of plants was recognised to be high, no deliberate study has been carried out, except for S.

halepense, to characterize the extent of crossing and nature of hybrid progenies among these weedy species.

Pollen from agricultural crops often reaches wild plants growing nearby, and when the wild species are closely related to the crops, hybridisation often ensues (Ellstrand *et al.,* 1999; Hancock *et al.,* 1996). Gene-flow from crops to wild relatives has been happening for millennia (Ellstrand *et al.,* 1999; Hancock *et al.,* 1996), but concern about it has become widespread with the increasing cultivation of transgenic crops (Ellstrand, 2001; Snow and Moran-Palma, 1997), especially in view of reports of escaped transgenes (Brasher and Snow, 2002; Quist and Chapela, 2001). Regardless of whether transgenes are involved, the consequences of crop–wild gene-flow can be problematic. One possible consequence is that crop genes may replace wild ones; this is known as genetic assimilation (Wolf *et al.,* 2001). Obviously, this is a conservation problem because it threatens the genetic diversity of wild populations. Another possible consequence is that if hybrids are less fertile than their wild parents, then wild populations may shrink; this is known as demographic swamping (Wolf *et al.,* 2001). It is a conservation problem, because smaller populations are more vulnerable to habitat disruption, inbreeding depression and other risks (Ellstrand and Elam, 1993). Demographic swamping can aggravate genetic assimilation, in that as a wild population shrinks, it is likely to produce less pollen, so any pollen it receives from a crop becomes a higher fraction of the total (Ellstrand and Elam, 1993).

A critical impediment to field testing and deployment of transgenic sorghum is the risk of gene-flow to weedy relatives through pollen. Crop-to-weed gene-flow has

been documented from sorghum to jobson grass [*Sorghum halepense (L.) Pers*.] a noxious weed, at distances of up to 100 m (Arriola and Ellstrand, 1996). Although the triploid progeny of Johnson grass crossed with sorghum hybrids would be expected to be sterile, viable seed production on Johnson grass crossed sorghum hybrids has been reported (Arriola and Ellstrand, 1997; Hoang-Tang and Lian, 1988) and the fitness of hybrid progeny found to be equivalent to Johnson grass (Arriola and Ellstrand, 1997), causing those authors to conclude that a trans-gene that is either neutral or beneficial to Johnson grass would likely persist in populations growing in agricultural conditions under continued gene-flow from the crop.

Shatter cane, a noxious weed of economic importance to commercial corn (*Z. mays* L.), soybean (*Glycine max* L. Merrill*)* and sorghum production, crosses freely with cropped sorghum (De Wet and Harlan, 1971). Previous field research, involving Sudan grass, a cultivated form of *S. bicolor subsp. drummondii* showed natural out-crossing averaging 39% and 57% in each of two years. Similarly out-crossing is present in shatter cane, with gene-flow from cropped sorghum to shatter cane populations being rapid.

The use of cytoplasmic male sterility to prevent the release of viable pollen from transgenic corn has been recommended by different researchers. In their system, transgenic plants are male sterile and are grow in a mixture with fertile non transgenic pollen donors. Male fertile pollinator mixtures have already proven commercially successful for the production of high oil maize and high grain quality maize (Bergquist *et al*., 1998). Apparently, It appears male sterile: male fertile pollinator mixtures could be exploited to reduce the threat of trans-gene-flow through

pollen in sorghum production could be commercially viable. Nearly all commercial sorghum hybrids are currently produced by means of cytoplasmic male sterile seed parents. This is at least partially due to the availability of many lines known to restore fertility R-line when crossed to A.sub.1 cytoplasm (Torres-Cardona *et al.,* 1990). Since many lines are known to restore fertility in A.sub.1 cytoplasm, the probability of male fertile volunteer escapes following sorghum harvest is also high. The risk of trans-gene-flow from fertile volunteer escapes is unacceptable for sorghum hybrids based on A.sub.1 male sterile cytoplasm. Even if lines known to maintain A.sub.1 cytoplasmic male sterility B.sub.1lines were to be used as pollinators in the mixture of stray pollen from shatter cane or neighbouring sorghum fields with the capacity to restore fertility to A. sub.1 cytoplasmic male sterile could be expected to pollinate some of the transgenic sorghum females and introduce male fertile transgenic plants into the population of volunteer escaped plants (Pedersen *et al.,* 2003).

2.2.13 Wild relatives of crops

Ellstrand (2003b) reported that the wild relatives of modern crops are critical in the search for new genes to improve the quantity and quality of agricultural production and reduce risk of crop losses by providing resistance to biotic and abiotic disturbances. Valuable genes obtained from crop's wild relatives are used to improve yield, resistance against specific pests and diseases, tolerance to abiotic stresses and nutritional qualities.

Modern cultivars of most crops now contain some genes that are derived from a wild relative, whether through natural gene-flow or human-induced processes. Some crops have sexually compatible relatives in the form of wild plants and arable weeds

that grow in the same geographic region (Ellstrand, 2003b). Weedy relatives are those that are undomesticated but invade disturbed areas such as cultivated plots and roadsides. In agro-ecosystems, weedy relatives of crops tend to have innate ruder characteristics such as the ability to rapidly establish vegetative cover, produce large amounts of seeds that can be stored in the soil for the next growing season or seasons, and to persist under difficult growing conditions. A crop's weedy forms can act as a natural bridge relaying genes from crops to wild populations and from crops to crops by pollen and seed at a landscape level. Companion weeds and related crops may overlap at the boundaries of cultivated plots, thus may continuously give rise to new forms (Ellstrand, 2003b).

2.2.14 Barriers of gene escape from transgenic plants

Both agronomic and environmental concerns mandate the development of effective strategies for isolating transgenic varieties from related non-transgenic varieties or cross fertile weeds. Morris and Kareiva (1994) presented the results of the first field experiment designed to test the effectiveness of two containment strategies that are commonly used in field trials of transgenic crops: (1) an isolation zone devoid of vegetation to discourage emigration of insect pollinators from transgenic plots; and (2) trap crops (non-transgenic pollen). In conjunction with field trials of genetically engineered canola (*Brassica napus*) conducted by Cal gene in California and Georgia, they varied both the width of the barren zone and the presence or absence of a trap crop, and measured the effects on gene escape.

Escape was easily detected since the genetic construct inserted into the transgenic canola contained a gene that rendered seedlings resistant to the normally lethal

antibiotic kanamycin **(**Morris and Kareiva, 1994). Their results suggested that barren zones 4-8 m in width may actually increase seed contamination over what would be expected if intervening ground were instead planted entirely with a trap crop. When trap crops occupied a limited portion of the isolation zone separating transgenic and non-transgenic varieties, the effectiveness of the trap depended on the width of the isolation zone: They reduced gene escape when the two varieties were separated by 8 m, but increased escape across a 4 m isolation zone. Morris and Kareiva (1994) concluded that, for the relatively short isolation distances they examined, the most effective strategy for reducing the escape of transgenic pollen is to devote the entire region between transgenic and non-transgenic varieties to a trap crop.

Barriers to gene-flow: Barriers that prevent gene-flow via pollen include physical, biological, mechanical, spatial and temporal barriers.

Physical barriers to gene-flow: Physical barriers are barriers that will physically prevent pollen from moving out of the zone of production. An obvious physical barrier is a glass house. If a crop is grown inside of a glass house, then pollen movement is severely restricted, but there is still a finite possibility of escape. Under field conditions, 30 ha of field is surrounded by trees or other fast growing species that are taller than sorghum and would provide both a wind break and a physical barrier to the movement of pollen from the field (Morris and Kareiva, 1994).

Biological barriers to gene-flow: There have been several reports written about biological barriers to gene-flow. The most comprehensive is the eight molecular techniques for transgene containment. Male sterility, particularly cytoplasmic male

sterility, is an example of a technology that could be used to biologically suppress gene-flow. The use of this technology has already been demonstrated in maize with the Top Cross high-oil production method. Essentially the transgene would be introduced into a male sterile hybrid which would be pollinated by a non-transgenic pollinator. There are also transgenes that are known to render the pollen grains in which they are expressed unviable. These transgenes could then be linked to the transgene that is of interest to effectively block unwanted gene-flow (Morris and Kareiva, 1994).

Mechanical barriers to gene-flow: A mechanical barrier in the case of maize would be through the removal of the tassel prior to pollen shed (Morris and Kareiva, 1994). When properly done in a timely fashion, tassel removal is very effective. However for crops with perfect flowers, mechanical barriers would not be an option.

Spatial barriers to gene-flow: Spatial barriers are the most commonly prescribed treatment to prevent gene-flow. The rationale is that if enough distance is placed between the unwanted pollen source giving zero or very small levels of contamination. Unfortunately, it is also the method over which we have the least control. Crop certification requirements are frequently cited as justification for the use of spatial barriers. Crop certification requirements, however, have built in levels of acceptable contamination. In fact, rather high levels (up to 5%) of contamination can be found in seed production fields. These levels can be tolerated because it is very difficult for producers to identify the contamination. The present study focused estimating seed set as affected by the distance and direction of planting from the pollen source (Morris and Kareiva, 1994).

Temporal barriers to gene-flow: Temporal barriers to gene-flow can be quite effective. In order for there to be gene-flow via pollen movement there must be receptive and un-pollinated stigmas. By waiting until neighbouring crops have been pollinated or planting well before the neighbouring crops are planted, the whole issue of crop-to-crop gene-flow can be avoided (Morris and Kareiva, 1994). Taken individually, none of the five methods listed above are sufficient to prevent gene-flow via pollen movement. Some aspects of all five methods should therefore be employed. Stacking of these barriers would most likely reduce the probability of pollen movement by the product of each independent barrier. Thus, stacking individual barriers would rapidly reduce the probability of pollen movement to negligible levels. Animal and plant health inspection service (APHIS) currently has the following requirements for the field testing of corn for the production of pharmaceuticals. Transgenic corn must be planted at sites that are at least 1.6 km away from corn seed production. Maize from previous seasons must be harvested and removed in a radius of 4 km of the transgenic maize plot, before the transgenic maize is sown. The land within 7.6 m of the transgenic plant area must remain fallow during test. No other maize plants are grown in within a radius of 0.4 - 0.8 km if a buffer is used on the transgenic test plants, at any time during the field test. Transgenic maize must be planted no less than 21 days before or 21 days (14 days with a buffer) after the planting dates of any other corn that is growing within a zone extending from 0.8 km to 1.6 km (0.4 to 0.8 km with a buffer of the transgenic test plants.

The listed regulations only encompass two of the five recommended barriers to pollen movement, spatial and temporal. There are no requirements that the applicants also use mechanical and biological means of controlling pollen movement. Adding these mechanisms as an additional requirement would be prudent given that the tolerance to the products of these transgenes in the environment is currently zero. The reality is, if pollen movement is effectively kept to zero, there are other ways that the GMO crops can become co-mingled with non-GMO crops or other GMO crops producing other products (Morris and Kareiva, 1994). Volunteer plants are an issue in the following season as there is no perfect method of harvesting agronomic crops. The technology could be misappropriated in a variety of ways and contaminate the crop. Human errors and accidents during transportation and handling of the crop could also lead to contamination events that effectively would have the same result as contamination via pollen movement.

2.3 Work not yet done to determine good isolation distance

To introduce sorghum transgenes among farmers, in particular small scale farmers need to put in place precautionary measures to avoid contamination of existing cultivars and this could be done by using cytoplasmic sterile varieties.

CHAPTER 3

DETERMINATION OF POLLEN-MEDIATED GENE-FLOW IN SORGHUM USING SEED SET AT VARIOUS DISTANCES AND DIRECTIONS FROM THE POLLEN SOURCE

3.1Introduction

Sorghum is one of the most important crops in the world with over 40 million hectares dedicated to its cultivation. In Africa, sorghum represents about half the total cereal production on the continent and as such is a major food staple for the population (Belton and Taylor, 2003). Therefore, it is a vitally important cereal for the maintenance of food security in Africa especially due to its adaptation to harsh environmental conditions of the arid and semi-arid climate and its good yield in such conditions (Dicko *et al.,* 2006). Also, there is a recent debate which suggested that sorghum, especially the sweet sorghum varieties, could be used as a major source of biofuel to ease overdependence of Africa on fossil fuels (Reddy and Sharma, 2007).

A wide range of varieties and races of sorghum exist owing to their biology as well as their drought tolerance and adaptation to several environmental conditions. More than 7000 varieties of sorghum have been identified (Kangama and Rumei, 2005). Most of these varieties as well as the wild sorghum species exist in Africa making the continent the centre of greatest diversity for sorghum. The crop like most others continue to benefit from innovations through science and technology especially in attaining productivity gains to satisfy increased demand for food and agricultural products. Sorghum is a drought tolerant crop that is mainly grown in drier parts of the

region where maize does not perform well (Schimidt and Bothma, 1993). Throughout the history of agriculture sorghum has become adapted to a wide range of environments and farming systems, giving rise to wide varieties and races. These varieties are useful as sources of genes that confer resistance against various biotic and abiotic stresses (Schmidt and Bothma, 2006).

The diversity of sorghum is likely to be threatened by gene-flow-from improved transgenic varieties from research stations to farmers' fields. Given such reason it is very likely that traditional genetic resources may be lost. In addition, the release of transgenic sorghum that is being developed in various research programs will lead to contamination of the traditional cultivars through gene-flow. One ecological concern about the wide spread commercial use of transgenic crops is that transgenes might move into natural populations. For crops that are resistant to be affected by genetically modified varieties, there is potential for gene-flow via two mechanisms: 1. Recombination of transgenes with wild types and 2. Introgression of transgenes into population of wild crop relatives, both the recombination and transgenes introgression into wild plant population appear to be highly probable. Visarada and Kishore (2007) provided information about the likelihood that transgenes would spread to wild relatives of the crop. Introgression of transgenes that enhances the fitness of the weedy relatives could possibly make them more difficult to manage.

One of the most important issues related to biosafety concerns in sorghum is pollen mediated gene-flow to the wild relative species *S. halepense* (Johnson grass), crop to wild gene-flow will not occur because hybridisation of *S. hapelense* and cultivated sorghum *S. bicolor*, would produce unviable hybrids. Interestingly enough De Wet

and Harlan (1971) postulated that gene-flow to wild and weedy relatives of sorghum are expected to be extensive. According to Arriola and Ellstrand (1996), Johnson grass is self-incompatible but hybrids affected by gene-flow from crop to wild occurred 100 m away from the crop, being the greatest distance so far examined.

Various concerns exist about gene-flow from one crop to the other which may lead to cross pollination between different crops. However, there are many factors that naturally limit cross pollination between varieties, such as their tendency to self-pollinate and the limited duration of stigma reception. Farmers can enhance these limitations by growing different crop varieties at recommended distances from each other using border rows that have greater capacity for cross pollination. If sorghum trans-genetics such as bio-fortified sorghum varieties are introduced in the proximity of farmer's varieties, factors such as pollen size, the distance pollen travels, pollen competition (a function of the relative amount of pollen of two competitor sources) and the mode of pollination cause cross pollination between the varieties. Other environmental factors that can influence cross pollination between existing crops and newly introduced varieties are wind speed and direction, presence of barriers between fields, percentage of relative humidity and temperature.

In order to assess the likelihood of gene-flow from any crop to its wild relative, it is essential first to determine whether the crop is growing in a habitat that contains wild relatives and if not the likelihood of gene transfer is zero. If wild relatives are growing in close proximity to the crop then a number of factors influencing gene-flow will determine the gene transfer.

Sorghum is an important crop in Limpopo Province. Since its nutritional value is low an attempt is being made to release bio-fortified transgenic sorghum cultivars. Before, release of such cultivars detailed research is needed on the rate and distance of pollen-mediated gene-flow in sorghum at various locations using non transgenic baseline environments. This preliminary study would help in risk assessment and to provide data guiding the safe introduction and deployment of the transgenic varieties. Such information is valuable towards protecting the vulnerability of traditional germplasm or farmers' varieties from genetic erosion. The broad objective of this study was to determine the potential of out-crossing and gene-flow in sorghum. The specific objective of this study was to determine the distance and rates of pollen flow in sorghum using male sterile sorghum varieties (MSV) and the seed set as an indicator of pollen mediated gene-flow in the species.

3.2 Materials and methods

3.2.1 Study sites

The study was conducted at two locations *viz.* Syferkuil and Roedtan, in Limpopo Province. Both experiments were conducted under dry land conditions. Syferkuil is the experimental station of the University of Limpopo which is situated about 35 km east of Polokwane (23˚ 50' 24.33 S and 29˚ 41' 15.13" E) with altitude of 1 232 m above sea level (as per goggle earth). The site has an average temperature of 22°C with a long term average annual rainfall of 325 mm. The soil at the farm is sandy loam of Hutton form, Glenrosa family, with the pH ranging from 6-6.2. While Roedtan (24°70′35.33"S; 29°10′13.12"E) has an altitude of 980 m with an annual average rainfall of 600 mm. At Roedtan the annual mean temperature is 22 ºC with clay-loam

soil. In general, soil, climatic, and biological conditions of the two locations varies considerably.

3.2.2 Experimental design and trial establishment

In both locations the study was conducted as 8 × 3 × 20 factorial experiment. The three treatments were eight directions of planting, three male sterile sorghum varieties (MSVs) and 20 distance intervals demarcated from the central pollen source. The eight directions of planting were N, NE, E, SE, S, SW, W and NW coordinates. The three sorghum varieties (Ps 213A, Ps 256A and Ps 278A) were planted in 100 m long rows across the eight coordinates. Seed set by the MSVs as indicator of the distance of pollen travelled was assessed at 20 points marked at 5.0 m intervals along each row starting at the pollen source (Figure 3.1). Assumptions were that if seed set was not detected at the 100-m mark, then it would be safe to introduce genetically modified sorghum varieties among sorghum farmers without fear contamination. The central pollinator was R8602, a fertile sorghum variety. Seeds of the MSVs and R8602 were kindly supplied by International Crop Research Institute for Semi-Arid Tropics (ICRISAT). R8602 was planted in the centre of the field on an area of 400 m^2 (20 m × 20 m). Pollen from the square area was expected to be carried by wind in all directions to pollinate the MSVs. Along the eight directions three rows of MSVs were randomly assigned and planted with spacing of 50 cm inter-row and 30 cm intra-row. The actual field establishment of the study at Syfekuill is shown in Figure 3.2. Fertilizer was applied at the rate of 100 kg ha^{-1} ammonium phosphate (DAP) and 50 kg ha^{-1} urea as recommended for sorghum. All the DAP was applied at the time of planting, while urea was applied in a split application. Other standard agronomic practices were followed as required. Biasness

was minimised by ensuring that experimental errors were reduced by planting the experiment using eight arms direction, three different sorghum varieties and pollen travelled was assessed at 20 intervals from the pollen source.

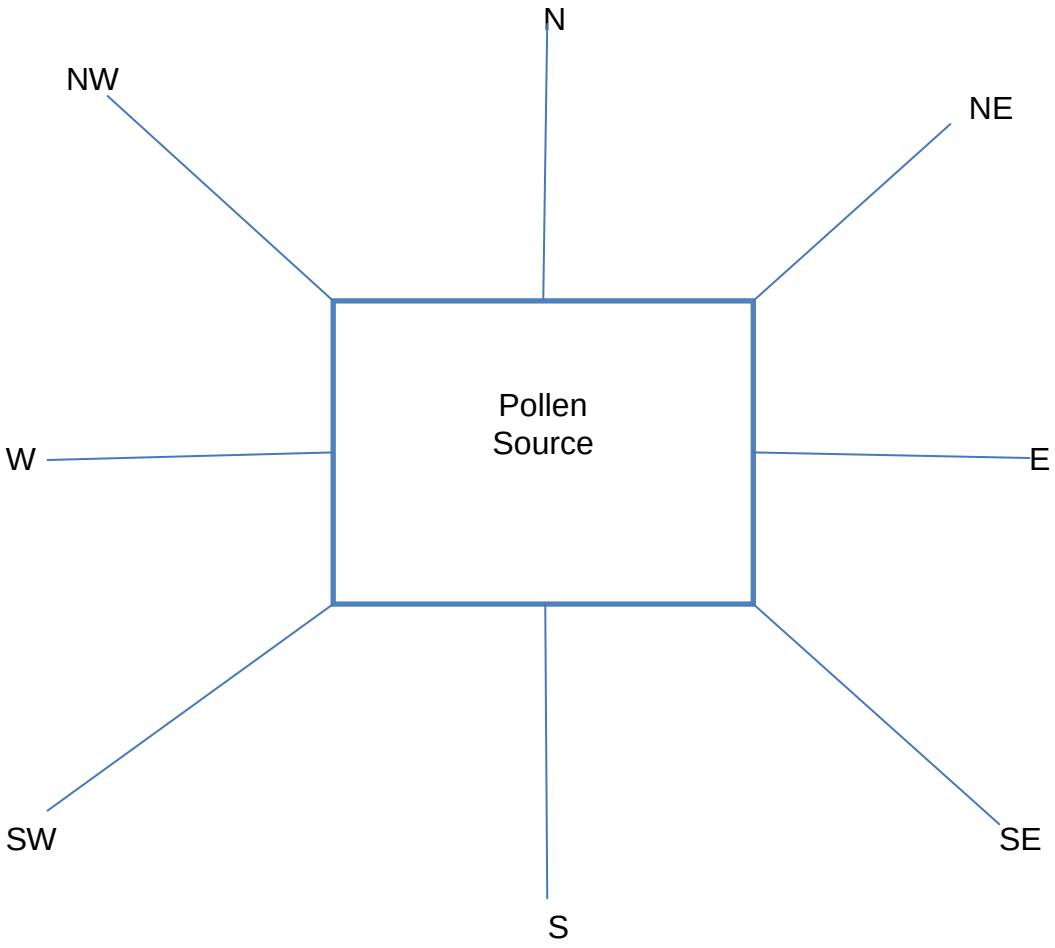

Fig. 3.1 Lay-out of the study areas, with coordinates being eight arms the central square being the pollinator variety (R8602).

Fig. 3.2 Actual trial planted at the University of Limpopo.

3.2.3 Data collection and analysis

In both localities quantitative data were collected. Fertile and sterile panicles were counted per plot per variety during maturity. Panicle length was measured and number of seeds per panicle counted from eight randomly selected tagged plants after harvest. Plot seed yield was measured in kg per 5 m^2. Hundred seed mass was measured in gram by randomly sampling seeds per variety after harvest. All data were subjected to the analysis of variance (ANOVA) and principal component analysis procedures of SAS (SAS, 1996). When found significant from the ANOVA, treatment means were compared using the Duncan multiple range test procedure at 5% probability level. After threshing seeds were counted and weighed on the scale to determine the exact seed set in all three cultivars at different points and directions.

3.3 Results

3.3.1 Number of fertile panicle

The second order interaction was highly significant ($P \leq 0.005$) for the number of fertile panicle at Syferkuil while at Roedtan the interaction was not significant (Table 3.1). At Roedtan, the first order interaction, distance × direction, was significant. At Syferkuil, variety, direction, distance × direction and variety × distance × direction contributed 42%, 29%, 18% and 8%, respectively, to the total treatment variation (TTV) in the number of fertile panicle. At Roedtan, distance, direction and distance × direction contributed 64%, 19% and 11%, respectively, to the TTV.

3.3.2 Number of sterile panicle

At Syferkuil the second order interaction was slightly significant for the number of sterile panicle while at Roedtan interaction was not significant. The first order

interaction at Syferkuil for distance × direction was highly significant and it was the same at Roedtan. At Syferkuil, distance, direction, distance x direction, and variety × distance × direction contributed 13%, 44%, 27% and 12%, respectively, to the TTV in the number of sterile panicle and at Roedtan, direction, distance × direction and variety × distance × direction contributed 14%, 48% and 24%, respectively, to the TTV (Table 3.1). At Syferkuil the number of fertile panicles varied from 1-12 but the number of sterile panicle varied from 0-11 (Table 3.2). All varieties showed high number of fertile panicles that decreased with increasing distance from the pollen source. Considerable number of sterile plants still existed hundred meters away from the pollen source (Figure 3.3C; Figure 3.3D). Invariably the three cultivars showed clear differences in the number of fertile and sterile panicle. Varieties Ps213A showed high number of fertile panicles followed by Ps256A and Ps278A. The numbers of fertile panicles were high at NE and followed by E, SE, and Westerly directions (Figure 3.3B). Relatively little number of fertile plants were observed in NW coordinates and the highest number of fertile panicle were also observed in NW and SW. The number of fertile and sterile panicles in the experiment showed relatively low CV at 1.6% (Table 3.2) and 1%, respectively. Appendix 6.1 shows that number of fertile panicles per 5 m^2 among three cytoplasmic sterile sorghums when evaluated at 20 distance intervals and eight arm directions from pollen source. The P- value for Variety, Distance, Arm direction, Variety by distance, Variety by arm direction, distance by arm direction and variety were all less than 5%, suggesting the existence of differences. The results show that numbers of fertile panicles per 5 m^2 among three cytoplasmic sterile sorghum when evaluated at 20 distance intervals and eight arm directions from pollen source affected by variety, distance, arm

direction, variety by distance, variety by arm direction, distance by arm direction and variety (Appendix 6.2).

Table 3.2 Mean number of fertile and sterile panicles per 5 m^2 plot among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals (5 to 100 m) and eight arm directions from pollen source.

Variety	Distance (m)	Number of fertile panicles								Number of sterile panicles							
		Arm Direction								Arm Direction							
		N	NE	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW
Ps213A	5	10	7	9	8	3	3	10	10	5	6	2	2	9	10	5	5
	10	5	7	8	6	5	4	9	7	1	4	3	3	9	8	2	4
	15	6	8	9	6	4	2	5	4	2	3	2	1	5	8	2	3
	20	4	8	6	4	4	4	7	6	3	1	2	3	5	7	5	3
	25	7	6	6	7	3	3	5	2	3	1	1	3	5	4	2	5
	30	6	8	5	5	4	3	8	3	3	3	2	3	6	5	2	7
	35	5	10	7	6	4	4	5	3	1	1	2	2	5	3	2	5
	40	3	4	4	3	3	3	6	2	3	1	1	2	7	5	2	6
	45	4	6	5	4	3	3	4	1	2	5	5	2	6	6	1	4
	50	5	6	6	3	3	3	3	1	2	1	4	2	4	6	3	5
	55	6	6	5	5	4	4	3	2	2	2	2	4	5	4	4	5
	60	6	5	7	5	3	5	2	1	1	1	3	2	3	3	5	6
	65	4	4	3	2	3	2	3	2	0	1	1	1	3	6	3	4
	70	3	4	5	6	2	4	1	1	0	0	6	2	3	5	3	3
	75	5	5	4	3	5	2	2	2	5	2	1	2	3	4	6	6
	80	3	4	4	4	2	2	2	1	0	0	2	2	1	4	3	4
	85	4	4	4	4	1	2	1	1	2	1	1	1	3	4	4	4
	90	5	3	4	6	1	2	2	2	2	1	3	2	4	5	4	4
	95	4	2	3	2	1	1	2	1	2	1	0	1	3	3	5	6
	100	7	7	10	11	5	1	1	1	0	1	1	2	2	3	3	3
Ps256A	5	8	7	9	7	4	2	9	10	6	5	3	2	7	11	4	3
	10	7	7	8	4	4	3	9	6	3	5	2	4	8	9	2	5
	15	5	8	7	6	3	4	8	5	3	2	2	6	5	5	2	5
	20	5	8	6	5	4	3	5	4	2	1	1	1	6	5	2	3
	25	4	6	6	5	4	3	6	3	3	2	1	3	6	4	2	5
	30	7	8	5	7	5	2	4	2	2	2	3	1	4	7	2	4
	35	5	8	6	5	3	4	7	3	2	2	2	2	5	3	2	6
	40	5	6	6	3	4	2	5	2	1	1	1	1	6	5	3	4
	45	3	5	4	3	3	3	4	2	1	3	2	3	5	5	2	4
	50	4	5	7	4	3	3	3	2	3	1	3	3	3	4	3	4
	55	5	7	6	5	4	4	3	1	2	1	2	2	3	5	3	5
	60	4	4	4	2	3	3	3	2	2	1	2	2	4	5	4	5
	65	5	5	4	1	2	1	4	2	1	2	3	3	3	6	2	3
	70	2	5	4	4	2	3	2	2	0	1	3	4	4	4	3	3
	75	4	5	4	3	3	2	1	1	4	1	3	2	4	5	5	5
	80	5	5	3	4	2	1	1	2	1	1	1	2	3	4	4	3
	85	4	5	4	3	1	1	1	1	1	1	2	2	3	5	5	5
	90	5	4	2	2	1	2	2	2	2	1	1	3	5	3	4	4
	95	3	3	2	2	4	1	2	1	1	0	1	4	3	2	4	5
	100	9	8	10	11	4	1	1	1	1	2	1	2	1	2	3	3
Ps278A	5	6	8	12	5	4	1	10	7	4	3	1	2	7	10	3	5
	10	9	7	8	6	3	4	6	6	2	4	2	3	8	10	2	2
	15	6	9	8	3	5	3	7	6	3	1	2	6	5	7	5	6
	20	7	7	7	7	3	4	6	3	2	2	1	5	6	5	2	5
	25	5	7	5	7	3	2	7	2	2	1	1	2	5	6	2	7
	30	5	9	7	5	4	3	6	2	2	1	3	1	5	5	2	6
	35	6	9	5	5	3	4	4	2	1	2	2	1	7	5	2	4
	40	6	7	3	4	3	2	4	1	1	1	3	2	7	6	3	6

	45	4	5	5	4	3	3	5	2		1	2	5	3	5	5	2	5
	50	4	5	6	4	3	2	5	2		1	1	2	1	3	5	0	3
	55	6	5	6	6	4	4	2	1		1	2	1	1	4	3	3	4
	60	4	4	3	2	4	2	3	2		2	2	1	2	3	5	5	6
	65	5	5	5	3	2	1	1	1		1	3	4	3	3	6	4	4
	70	3	5	5	2	2	3	2	1		2	0	3	4	3	5	5	6
	75	3	6	3	3	2	3	1	1		1	1	2	2	4	4	3	3
	80	3	4	3	4	1	2	2	2		2	0	3	2	4	4	3	3
	85	4	6	4	4	3	2	2	1		2	1	3	1	4	4	3	4
	90	3	4	2	1	2	2	1	1		0	1	1	0	4	3	3	3
	95	2	4	3	2	2	1	1	1		2	0	1	4	2	2	4	4
	100	10	7	9	8	3	3	10	10		2	0	1	2	2	3	3	3
	LSD (0.05) V x D x AD = 0.13										LSD (0.05) V x D x AD = 0.06							
	CV% = 1.6										CV% = 1.00							

At Roedtan, highly significant ($P \leq 0.01$) interactions were observed among varieties, distance and arm direction both on sterile and fertile panicles (Appendices 6.7, 6.8). The number of fertile panicles at Roedtan varied from 1-15 (Table 3.4). While the number of sterile panicle ranged from 0 to 8. The MSVs had high number of fertile panicles close to the pollen source. The MSVs showed differences in the number of fertile and sterile panicles. Variety Ps213A showed high number of fertile panicles followed by Ps256A and Ps278A. The numbers of fertile panicles were frequent at E followed by SE, SW and North Easterly directions (Figure 3.4 A and B). Relatively few numbers of fertile plants were observed in W coordinate suggesting limited wind movement at this direction. The number of fertile and sterile panicles in the experiment showed relatively low CV at 1.2% and 1.5%, respectively (Table 3.6). In this locality considerably high number of fertile plants prevailed hundred meters away from the pollen source (Figure 3.4 A and B). The highest number of sterile panicle was also observed in NE and SW (Figure 3.4 D).

3.3.3 Number of seeds per panicle

The second order interaction was significant for the number of seeds per panicle at Syferkuil while at Roedtan the interaction was not significant (Table 3.1). At Roedtan, the first order interaction in all variables was not significant. At Syferkuil, variety × direction and distance × direction, contributed 14% and 11% to the TTV in the number of seeds per panicle respectively. At Roedtan only variety contributed 67% to the TTV (Table 3.1).

At Syferkuil and Roedtan only variety was highly significant and at both localities variety contributed more than 99% to the TTV in panicle length and all other sources of variation were not significant (Table 3.1). Table 3.2 summarizes the mean panicle length of the three varieties. Ps213A and Ps278A have a panicle length of 25 cm while Ps256A has 30 cm. Ps256A had relatively the fewest number of seeds per panicle as compared to Ps213A and Ps278A, which were more fertile than this long panicle variety. According to Appendix 6.3, only Variety had P values less than 0.05 and distance, arm direction, variety by distance, variety by arm direction, distance by arm direction and variety were all above and did not show significant differences. Table 3.6 summarises the mean panicle length of the three varieties. Ps213A and Ps278A have a panicle length of 25 cm while Ps256A has 30 cm.

Table 3. 3 Mean number of fertile and sterile panicles among three CMS sorghum varieties at Roedtan when evaluated at 20 distance intervals (5 to 100 m) and eight arm directions from pollen source.

Variety	Distance (m)	Number of fertile panicles								Number of sterile panicles							
		Arm Direction								Arm Direction							
		N	NE	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW
Ps213A	5	14	13	15	13	12	13	10	10	1	1	0	2	0	0	5	5
	10	10	12	14	12	11	11	9	10	4	2	1	2	0	1	2	1
	15	13	10	13	9	9	10	5	10	1	3	1	3	1	0	2	1
	20	8	9	10	12	11	4	7	9	7	3	5	3	2	7	5	0
	25	5	8	9	9	9	10	5	5	8	1	4	3	0	3	2	2
	30	7	6	12	9	8	8	8	7	3	3	2	1	1	0	2	3
	35	6	8	10	9	9	7	5	7	1	1	3	2	0	0	2	0
	40	5	10	11	11	10	7	6	7	3	1	3	1	1	1	2	1
	45	3	4	10	9	8	8	4	5	2	5	1	3	1	1	1	0
	50	4	6	10	10	7	9	3	4	2	1	3	2	0	0	3	2
	55	5	6	9	8	8	8	3	6	2	2	3	3	0	0	4	1
	60	6	6	8	6	9	8	2	1	1	1	4	1	3	0	5	6
	65	6	5	10	8	6	6	3	2	0	1	3	1	0	2	3	4
	70	4	4	9	9	3	8	1	1	0	0	4	1	3	1	3	3
	75	3	4	8	7	2	6	2	2	5	2	6	1	3	0	6	6
	80	5	5	7	3	5	5	2	1	0	0	2	2	0	1	3	4
	85	3	4	7	4	2	4	1	1	2	1	1	1	3	1	4	4
	90	4	4	4	4	1	7	2	2	2	1	3	2	4	0	4	4
	95	5	3	4	6	1	1	2	1	2	1	0	1	3	3	5	6
	100	4	2	3	2	1	1	1	1	0	1	1	2	2	3	3	3
Ps256A	5	15	14	15	11	12	13	9	10	0	1	0	2	0	0	4	3
	10	11	14	14	11	12	11	9	9	3	1	1	2	0	0	2	2
	15	12	11	13	8	9	12	8	10	3	3	2	2	0	1	2	0
	20	11	10	9	8	9	10	5	7	4	3	4	2	0	3	2	0
	25	8	8	10	8	10	10	6	7	6	2	4	5	0	0	2	1
	30	8	6	13	10	8	9	4	8	5	2	2	3	0	0	2	1
	35	7	8	9	7	10	6	7	8	2	2	4	2	0	1	2	1
	40	5	8	10	9	7	7	5	5	1	1	1	5	2	0	3	2
	45	5	6	12	8	9	8	4	4	1	3	2	3	0	0	2	2
	50	3	5	11	9	8	7	3	5	3	1	1	4	1	3	3	1
	55	4	5	8	10	9	8	3	4	2	1	5	2	0	1	3	5
	60	5	7	8	7	8	8	3	2	2	1	4	0	0	0	4	5
	65	4	4	10	8	6	6	4	2	1	2	2	2	0	1	2	3
	70	5	5	8	7	2	6	2	2	0	1	3	2	4	1	3	3
	75	2	5	9	7	2	5	1	1	4	1	1	2	4	2	5	5
	80	4	5	8	3	3	5	1	2	1	1	1	2	3	0	4	3
	85	5	5	7	4	2	5	1	1	1	1	2	2	3	0	5	5
	90	4	5	4	3	1	2	2	2	2	1	1	3	5	3	4	4
	95	5	4	2	2	1	1	2	1	1	0	1	4	3	2	4	5
	100	3	3	2	2	4	1	1	1	1	2	1	2	1	2	3	3
Ps278A	5	13	15	15	11	10	10	10	11	2	0	0	2	1	1	3	2
	10	10	12	14	10	12	12	6	9	5	2	0	4	0	2	2	1
	15	9	10	14	10	10	10	7	9	5	5	1	2	0	11	5	3
	20	9	9	10.5	9	10	11	6	7	5	2	4	2	1	1	2	1
	25	7	7	10	9	12	8	7	8	6	1	5	3	1	1	2	1
	30	6	7	10	10	8	7	6	7	2	1	5	2	0	1	2	2
	35	5	9	7	9	9	8	4	4	1	2	2	1	2	1	2	2
	40	6	9	10	10	9	7	4	6	1	1	2	4	1	1	3	1
	45	6	7	9	9	7	8	5	5	1	2	5	4	1	0	2	2
	50	4	5	9	7	8	9	5	4	1	1	4	3	2	2	0	1
	55	4	5	9	7	10	9	2	4	1	2	5	2	1	1	3	1

	60	6	5	9	7	6	6	3	2		2	2	4	1	1	1	5	6
	65	4	4	10	9	6	5	1	1		1	3	1	2	1	2	4	4
	70	5	5	8	7	2	6	2	1		2	0	2	0	3	2	5	6
	75	3	5	9	2	2	6	1	1		1	1	4	2	4	1	3	3
	80	3	6	8	3	2	5	2	2		2	0	2	2	4	1	3	3
	85	3	4	8	4	1	7	2	1		2	1	1	1	4	2	3	4
	90	4	6	4	4	3	4	1	1		0	1	1	0	4	1	3	3
	95	3	4	2	1	2	1	1	1		2	0	1	4	2	2	4	4
	100	3	4	3	2	2	1	1	1		2	0	1	2	2	3	3	3
	LSD (0.05) V x D x AD = 0.16										LSD (0.05) V x D x AD = 0.06							
	CV% = 1.2										CV% = 1.5							

Only variety was significant ($P \leq 0.05$) (Appendix 6.3). However, distance, arm direction, variety by distance, variety by arm direction, distance by arm direction and variety were not significant at the 5% level (Appendix 6.3).

Syferkuil

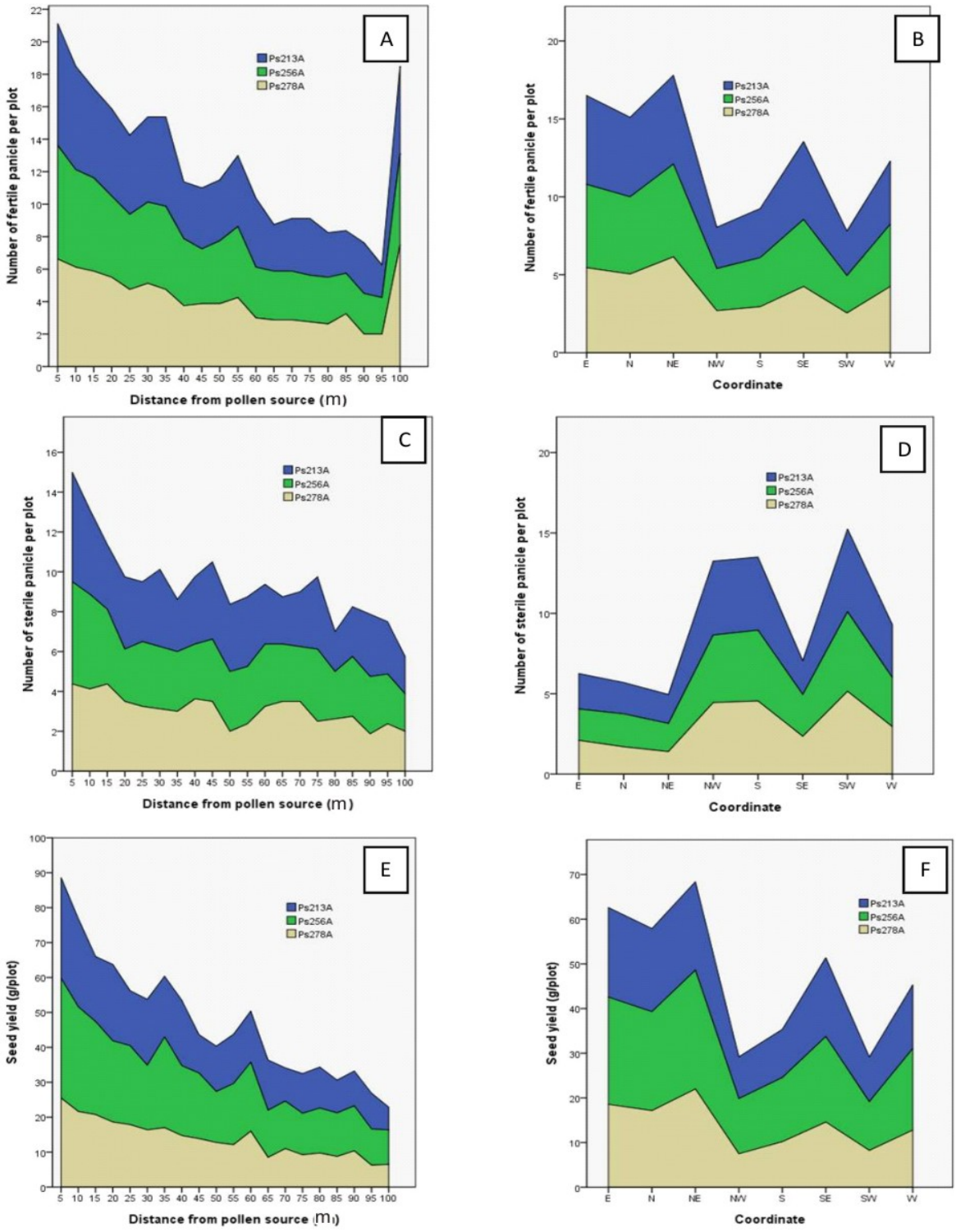

Fig: 3.3 A to F. The number of fertile (A and B) and sterile panicles (C and D) and seed yield (E and F) among the three CMS sorghum varieties grown at Syferkuil at 20 distances and eight coordinates from the pollen source.

Roedtan

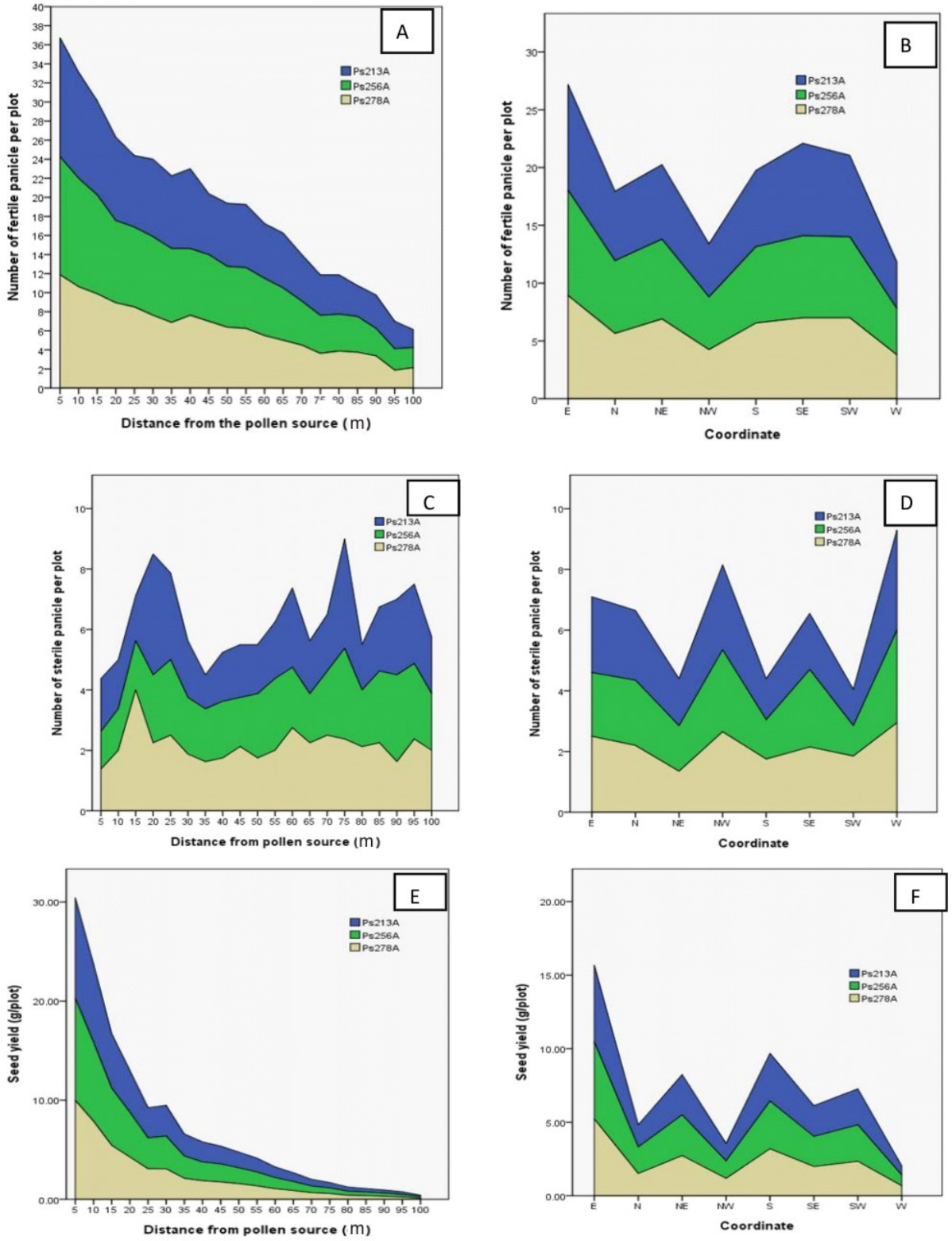

Figure 3.4 The number of fertile (A and B) and sterile panicles (C and D) and seed yield (E and F) among the three CMS sorghum varieties grown at Roedtan at 20 distances and eight coordinates from the pollen source

Table 3.4 Mean panicle length and number of seeds per panicle among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals (5 to 100 m) and eight arm directions from pollen source.

Variety	Distance (m)	Panicle length (cm)								Number of seeds per panicle							
		Arm Direction								Arm Direction							
		N	NE	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW
Ps213A	5	25	25	25	25	25	25	25	25	1146	1166	1240	1433	1467	1700	1175	1251
	10	25	25	25	25	25	25	25	25	1135	1154	1228	1419	1452	1683	1163	1238
	15	25	25	25	25	25	25	25	25	1112	1131	1203	1390	1423	1649	1140	1214
	20	25	25	25	25	25	25	25	25	1078	1097	1167	1349	1381	1600	1106	1177
	25	25	25	25	25	25	25	25	25	1068	1086	1155	1335	1367	1584	1095	1166
	30	25	25	25	25	25	25	25	25	1057	1075	1144	1322	1353	1568	1084	1154
	35	25	25	25	25	25	25	25	25	1046	1065	1132	1309	1340	1552	1073	1142
	40	25	25	25	25	25	25	25	25	1036	1054	1121	1295	1326	1537	1062	1131
	45	25	25	25	25	25	25	25	25	1026	1044	1110	1282	1313	1521	1052	1120
	50	25	25	25	25	25	25	25	25	985	1002	1065	898	1234	1461	1031	1075
	55	25	25	25	25	25	25	25	25	975	992	1023	889	1222	1446	1000	1043
	60	25	25	25	25	25	25	25	25	965	982	1013	880	1210	1432	990	1032
	65	25	25	25	25	25	25	25	25	955	972	1002	871	1197	1417	980	1022
	70	25	25	25	25	25	25	25	25	946	962	992	862	1186	1403	970	1012
	75	25	25	25	25	25	25	25	25	936	953	982	854	1174	1389	960	1001
	80	25	25	25	25	25	25	25	25	899	915	943	803	1127	1333	922	961
	85	25	25	25	25	25	25	25	25	827	841	868	738	1037	1213	839	885
	90	25	25	25	25	25	25	25	25	744	757	781	664	933	1092	755	796
	95	25	25	25	25	25	25	25	25	149	151	156	133	187	218	151	159
	100	25	25	25	25	25	25	25	25	45	45	16	40	56	66	60	80
Ps256A	5	30	30	30	30	30	30	30	30	1413	1288	1188	1146	1386	1166	1700	1688
	10	30	30	30	30	30	30	30	30	1399	1275	1176	1135	1372	1154	1683	1671
	15	30	30	30	30	30	30	30	30	1371	1250	1153	1112	1345	1131	1649	1638
	20	30	30	30	30	30	30	30	30	1330	1212	1118	1078	1304	1097	1600	1589
	25	30	30	30	30	30	30	30	30	1316	1200	1107	1068	1291	1086	1584	1573
	30	30	30	30	30	30	30	30	30	1303	1188	1096	1057	1278	1075	1568	1557
	35	30	30	30	30	30	30	30	30	1290	1176	1085	1046	1266	1065	1552	1541
	40	30	30	30	30	30	30	30	30	1277	1164	1074	1036	1253	1054	1537	1526
	45	30	30	30	30	30	30	30	30	1265	1153	1063	1026	1240	1044	1521	1511
	50	30	30	30	30	30	30	30	30	1214	1107	1021	718	1166	1002	1491	1450
	55	30	30	30	30	30	30	30	30	1202	1096	980	711	1154	992	1446	1407
	60	30	30	30	30	30	30	30	30	1190	1085	970	704	1143	982	1432	1393
	65	30	30	30	30	30	30	30	30	1178	1074	960	697	1131	972	1418	1379
	70	30	30	30	30	30	30	30	30	1166	1063	951	690	1120	962	1403	1365
	75	30	30	30	30	30	30	30	30	1155	1052	941	683	1109	953	1389	1351
	80	30	30	30	30	30	30	30	30	1108	1010	904	642	1065	915	1334	1297
	85	30	30	30	30	30	30	30	30	1020	929	831	590	979	832	1214	1194
	90	30	30	30	30	30	30	30	30	918	837	748	531	881	749	1092	1074
	95	30	30	30	30	30	30	30	30	184	167	150	106	176	150	218	215
	100	30	30	30	30	30	30	30	30	55	50	15	32	53	45	87	107
Ps278A	5	25	25	25	25	25	25	25	25	1433	1467	1700	1412	1243	1188	1166	1240
	10	25	25	25	25	25	25	25	25	1419	1452	1683	1398	1231	1176	1154	1228
	15	25	25	25	25	25	25	25	25	1390	1423	1649	1370	1206	1153	1131	1203
	20	25	25	25	25	25	25	25	25	1349	1381	1600	1329	1170	1118	1097	1167
	25	25	25	25	25	25	25	25	25	1335	1367	1584	1316	1158	1107	1086	1155
	30	25	25	25	25	25	25	25	25	1322	1353	1568	1302	1147	1096	1075	1144
	35	25	25	25	25	25	25	25	25	1309	1340	1552	1289	1135	1085	1065	1132
	40	25	25	25	25	25	25	25	25	1295	1326	1537	1276	1124	1074	1054	1121
	45	25	25	25	25	25	25	25	25	1282	1313	1521	1264	1112	1063	1044	1110
	50	25	25	25	25	25	25	25	25	1231	1260	1461	885	1046	1021	1023	1065

	55	25	25	25	25	25	25	25	25		1219	1248	1402	876	1035	1010	992	1033
	60	25	25	25	25	25	25	25	25		1207	1235	1388	867	1025	1000	982	1023
	65	25	25	25	25	25	25	25	25		1195	1223	1374	858	1015	990	972	1013
	70	25	25	25	25	25	25	25	25		1183	1211	1361	850	1004	980	963	1003
	75	25	25	25	25	25	25	25	25		1171	1199	1347	841	994	971	953	993
	80	25	25	25	25	25	25	25	25		1124	1151	1293	791	955	932	915	953
	85	25	25	25	25	25	25	25	25		1034	1059	1190	728	878	848	832	877
	90	25	25	25	25	25	25	25	25		931	953	1071	655	790	763	749	789
	95	25	25	25	25	25	25	25	25		186	191	214	131	158	153	150	158
	100	25	25	25	25	25	25	25	25		56	57	21	39	47	46	60	79
	LSD (0.05) V = 0.01										LSD (0.05) V x AD = 268.8							
	CV% = 0.1										CV% = 43.6							

Panicle length: At Roedtan highly significant differences (P ≤ 0.01) were only detected among varieties for panicle length (Appendix 6.9). Similar to Syferkuil varieties Ps213A and Ps278A had a panicle length of 25 cm and Ps256A with 30 cm (Table 3.6).

Number of seeds per panicle: At Syferkuil the number of seeds per panicles varied from 15 at 100 m (Ps256A) to 1700 in all the MSVs (Table 3.2). Ps213A had the highest number of seeds per panicle at a south westerly direction while Ps256A at westerly and Ps 278A at easterly (Table 3.4). In all the three tested varieties the numbers of seeds per panicle are significantly reduced furthest from the pollen source.

Number of seeds per panicle: Variety and arm direction had significant interaction (p ≤ 0.00) on this trait (Appendix 6.10). At Roedtan the number of seeds per panicles varied from 14 (Ps213A, 100 m, E) to 1739 (Ps278A, 5 m, E). Ps 213A had the highest number of seeds per panicle at a southerly and Ps256A at easterly directions (Table 3.6). While Ps278A had the highest number of seed set per panicle at south easterly direction, while the numbers of seed set per panicle shows the same trend

in all three varieties by decreasing with furthest distance from the pollen source. In general, the highest number of seed set was observed in variety Ps278A.

Table 3. 5 Mean panicle length and number of seeds per panicle among three CMS sorghum varieties Roedtan farm when evaluated at 20 distance intervals (5 to 100 m) and eight arm directions from pollen source.

Variety	Distance (m)	Panicle length (cm)								Number of seeds per panicle							
		Arm Direction								Arm Direction							
		N	NE	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW
Ps213A	5	25	25	25	25	25	25	25	25	1154	1234	1100	1200	1243	1211	1232	1166
	10	25	25	25	25	25	25	25	25	1142	1222	1089	1188	1231	1199	1220	1154
	15	25	25	25	25	25	25	25	25	1120	1197	1067	1164	1206	1175	1195	1131
	20	25	25	25	25	25	25	25	25	1086	1161	1035	1129	1170	1140	1159	1097
	25	25	25	25	25	25	25	25	25	1075	1150	1025	1118	1158	1128	1148	1086
	30	25	25	25	25	25	25	25	25	1064	1138	1015	1107	1147	1117	1136	1075
	35	25	25	25	25	25	25	25	25	1054	1127	1004	1096	1135	1106	1125	1065
	40	25	25	25	25	25	25	25	25	1043	1116	994	1085	1124	1095	1114	1054
	45	25	25	25	25	25	25	25	25	1033	1104	984	1074	1112	1084	1103	1044
	50	25	25	25	25	25	25	25	25	991	1060	945	752	1046	1040	1081	1002
	55	25	25	25	25	25	25	25	25	982	1050	907	744	1035	1030	1048	972
	60	25	25	25	25	25	25	25	25	972	1039	898	737	1025	1020	1038	962
	65	25	25	25	25	25	25	25	25	962	1029	889	729	1015	1010	1027	952
	70	25	25	25	25	25	25	25	25	952	1018	880	722	1004	999	1017	943
	75	25	25	25	25	25	25	25	25	943	1008	872	715	994	989	1007	933
	80	25	25	25	25	25	25	25	25	905	968	837	672	955	950	967	896
	85	25	25	25	25	25	25	25	25	833	890	770	618	878	864	880	824
	90	25	25	25	25	25	25	25	25	749	801	693	556	790	778	792	742
	95	25	25	25	25	25	25	25	25	150	160	139	111	158	156	158	148
	100	25	25	25	25	25	25	25	25	45	48	14	33	47	47	63	74
Ps256A	5	30	30	30	30	30	30	30	30	1125	1232	1234	1126	1100	1067	1123	1124
	10	30	30	30	30	30	30	30	30	1114	1220	1222	1115	1089	1056	1112	1113
	15	30	30	30	30	30	30	30	30	1091	1195	1197	1092	1067	1035	1090	1091
	20	30	30	30	30	30	30	30	30	1059	1159	1161	1060	1035	1004	1057	1058
	25	30	30	30	30	30	30	30	30	1048	1148	1150	1049	1025	994	1046	1047
	30	30	30	30	30	30	30	30	30	1038	1136	1138	1039	1015	984	1036	1037
	35	30	30	30	30	30	30	30	30	1027	1125	1127	1028	1004	974	1025	1026
	40	30	30	30	30	30	30	30	30	1017	1114	1116	1018	994	965	1015	1016
	45	30	30	30	30	30	30	30	30	1007	1103	1104	1008	984	955	1005	1006
	50	30	30	30	30	30	30	30	30	967	1059	1060	705	925	917	985	966
	55	30	30	30	30	30	30	30	30	957	1048	1018	698	916	908	955	937
	60	30	30	30	30	30	30	30	30	947	1037	1008	691	907	898	946	927
	65	30	30	30	30	30	30	30	30	938	1027	998	684	898	890	936	918
	70	30	30	30	30	30	30	30	30	928	1017	988	678	889	881	927	909
	75	30	30	30	30	30	30	30	30	919	1007	978	671	880	872	918	900
	80	30	30	30	30	30	30	30	30	882	966	939	631	845	837	881	864
	85	30	30	30	30	30	30	30	30	812	889	864	580	777	762	802	795
	90	30	30	30	30	30	30	30	30	731	800	777	522	700	685	722	715
	95	30	30	30	30	30	30	30	30	146	160	155	104	140	137	144	143
	100	30	30	30	30	30	30	30	30	44	48	16	31	42	41	58	72
Ps278A	5	25	25	25	25	25	25	25	25	1700	1734	1655	1739	1723	1688	1569	1689
	10	25	25	25	25	25	25	25	25	1683	1717	1638	1722	1706	1671	1553	1672
	15	25	25	25	25	25	25	25	25	1649	1682	1606	1687	1672	1638	1522	1639
	20	25	25	25	25	25	25	25	25	1600	1632	1558	1637	1622	1589	1477	1590
	25	25	25	25	25	25	25	25	25	1584	1616	1542	1620	1605	1573	1462	1574
	30	25	25	25	25	25	25	25	25	1568	1599	1527	1604	1589	1557	1447	1558

	35	25	25	25	25	25	25	25	25		1552	1583	1511	1588	1573	1541	1433	1542
	40	25	25	25	25	25	25	25	25		1537	1568	1496	1572	1558	1526	1418	1527
	45	25	25	25	25	25	25	25	25		1521	1552	1481	1556	1542	1511	1404	1512
	50	25	25	25	25	25	25	25	25		1461	1490	1422	1089	1450	1450	1376	1451
	55	25	25	25	25	25	25	25	25		1446	1475	1365	1079	1435	1436	1335	1408
	60	25	25	25	25	25	25	25	25		1432	1460	1351	1068	1421	1421	1321	1394
	65	25	25	25	25	25	25	25	25		1417	1446	1338	1057	1406	1407	1308	1380
	70	25	25	25	25	25	25	25	25		1403	1431	1325	1047	1392	1393	1295	1366
	75	25	25	25	25	25	25	25	25		1389	1417	1311	1036	1378	1379	1282	1352
	80	25	25	25	25	25	25	25	25		1333	1360	1259	974	1323	1324	1231	1298
	85	25	25	25	25	25	25	25	25		1227	1251	1158	896	1217	1205	1120	1194
	90	25	25	25	25	25	25	25	25		1104	1126	1042	806	1096	1084	1008	1075
	95	25	25	25	25	25	25	25	25		221	225	208	161	219	217	202	215
	100	25	25	25	25	25	25	25	25		66	68	21	48	66	65	81	107
	LSD (0.05) V = 0.04										LSD (0.05) V x D = 120.94							
	CV% = 0.1										CV% = 13.20							

3.3.4 Seed yield

The second order interaction at Syferkuil was not significant for seed yield while at Roedtan the interaction was highly significant. At both Syferkuil and Roedtan, the first order interaction, distance × direction was significant. At Syferkuil, distance, direction, distance × direction contributed at 40%, 28% and 17% to the TTV in seed yield respectively. At Roedtan, distance x direction and variety × distance × direction contributed 29% and 50% to the TTV, respectively (Table 3.1). Seed yield varied from 3 to 49 g per 5 m^2 (Table 3.4). All varieties showed high seed yield close to the pollen source. Seed set considerably decreased with increasing distance from the pollen source. Remarkably high seed set was noted at 100 m further from the designated pollen source (Figures 3.3 E and F) requiring greater distance to effectively minimize cross pollination. The three MSVs showed clear differences in seed yield per plot accounted by arm direction and distance from the pollen source. Variety Ps213A showed high seed yield followed by Ps256A. Seed yield was the highest at NE direction for varieties Ps213A and Ps256A. Relatively low seed yield was observed in a south and north westerly (Figure 3.3 E and F). This suggests that

at Syferkuil and growing season there was increased wind movement at NE, followed by E, SE, and W directions (Figure 3.3 F) rendering pollen dispersal and fertilisation. Seed yield in the experiment showed relatively low CV at 0.2% (Table 3.4). Figure 4.1 shows the assessment of seed set during the experiment at Syferkuil Seed mass showed the same trend of significance as in panicle length as far as contribution towards TTV is concerned. At both localities, that is Syferkuil and Roedtan the only significant source of variation was variety that contributed to 96% and 99%, respectively (Table 3.1). Variety Ps213A had a hundred seed mass of 6 g, Ps256A 7 g and Ps278A 5 g (Table 3.6) which showed significant difference from each other. Hundred seed mass was consistent across all directions except varietal differences.

Seed yield: Seed yield was significantly influenced ($P \leq 0.01$) due to the interactive effect of variety, distance, and arm direction (Appendix 6.11). All varieties showed high number of seed yield. But seed yield decreased with increasing distance from the pollen source. Seed yield varied from 0.03 - 29.02 g per 5 m^2 (Table 3.7). There existed considerable number of seeds at hundred meters away from the pollen source (Figure 3.4 E and F). Also, the three MSVs had marked differences in seed yield per plot. Ps213A showed high number of seed yield followed by Ps256A and Ps278A. Seed yield was high at easterly direction for all the varieties. But low seed yield was observed at a westerly direction (Figure 3.4F). Seed yield in the experiment showed relatively low CV at 0.1% (Table 3.5).

Table 3.6 Mean seed yield (g/5 m^2) and hundred seed mass among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals (5 to 100 m) and eight arm directions from pollen source.

Variety	Distance (cm)	Seed yield (g/5 m^2)								Hundred seed mass (g/100 seed)							
		Arm Direction								Arm Direction							
		N	NE	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW
Ps213A	5	35	21	39	45	10	11	35	35	6	6	6	6	6	6	6	6
	10	35	25	32	28	11	14	32	25	6	6	6	6	6	6	6	6
	15	18	25	28	21	18	7	18	14	6	6	6	6	6	6	6	6
	20	22	28	31	21	14	14	24	21	6	6	6	6	6	6	6	6
	25	14	28	21	14	14	11	17	7	6	6	6	6	6	6	6	6
	30	24	21	21	24	11	11	28	11	6	6	6	6	6	6	6	6
	35	21	28	17	17	14	14	17	11	6	6	6	6	6	6	6	6
	40	18	35	24	21	14	10	21	7	6	6	6	6	6	6	6	6
	45	11	14	14	10	11	10	14	4	6	6	6	6	6	6	6	6
	50	14	21	18	14	11	11	11	4	6	6	6	6	6	6	6	6
	55	18	21	21	10	11	14	11	7	6	6	6	6	6	6	6	6
	60	21	21	17	17	14	17	7	3	6	6	6	6	6	6	6	6
	65	21	17	24	17	11	7	11	7	6	6	6	6	6	6	6	6
	70	14	14	10	7	10	14	4	3	5	6	6	6	6	6	6	6
	75	11	14	17	21	7	7	7	7	6	6	6	6	6	6	6	6
	80	18	17	14	10	17	7	7	4	6	6	6	6	6	6	6	6
	85	11	14	14	14	7	7	4	4	6	6	6	6	6	6	6	6
	90	14	14	14	14	3	7	7	7	6	6	6	6	6	6	6	6
	95	18	11	14	21	4	4	7	3	6	6	6	6	6	6	6	6
	100	14	7	11	7	4	3	3	3	6	6	6	6	6	6	6	6
Ps256A	5	32	32	45	49	22	9	40	45	7	7	7	7	7	7	7	7
	10	36	32	41	31	18	14	41	27	7	7	7	7	7	7	7	7
	15	32	32	36	18	18	18	36	23	7	7	7	7	7	7	7	7
	20	23	36	31	27	14	14	23	18	7	7	7	7	7	7	7	7
	25	23	36	27	22	18	14	27	14	7	7	7	7	7	7	7	7
	30	18	27	27	22	18	9	18	9	7	7	7	7	7	7	7	7
	35	32	36	22	31	23	18	32	14	7	7	7	7	7	7	7	7
	40	23	36	27	22	13	7	23	9	7	7	7	7	7	7	7	7
	45	23	27	27	14	18	14	18	9	7	7	7	7	7	7	7	7
	50	14	23	18	13	13	13	14	9	7	7	7	7	7	7	7	7
	55	18	23	31	18	13	18	14	5	7	7	7	7	7	7	7	7
	60	23	32	27	22	18	13	14	9	7	7	7	7	7	7	7	7
	65	18	18	18	9	13	5	18	9	7	7	7	7	7	7	7	7
	70	23	22	18	5	9	14	9	9	7	7	7	7	7	7	7	7
	75	9	22	18	18	9	9	5	5	7	7	7	7	7	7	7	7
	80	18	22	18	13	13	5	5	9	7	7	7	7	7	7	7	7
	85	23	22	13	18	9	5	5	5	7	7	7	7	7	7	7	7
	90	18	22	18	13	5	9	9	9	7	7	7	7	7	7	7	7
	95	23	18	9	9	5	5	9	5	7	7	7	7	7	7	7	7
	100	14	14	9	9	18	5	5	5	7	7	7	7	7	7	7	7
Ps278A	5	31	27	34	37	14	3	34	24	5	5	5	5	5	5	5	5
	10	20	27	41	17	14	14	20	20	5	5	5	5	5	5	5	5
	15	31	24	27	20	10	10	24	20	5	5	5	5	5	5	5	5
	20	20	31	27	10	17	14	20	10	5	5	5	5	5	5	5	5
	25	24	24	24	23	10	7	24	7	5	5	5	5	5	5	5	5
	30	20	24	17	23	10	10	20	7	5	5	5	5	5	5	5	5
	35	17	31	23	17	14	13	14	7	5	5	5	5	5	5	5	5
	40	20	31	17	17	10	7	13	3	5	5	5	5	5	5	5	5
	45	20	24	10	13	10	10	17	7	5	5	5	5	5	5	5	5
	50	14	17	17	13	10	7	17	7	5	5	5	5	5	5	5	5

	55	14	17	20	13	10	13	7	3		5	5	5	5	5	5	5	5
	60	20	31	20	20	13	7	10	7		5	5	5	5	5	5	5	5
	65	14	14	10	7	14	3	3	3		5	5	5	5	5	5	5	5
	70	17	17	17	10	7	10	7	3		5	5	5	5	5	5	5	5
	75	10	17	17	7	7	10	3	3		5	5	5	5	5	5	5	5
	80	10	20	10	10	7	7	7	7		5	5	5	5	5	5	5	5
	85	10	17	10	13	3	7	7	3		5	5	5	5	5	5	5	5
	90	14	20	13	13	10	7	3	3		5	5	5	5	5	5	5	5
	95	10	14	7	3	7	3	3	3		5	5	5	5	5	5	5	5
	100	7	13	10	6	7	3	3	3		5	5	5	5	5	5	5	5
	LSD (0.05) V x D x AD = 0.06										LSD (0.05) V = 0.03							
	CV% = 0.2										CV% = 2.8							

Hundred seed mass: At Roedtan only varietal differences were significant ($P \leq 0.01$) on hundred seed mass (Appendix 6.12). Variety Ps213A had seed mass at 6 g, Ps256A 7 g and variety Ps278A at 5 g (Table 3.7). This did not have any significant difference as far as arm directions were concerned and the only significant difference was among the varieties. Appendix 6.12 suggests that of all the sources of variation only variety had its p value less than 0.05.

Table 3.7 Mean seed yield and hundred seed mass among three CMS sorghum varieties Roedtan farm when evaluated at 20 distance intervals (5 to 100 m) and eight arm directions from pollen source.

Variety	Distance (m)	Seed yield (g/ 5m^2)								Thousand seed mass (g/100 seed)							
		Arm Direction								Arm Direction							
		N	NE	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW
Ps213A	5	9.80	10.82	28.91	5.25	12.71	6.84	2.67	4.22	6	6	6	6	6	6	6	6
	10	3.83	11.05	20.25	5.09	10.11	6.56	2.45	3.99	6	6	6	6	6	6	6	6
	15	5.35	5.52	10.02	3.99	8.07	5.99	2.09	3.09	6	6	6	6	6	6	6	6
	20	3.05	3.05	8.97	3.50	5.99	5.05	2.09	2.09	6	6	6	6	6	6	6	6
	25	0.80	2.40	5.90	3.09	5.03	5.00	0.17	1.98	6	6	6	6	6	6	6	6
	30	2.41	2.41	5.54	2.98	4.99	3.56	1.08	1.87	6	6	6	6	6	6	6	6
	35	0.47	2.06	3.98	2.45	2.99	3.22	0.98	1.67	6	6	6	6	6	6	6	6
	40	0.18	1.39	3.76	3.67	2.87	3.08	0.08	1.23	6	6	6	6	6	6	6	6
	45	0.51	1.98	2.99	2.32	2.45	2.87	0.14	1.09	6	6	6	6	6	6	6	6
	50	0.26	2.14	2.78	2.09	2.22	2.09	0.11	0.99	6	6	6	6	6	6	6	6
	55	0.40	1.73	2.09	1.98	2.04	1.99	0.11	0.78	6	6	6	6	6	6	6	6
	60	0.89	1.42	1.98	1.06	1.89	1.09	0.07	0.23	6	6	6	6	6	6	6	6
	65	0.43	1.38	1.76	0.97	1.23	1.06	0.11	0.19	6	6	6	6	6	6	6	6
	70	0.31	1.34	1.45	0.89	0.98	0.19	0.04	0.11	6	6	6	6	6	6	6	6
	75	0.30	1.19	1.21	0.76	0.67	0.11	0.07	0.02	6	6	6	6	6	6	6	6
	80	0.29	1.18	0.98	0.45	0.22	0.05	0.07	0.04	6	6	6	6	6	6	6	6
	85	0.29	1.07	0.78	0.34	0.19	0.07	0.04	0.04	6	6	6	6	6	6	6	6
	90	0.28	1.02	0.54	0.32	0.11	0.07	0.07	0.07	6	6	6	6	6	6	6	6
	95	0.18	0.98	0.23	0.45	0.08	0.04	0.07	0.03	6	6	6	6	6	6	6	6
	100	0.14	0.54	0.11	0.22	0.05	0.03	0.03	0.03	6	6	6	6	6	6	6	6
Ps256A	5	10.40	11.09	29.02	5.28	12.77	6.88	2.65	4.23	7	7	7	7	7	7	7	7
	10	4.54	10.97	20.27	5.07	10.01	6.55	2.34	3.98	7	7	7	7	7	7	7	7
	15	6.43	5.59	10.09	4.00	8.05	6.00	3.01	3.05	7	7	7	7	7	7	7	7
	20	4.56	3.07	8.99	3.59	5.87	5.99	2.07	2.04	7	7	7	7	7	7	7	7
	25	1.09	2.49	5.98	3.22	5.06	4.09	1.09	1.97	7	7	7	7	7	7	7	7
	30	3.45	2.48	5.57	2.99	4.98	3.99	1.11	1.88	7	7	7	7	7	7	7	7
	35	0.50	2.09	3.99	2.50	2.99	3.29	0.99	1.65	7	7	7	7	7	7	7	7
	40	0.23	1.43	3.79	2.45	2.88	3.09	0.06	1.23	7	7	7	7	7	7	7	7
	45	0.59	2.00	3.00	2.34	2.46	2.99	0.18	1.08	7	7	7	7	7	7	7	7
	50	0.31	2.16	2.79	2.08	2.29	2.09	0.14	0.90	7	7	7	7	7	7	7	7
	55	0.49	1.75	2.09	1.99	2.07	1.98	0.14	0.77	7	7	7	7	7	7	7	7
	60	0.95	1.48	1.99	1.07	1.99	1.09	0.14	0.22	7	7	7	7	7	7	7	7
	65	0.49	1.39	1.79	0.99	1.22	1.04	0.18	0.17	7	7	7	7	7	7	7	7
	70	0.39	1.37	1.49	0.87	0.99	0.14	0.09	0.12	7	7	7	7	7	7	7	7
	75	0.37	1.24	1.28	0.77	0.65	0.12	0.05	0.09	7	7	7	7	7	7	7	7
	80	0.32	1.19	0.99	0.46	0.23	0.04	0.05	0.09	7	7	7	7	7	7	7	7
	85	0.31	1.09	0.79	0.43	0.19	0.05	0.05	0.05	7	7	7	7	7	7	7	7
	90	0.29	1.05	0.57	0.33	0.12	0.09	0.09	0.09	7	7	7	7	7	7	7	7
	95	0.23	0.99	0.29	0.33	0.07	0.05	0.09	0.05	7	7	7	7	7	7	7	7
	100	0.17	0.56	0.17	0.12	0.04	0.05	0.05	0.05	7	7	7	7	7	7	7	7
Ps278A	5	8.70	10.76	28.90	5.22	12.34	6.87	2.78	4.22	5	5	5	5	5	5	5	5
	10	3.97	10.94	20.24	5.05	10.09	6.45	2.33	3.99	5	5	5	5	5	5	5	5
	15	5.43	5.53	10.01	3.08	8.03	5.45	2.98	3.02	5	5	5	5	5	5	5	5
	20	3.57	3.04	8.96	3.55	5.88	5.07	2.02	2.03	5	5	5	5	5	5	5	5
	25	0.95	2.45	5.97	3.09	5.02	4.09	1.09	1.96	5	5	5	5	5	5	5	5
	30	2.32	2.43	5.55	2.97	4.89	3.45	1.12	1.88	5	5	5	5	5	5	5	5
	35	0.49	2.04	3.98	2.51	2.93	3.23	0.08	1.65	5	5	5	5	5	5	5	5
	40	0.20	1.40	3.77	2.34	2.85	3.09	0.13	1.20	5	5	5	5	5	5	5	5
	45	0.57	1.95	2.98	2.31	2.45	2.45	0.17	1.07	5	5	5	5	5	5	5	5
	50	0.28	2.11	2.77	2.09	2.20	2.09	0.17	0.97	5	5	5	5	5	5	5	5

	55	0.47	1.72	2.07	1.87	2.01	1.90	0.07	0.67		5	5	5	5	5	5	5	5
	60	0.91	1.43	1.97	1.08	1.78	1.08	0.10	0.21		5	5	5	5	5	5	5	5
	65	0.45	1.37	1.77	0.97	1.11	1.07	0.03	0.32		5	5	5	5	5	5	5	5
	70	0.37	1.33	1.46	0.88	0.97	0.17	0.07	0.10		5	5	5	5	5	5	5	5
	75	0.32	1.20	1.27	0.98	0.67	0.10	0.03	0.07		5	5	5	5	5	5	5	5
	80	0.28	1.17	0.98	0.44	0.21	0.03	0.07	0.07		5	5	5	5	5	5	5	5
	85	0.29	1.11	0.76	0.45	0.16	0.07	0.07	0.03		5	5	5	5	5	5	5	5
	90	0.28	1.01	0.55	0.34	0.11	0.07	0.03	0.03		5	5	5	5	5	5	5	5
	95	0.19	0.96	0.27	0.34	0.06	0.03	0.03	0.03		5	5	5	5	5	5	5	5
	100	0.11	0.53	0.10	0.11	0.04	0.03	0.03	0.03		5	5	5	5	5	5	5	5
	LSD (0.05) V x D x AD = 0.01										LSD (0.05) V = 0.02							
	CV% = 0.1										CV% = 1.6							

Principal component analysis: The data from Syferkuil was subjected to the principal component analysis (PCA) using the number of fertile and sterile panicles, panicle length, number of seeds per panicle, seed yield and hundred seed mass to identify the most influential or representative trait(s) in the experiment. The PCA identified three principal components (PCs) that contributed to 86% of the total variation. PC1, PC2 and PC3 contributed to 35.55, 29.24 and 20.79% of the total variation; respectively. Three traits, that is, seed yield, panicle length and number of seed per panicle correlated well with PC1, PC2 and PC3, respectively. Thus, seed yield is the most important contributor at 36% to the total variation exhibited by the MSVs, distance and direction of planting.

The analysis of variance indicated significant interactions among varieties, distance and direction from the pollen source on the number of fertile and sterile panicles (Appendices 6.7, 6.8) and, seed yield (Appendix 6.11). There were significant differences only among varieties for panicle length (Appendix 6.9) and hundred seed mass (Appendix 6.12). The number of seeds per panicle showed significant interaction among variety and planting or arm directions at Roedtan. Results on each agronomic trait are summarized below (Table 3.1).

Data collected from Roedtan was also subjected to the principal component analysis (PCA). The first two principal components (PC1 and PC2) accounted for 62.14% of the total variation. PC1 alone explained 35.98% of the total variability among the varieties and was mainly due to variation in panicle length, while PC2 accounted for 16% of the total variation was a function of number of fertile panicles. PC1 correlated with panicle length and PC2 with number of fertile panicle. Panicle length was also found the most important traits in Syferkuil contributing to the total variation.

Table 3.1. Analysis of variance for six agronomic traits among three CMS sorghum varieties at Syferkuil and Roedtan, evaluated at 20 distance intervals and eight arm directions from pollen source.

Source of		No. fertile panicle		No. sterile panicle		Seed yield		No. seed/ panicle		Panicle length		Seed mass	
variation	DF	SS	Impact	SS	Impact	SS	Impact	SS	Impact	SS	Impact	SS	Impact
Syferkuil													
Variety (A)	2	9.66	0.2^{ns}	2.63	0.0^{ns}	3633.00	4.7^{ns}	2969.98	0.0^{ns}	5337.00	99.9***	636.0	96.0***
Distance (B)	19	2132.0	41.9***	427.20	13.0***	30840.00	40.0***	66138.60	0.6^{ns}	0.02	0.0^{ns}	0.511	0.1^{ns}
Direction (C)	7	1478.0	29.0***	1525.00	44.0***	21490.00	28.0***	49730.63	0.1^{ns}	0.01	0.0^{ns}	0.357	0.0^{ns}
A x B	38	104.20	2.0^{ns}	126.80	3.6^{ns}	1903.00	2.5^{ns}	129176.41	0.3^{ns}	0.04	0.0^{ns}	1.116	0.2^{ns}
A x C	14	30.14	0.6^{ns}	18.08	0.4^{ns}	589.80	0.8^{ns}	580369.39	14.0**	0.01	0.0^{ns}	0.058	0.0^{ns}
B x C	133	922.20	18.1**	934.60	27.0***	13210.00	17.0**	462756.83	11.0*	0.14	0.0^{ns}	3.580	0.5^{ns}
A x B x C	266	416.00	8.2*	427.90	12.0***	5631.00	7.0^{ns}	1034101.64	25.0**	0.28	0.0^{ns}	7.816	1.2^{ns}
Residual	480	2.00	0.0	0.50	0.0	0.50	0.0	1796362.61	40.0***	0.50	0.1	13.5	2.0
Total	959	5094.2	100	3424.00	100	77300.00	100	4121606.10	100	5338.00	100	662.9	100
Roedtan													
Variety (A)	2	9.20	0.7^{ns}	2.85	0.0^{ns}	453.20	0.3^{ns}	58947170.0	67.3***	5313.41	98.9***	634.02	98.6***
Distance (B)	19	7329.0	63.7***	169.80	6.8^{ns}	10800.	8.3^{ns}	771918.00	0.9^{ns}	1.14	0.0^{ns}	0.18	0.0^{ns}
Direction (C)	7	2209.0	19.0**	339.20	13.6**	3426.	2.6^{ns}	37024.00	0.0^{ns}	1.05	0.0^{ns}	0.07	0.0^{ns}
A x B	38	70.13	0.6^{ns}	153.40	6.0^{ns}	9312.	7.2^{ns}	2071354.00	2.4^{ns}	2.51	0.0^{ns}	0.36	0.1^{ns}
A x C	14	34.28	0.0^{ns}	36.46	1.5^{ns}	3392.	2.6^{ns}	72516.00	0.0^{ns}	0.53	0.0^{ns}	0.13	0.0^{ns}
B x C	133	1280.0	11.0**	1204.00	48.5***	37260.	29.0**	3292498.00	3.8^{ns}	7.99	0.0^{ns}	1.25	0.2^{ns}
A x B x C	266	579.70	5.0^{ns}	587.00	23.6***	64990.	50.0***	7801062.00	8.9^{ns}	17.56	0.3^{ns}	2.49	0.4^{ns}
Residual	480	3.00	0.0	0.50	0.0	0.01	0.0	14547814.0	16.7	31.00	0.6	4.50	0.7
Total	959	11510.	100	2485.00	100	129600.	100	87541355.0	100	5375.18	99.75	642.99	100

Ns = Not significant *** = Significant P ≤ 0.05** *** * = Lightly significant P ≤ 0.01** ***** = highly significant P ≤ 0.001%**

3.4 Discussion

According to Pedersen *et al*. (2003), the possibility of impeding gene-flow whether by wind or bees is the use of cytoplasmic male sterility in transgenic sorghum together with male fertile conventional lines. The authors further stated that this kind of production in sorghum could prevent gene-flow in transgenic sorghum as pollen is dispersed by wind and no bees in this case are attracted to the sterile lines because there are no flowers. In this study three male sterile sorghum varieties (Ps 213A, Ps 256A and Ps 278A) were planted according to eight geographical directions (N, NE, E, SE, S, SW, W and NW) because wind can be blown in all directions. Seed set by the MSVs as indicator of the distance of pollen travelled was assessed at 20 intervals marked at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 m away from the central pollen source. Sorghum naturally is a self-pollinating crop and if we were to plant only male sterile varieties there wouldn't be any fertilization hence the pollinator (R8602) was planted in the centre of the trial as a fertile sorghum variety.

3.4.1 Distance

The observation in this study was that when evaluating the three male sterile varieties at 20 distance intervals from the pollen source, the mean number of fertile panicles per plot showed that the number of fertile panicles by arm directions were quiet significant. Even though pollen flow quickly falls off with distance, distance alone will not completely prevent gene-flow and hybridization between crop and wild relatives. In a specific set-up, Raybould (1993) showed that a gap between crops is an inefficient way of reducing pollination; an unplanted distance of 3-4 m had no more effect than

one of 1 m. At both localities in this study most fertile panicles were observed close to the pollen source with few abnormalities of fertile panicles in abundance at a distance far from the pollen source to confirm what Raybould (1993) observed, but in this study it was even observed some hundred meters away from the pollen source to suggest that even more space is needed to reduce contamination. All varieties showed high number of fertile panicles that decreased with increasing distance from the pollen source. Considerable number of sterile plants still existed hundred meters away from the pollen source.

In the forage industry it is believed that the male sterile hybrid forage sorghums eliminate the hazards of volunteer regrowth noting that nearby fertile sorghums can pollinate plants in the field, as such under this circumstance the recommended minimum isolation distance is set at 1.6 km to protect the sterility (Raybould, 1993). In the case of this study it showed that sterile panicles were less close to the pollen source because the number of fertile panicles was high close to the pollen source and for the fact that there were sterile panicles observed at 100 m away from the pollen source and there were also fertile panicles at that distance which suggest 1.6 m as good isolation distance.

Cytoplasmic male sterile sorghum varieties showed that arm direction x variety had no significant difference when evaluated at 20 distance interval of 100 m and eight arm directions on panicle length. It was also observed that there was no significant difference on the same variety as far as distance and direction is concerned. When varieties are compared to each other, Ps213A was found to be significantly different to

Ps256A and Ps276A. Regarding the mean panicle length in the three varieties, Ps213A and Ps278A have a panicle length of 25 cm while Ps256A has 30 cm. Ps256A had relatively the lowest number of seed yield maybe it is because of the two other varieties were more fertile than this long variety. It is a fact that outcrossing decreases significantly with distance.

Variety Ps213A showed high seed yield followed by Ps256A. Seed yield was the highest at NE direction for varieties Ps213A and Ps256A. Relatively low seed yield was observed in a south and north westerly. This suggests that at this locality and growing season there was increased wind movement at NE, followed by E, SE, and W directions rendering pollen dispersal and fertilization. The effects of gene-flow between domesticated varieties and their wild progenitors depend on the direction and potential of pollen transfer. Gene-flow can retard progress in breeding and also boost fitness in the cultivated crop/weed hybrids and even improve cultivated crop (Klinger and Elistrand, 1994). Seed yield in the experiment showed relatively low CV at 0.2%. Seed mass showed the same trend of significance as in panicle length as far as contribution towards TTV is concerned. At both localities, that is Syferkuil and Roedtan the only significant source of variation was variety and contributed by 96% and 99% respectively. Variety Ps213A had a hundred seed mass of 6 g, Ps256A 7 g and Ps278A 5 g, hundred seed mass was consistent across all directions except varietal differences. A sound resolution will need a better understanding that gene-flow between crops and weedy relatives has been occurring for as long as plants have been domesticated, weedy populations originated from wild relatives and were further

diversified by multidirectional and conducting research in this regard will bring good solutions.

Ecological studies conducted in the 1990s, when genetically modified crops were first introduced, concluded that genes will transfer to related species whenever crops and related wild and/or weedy species grow together. The movement of a transgene, or any modified gene, into a field that is planted with a traditional cultivar is a valid concern in countries where regulations require the segregation of GM crops from non-GM crops. This also means possible contamination of local landraces. Critical concerns have been raised about the potential risks posed by genetically modified (GM) crops on the environment (Ellstrand, 2003a). Foremost, among these concerns is the potential escape of trans-genes from cultivated crops to their wild and weedy relatives through gene-flow. The possible harmful consequences of such escape are the evolution of more aggressive weeds in agricultural systems. GM sorghum, being developed to solve agronomic or nutritional problems in developing countries. Often genetically modified sorghum (GMs) are being developed by publicly funded, non-profit institutions. Such developers and the regulatory authorities often have limited experience and resources for risk assessment and therefore increasing risks associated with gene-flow. Prediction of the extent and direction of introgression between sorghum varieties and wild weedy relatives is, thus, an important part of environmental risk assessment of transgenic sorghum. Before release of GM cultivars, detailed research is needed on the distance and direction of pollen-mediated gene-flow in sorghum at various locations using non-transgenic baseline environments.

The significance of the study was to contribute to the understanding of the gene-flow related environmental risks of releasing GM sorghum into Limpopo Province agro-ecosystem and contribute to biosafety, conservation and utilization decisions regarding sorghum in the Province. Understanding the distance and direction of movement of genes between domesticated and wild and/or weedy relative populations of sorghum is the first step in characterising the potential environmental risks of escaped transgenes. Such information is currently lacking, but is critically needed for science-based decision making by biosafety regulators. Ellstrand (2003b) indicated that the wild relatives of modern crops are critical in the search for new genes to improve the quantity and quality of agricultural production and reduce risk of crop losses by providing resistance to biotic and abiotic disturbances. Valuable genes obtained from crop's wild relatives are used to improved yield, resistance against specific pests and diseases, tolerance to abiotic stresses and nutritional qualities. The introduction of transgenic sorghum among farmers taking precautionary measures using eight coordinates for transgene containment male sterility, particularly cytoplasmic male sterility to avoid contamination of existing cultivars.

Whenever transgenic sorghum such as bio-fortified sorghum varieties are introduced in the proximity of farmers varieties, factors such as pollen size, the distance pollen travels, pollen competition (a function of the relative amount of pollen of two competitor sources) and the mode of pollination cause cross pollination between the varieties. Other environmental factors that can influence cross pollination between existing crops and newly introduced varieties are wind speed and direction, presence

of barriers between fields, percentage of relative humidity and temperature. Distances used in this study were not sufficient to avoid genetic contamination. Further studies with relatively longer distances from pollen sources are therefore, required to allow for sound conclusions on the least isolation distance to preclude seed set due to cross pollination.

CHAPTER 4

SUMMARY, CONCLUSION AND RECOMMENDATIONS

4.1 Summary

On determining pollen gene-flow three male sterile varieties were planted for the fact that self-pollination should not occur but pollen from the normal sorghum variety planted at the centre of the trial should be the one to determine how long and at what direction is likely to travel. Contamination of existing varieties by newly established genetically modified ones is of great concern in South Africa hence there are no transgenic sorghum crops under commercial cultivation to date in this country. Taking into consideration nutritional value of bio-fortified sorghum variety this study was necessary to be conducted, for issue related to biosafety. Concerns in sorghum, is pollen-mediated gene-flow to the wild species *Sorghum halepense* (Johnson grass), a wild weedy relative, reported to occur naturally at frequencies of 2.5% at a distance of 13 m (Schmidt and Bothma, 2006). The above recommended distance may be a point of departure for the deployment of sorghum transgenes in South Africa. Because our country policies does not allow establishment of such cultivars, much can be learned from Zambia and Kenya ICRISAT which have established some trials in order to find out how much of damage will these transgenes cause on the existing cultivars. This gave courage that one day transgenes could be planted side by side with other existing varieties among our sorghum farmers in South Africa.

The main observed characteristic during plant growth was that in all three varieties, growth patterns, in particular panicle height were affected neither by direction nor

fertilisation from pollen flow. With the information obtained from the study in the Province of Limpopo the deployment of sorghum transgenes is possible only if certain aspects, such as distance and wind direction are taken into consideration. From both localities under which these studies were conducted, it was obvious that pollen could be carried over a long distance, thus, threatening contamination of existing varieties. The data from both localities was subjected to the principal component analysis (PCA) using the number of fertile and sterile panicles, panicle length, number of seeds per panicle, seed yield and hundred seed mass to identify the most influential or representative trait(s) in the experiment. The PCA identified three principal components (PCs) that contributed to 86% of the total variation. PC1, PC2 and PC3 contributed to 35.55, 29.24 and 20.79% of the total variation; respectively. Three traits, that is, seed yield, panicle length and number of seed per panicle correlated well with PC1, PC2 and PC3, respectively. Thus, seed yield is the most important contributor at 36% to the total variation exhibited by the MSVs, distance and direction of planting.

The analysis of variance indicated significant interactions among varieties, distance and direction from the pollen source on the number of fertile and sterile panicles. There were significant differences only among varieties for panicle length and hundred seed mass. The number of seeds per panicle showed significant interaction among variety and planting or arm directions.

4.2 Conclusion

Gene-flow, however, can have undesirable ecological or evolutionary consequence and particularly in cultivated sorghum that is why there has been a need to conduct

non-transgenic baseline environmental risk assessment studies in targeted regions where the Africa Bio-Fortified sorghum (ABS) is planned to be introduced in South Africa, Limpopo province has been earmarked to form part of this. The assessment information generated in this study will assist to create data on potential outcrossing and gene-flow between cultivated sorghum varieties or wild relatives in target environments. For the fact that fertilization diminishes with distance away from pollen source, isolation distance can be established to avoid contamination. At 100 m distance from the pollen source seed set were also found, suggesting that 100 m as isolation distance when introducing transgenic is not enough.

Modelling to determine the lasting impact of gene-flow from crops to wild relatives is still in its early stages. What is clear is that gene-flow between crops and related wild taxa is widespread and gives rise to issues such as strategic release of new crop species, effects on genetic diversity of wild relatives populations, increased fitness of weedy relatives, consequently the quality of ecosystem services. Genes are likely to move quickly from cultivated crops into natural ecosystems whenever populations of compatible relatives are in close proximity (Hancock *et al.,* 1996). The issues that must be examined to estimate the probability of gene-flow and impact of introgression are: whether genetic or geographic barriers exist to gene-flow from a given crop into wild populations; whether the transferred gene is likely to increase in frequency and be selectively advantageous for wild populations. For any plant introduction, sufficient information is needed on the properties of the introduced organism and the potential for gene-flow to wild and weedy relatives.

Consequently, there are fears, that newly developed bio-fortified sorghum varieties might contaminate the existing farmers' varieties. Thus, detailed information is required on the rate and distance of pollen-mediated gene-flow at various locations using non transgenic baseline environments. Pollen travels quiet a long distance, as long as 13 m (Schmidt and Bothma, 2006), if not obstructed by any barriers. Consequently a study in this regard had to be conducted at two localities in Limpopo Province using three cytoplasmic male sterile sorghum varieties at Waterberg District (Roedtan) and Capricorn District (Syferkuil). This was done in order to determine seed set in sorghum as affected by direction and distance of pollen dissemination.

The results indicated significant interactions among varieties, distance and direction from the pollen source on the number of fertile and sterile panicles, seed yield, both at Syferkuil and Roedtan. The study established the presence of clear differences among the MSVs on the performance of agronomic traits. Variety Ps213A had the highest seed set and agronomic performances followed by Ps256A and Ps278A. Further, there was a considerable reduction in seed set and other agronomic attributes among all the varieties with increased distance from the pollen source. The results of the study suggested that sufficient distance would be required to minimize genetic drift from pollen flow of closely related sorghum including genetically modified varieties. Further studies with relatively longer distances from pollen source across different locations are required to draw sound conclusions.

4.3 Recommendations

The fact that there was a considerable reduction in seed set and other agronomic attributes among all the varieties with increase in distance from the pollen source,

suggesting that transgenic varieties can be deployed among sorghum farmers as long as considerable measures (particularly isolation by distance) to avoid contamination of existing cultivars are followed. Future gene-flow research in sorghum should aim at establishing the rate of transgene-flow from genetically engineered sorghum to non-transgenic varieties and wild/weedy relatives.

REFERENCES

ANDERSON, E. 1949**.** *Introgressive Hybridisation*. Wiley: New York.

ARRIOLA, P.E., and N.C. ELLSTRAND. 1996. Crop-to-weed gene-flow in the genus sorghum (Poaceae): Spontaneous interspecific hybridization between Johnson grass, *Sorghum halepense*, and crop sorghum, *S. bicolor*. *American Journal of Botany* 83:1153-1160.

ARRIOLA, P.E., and N.C. ELLSTRAND. 1997. Fitness of interspecific hybrids in the genus Sorghum: persistence of crop genes in wild populations. *Ecological Applications 7:512-518.*

AYOO, C. 2008. Economic instruments and the conservation of biodiversity*. Management of Environmental Quality: An International Journal* 19:550-564.

BAKER, H.G. 1974. The evolution of weeds. *Annual Review of Ecological Systems* 5:1-24.

BARNAUD, A., DEU, M., GARINE, E., JOLY, H.I., and D. McKEY. 2007. Local genetic diversity of sorghum in a village in northern Cameroon: structure and dynamics of landraces. *Theoretical and Applied Genetics 114: 237-248.*

BARRETT, S.C.H. 1983**.** Crop mimicry in weeds. *Economic Botany* 37:255-282.

BELTON, P.S., and J.N.R. TAYLOR. 2003. Sorghum and millets: Protein source for African Trends in food. *Science and Technology* 15:94-98.

BERGQUIST, R.R., NUBEL, D.S., and D.L. THOMSON. 1998**.** Production method for corn with enhanced quality traits, US. US Patent 5:706-803.

BRASHER, J.W., and N. SNOW. 2002. *Colorado Interactive Flora, Faze 1. Key to the Families*. American Society of Plant Taxonomists*:* Madison, WI.

BLANCAS, L. 2001. *Hybridization Between Rare and Common Plant Relatives: Implications for Plant Conservation Genetics*. University of California: Riverside.

BORRELL, A.K., and A.C.L. DOUGLAS. 1997**.** Maintaining green leaf area in grain sorghum increased nitrogen uptake under post-anthesis drought*.* International Sorghum and Millets Newsletter 38:*89-91*.

BORRELL, A.K., BIDINGER, F.R., and K. SUNITHA. 1999. Stay-green associated with yield in recombinant inbred sorghum lines varying in rate of leaf senescence*.* International Sorghum and Millets Newsletter 40*:*31-33*.*

BORRELL, A.K., DOUGLAS, A.C.L., and G.L. HAMMER. 2000**.** Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence*.* Crop Science 40*:*1026-1037*.*

CASAS, AM., KNONOWICZ, A.K., ZEHR, U.B., TOMES, D.T., AXTELL, J.D., BUTLER, LG., BRESSAN, R.A., and P.M. HASEGAWA. 1993**.** Transgenic sorghum plants via micro projectile bombardment. *Proceedings of the National Academy of Sciences* 90:11212-11216.

CASAS, A.M., KONONOWICZ, A.K., HAAN, T.G., ZHANG, L., TOMES, D.T., BRESSAN, R.A., and P.M. HASEGAWA. 1997. Transgenic sorghum plants obtained after micro projectile bombardment of immature inflorescences. *In Vitro Cell Development Biological* 33:92-100.

CONNER, A.J. 1997. Genetically Engineered Crops. *Environmental and Food Safety Issues.* The Royal Society of New Zealand, Miscellaneous Series 39: 1-34.

DE WET, J.M.J. 1978. Systematic and evolution of sorghum sect. Sorghum

(Graminae). Botany 65:477-484.

DE WET, J.M.J., and J.R. HARLAN. 1971. The origin and domestication of *Sorghum Bicolor. Economic Botany* 25:128-135.

DEVLIN, B., and N.C. ELLSTRAND. 1990. The development and application of a refined method for estimating gene-flow from angiosperm paternity analysis. *Evolution* 44:248-259.

DICKO, M.H., GRAPPEN, H., TRAORE, A.S., VORAGEN A.P.G., and W.J.H. VAN BERKEL. 2006. Sorghum grain as human food in Africa. Relevance of content of starch and amylase activities. *African Journal of Biotechnology* 5:384-395.

DILLON, S.L., LAWRENCE, P.K., HENRY, R.J., and H.J. PRICE. 2007. Sorghum resolved as a distinct genus based on combined ITS1, ndhF and Adh1 analyses. *Plant Systematics and Evolution.*

DOGGETT, H. 1970. *Sorghum.* Wiley: New York.

DOGGETT, H. 1988**.** *Sorghum. Tropical Agricultural Series*. Longman Scientific: Essex.

DOGGETT, H., and B.N. MAJISU. 1968. Disruptive selection in crop development. Heredity 23:1-23.

DOGGETT, H., and K.E. PRASADA RAO. 1995. *Sorghum*. In: J. SMARTT and N.W. SIMMONDS, (Eds.) Evolution of Crop Plants 173-180. Longman: Harlow.

ELLSTRAND, N.C. 1992. Gene-flow by pollen implications for plant conservation *Genetics Oikos* 63:77-86.

ELLSTRAND, N.C. 2001. When transgenic wander, should we worry? *Plant Physiology* 125:1543-1545.

ELLSTRAND, N.C. 2003a. Dangerous Liaisons when Cultivated Plants Mate with their Wild Relatives: *Syntheses in ecology and evolution.* The John Hopkins University Press: Baltimore, MD, USA.

ELLSTRAND, N.C. 2003b. Going to great lengths to prevent the escape of genes that produces specialty chemicals. *Plant Physiology* 132: 1770-1774.

ELLSTRAND, N.C., and D.L. MARSHALL. 1985. Interpopulation gene-flow by pollen in wild radish, *Raphanus sativus. The American Naturalist* 126:606-615.

ELLSTRAND, N.C., and D.R. ELAM. 1993. Population genetic consequences of small population size: implications for plant conservation. *Revolution, Ecology and System* 24:217-242.

ELLSTRAND, N.C., and K.A. SCHIERENBECK. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences* 97:7043-7050.

ELLSTRAND, N.C., PRENTICE, H.C., and J.F. HANCOCK. 1999. Gene-flow and introgression from domesticated plants into their wild relatives. *Annual Revolution Ecology System* 30:539-563.

EHRLICH. P.R. and P.H. Raven. 1969. Differentiation of populations. *Science* 165: 1228-1323.

ELLIS. R.H., CRUFURD, Q.I., SUMMERFIELD, P.P., and R.J. ROBERTS. 1997. Effects of photoperiod, temperature and asynchrony between thermoperiod and photoperiod on development to panicle initiation in *Sorghum.* Annals of Botany 79:169-178.

FREYRE, R., RIOS, R., GUZMAN, L., DEBOUCK, D.G., and P. GEPTS. 1996.

Ecogeographic distribution of *Phaseolus* spp. (Fabaceae) in Bolivia. *Economics Botany* 50:195-215.

GAO, Z., XIE, X., LING, Y., MUTHUKRISHNAN, S., and G.H. LIANG. 2005. *Agrobacterium tumefaciens*-mediated sorghum transformation using a mannose selection system*. Plant Biotechnology Journal* 3:591-599.

GEPTS, P., and R. PAPA. 2003. Asymmetry of gene-flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. *Theoretical and Applied Genetics* 106:239-250.

GIRJASHANKAR, V., SHARMA, H.C., SHARMA, K.K., SWATHISREE, V., PRASAD, L.S., BHAT, B.V., ROYER, M., SECUNDA, B.S., NARASU, M.L., ALTOSAAR, I., and N. SEETHARAMA. 2005. Development of transgenic sorghum for insect resistance against the spotted stemborer (*Chilo partellus*). *Plant Cell Replication* 24:513-522.

GRESSEL, J., and A.W. ROTTEVEEL. 2000**.** Risks from biotechnologically-derived herbicide-resistant crops: decision trees for assessment. *Plant Breeding Revolution* 18:251-303.

GURNEY, A.L., TAYLOR, A., MBWAGA. A., SCHOLES, J.D., and M.C. PRESS. 2002. Do maize cultivars demonstrate tolerance to the parasitic weed *Striga asiatica*? *Weed Research* 42:299-306.

HAIJAR, R., and T. HODGKIN. 2007**.** The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* 156:1-13.

HANCOCK. J.F., GRUMET, R., and S.C. HOKANSON. 1996. The opportunity for escape of engineered genes from transgenic crops. *Horticultural Science*

31:1080-1085.

HARLAN, J.R. 1992**.** *Crops and Man.* American Society of Agronomy and Crop Science Society of America: Madison, Wisconsin, USA.

HARLAN, J.R., and J.M.J. DE WET. 1971**.** Towards a rational classification of cultivated plants. *Taxonomy* 20:509-517.

HOANG-TANG, and G.H. LIANG. 1998. The genomics relationship between Cultivated sorghum, a revolution. *Theoretical and Applied Genetics* 76:277-284.

HOLM, L.G., PLUCKNETT, D.L., PANCHO, J.V., and J.P. HERBERGER. 1977. *The World's Worst Weeds.* University of Hawaii Press: Honolulu, USA.

HOUSE, L.R. 1985. A Guide to Sorghum Breeding. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics.

HOWE, A. 2000. Rapid and reproducible Agrobacterium-mediated transformation of sorghum. *Plant Cell Reports* 25:751-758.

JORDAN, D.R., TAO, Y.Z., GODWIN, I.D., HENZEL, R.G., COOPER, M., and C.L. MINTYRE. 1998. Loss of genetic diversity associated with selection for resistance to sorghum midge in Australian sorghum. *Euphytica* 102:1-7.

KAMALA, V., SINGH, S.D., BRAME, I.P.J., and D.M. RAO. 2002. Sources of resistance to downy mildew in wild and weedy sorghums. *Crop Science* 42:1357-1360.

KAMESWARA RAO, N.K., REDDY, L.J., and l.P.J. BRAME. 2003. Potential of wild species for genetic enhancement of some semi-arid food crops. *Genetic Resources and Crop Evolution* 50:707-721.

KOMOLONG, B., CHAKRABORTY, S., RYLEY, M., and D. YATES. 2002. Identity

and genetic diversity of the sorghum ergot pathogen in Australia. *Australian Journal of Agricultural Research* 53:621-628.

KANGAMA, C.O., and X. RUMEI. 2005. Introduction of Sorghum into China. *African Journal of Biotechnology* 4:575-579.

KLINGER, T., and N.C. ELISTRAND. 1994. Engineered genes in wild populations: fitness of weed crop hybrids *of Raphanus sativus. Ecological Applications* 4:117-120.

KRISHNAVENI, S., JEONG, J.M., MUTHUKRISHNAN, S., and G.H. LIANG. 2000. transgenic sorghum plants constitutively expressing a rice chitinase gene show improved resistance to stalk. *Journal of Genetics* 55:151-158.

LANGEVIN, S.A., CLAY, K., and J. GRACE. 1990. The incidence and effects of hybridization between cultivated rice and its related weed red rice *(Oryza sativa (*L*). Evolution* 44:1000-1008.

LINDER, C.R., TAHA, I., SEILER, G.J., SNOW, A.A., and L.H. RIESEBERG. 1998. Long-term introgression of crop genes into wild sunflower populations. *Theoretical and Applied Genetics* 96:339-347.

MARKUS, S., and B. GURLING. 2006. Risk assessment for transgenic sorghum in Africa crop-to-crop gene-flow in *Sorghum bicolor* (L.) Moench. *Crop Science* 46:790-798.

MAUNDER, A., and G. SHARP. 1963. Localization of outcrosses within panicle of fertile sorghum. *Crop Science* 3:449.

MORRIS, W.F., and P.M. KAREIVA. 1994. Ecological applications by the ecological society of America. *Ecological Applications* 4:157-165.

MUTEGI, E.F., SAGNARD, M., MURAYA, B., KANYENJI, B., RONO, C.,

MWONGERA, C., MARANGU, J., KAMAU, H., PARZIES, S., DE VILLIERS, K., TRAORE, P.S., and M. LABUSCHAGNE. 2009. Ecogeographical distribution of wild, weedy and cultivated *Sorghum bicolor* (L.) Moench in Kenya: implications for conservation and crop-to-wild gene-flow. *Genetic Resources* and *Crop Evolution.* Doi 10.1007/s10722-009-9466-7.

OKA, H.I., and W.T. CHANG. 1961. Hybrid swarms between wild and cultivated rice species, *Oryza perennis and Oryza sativa. Evolution* 15:418-430.

OTTAVIANO, E., SARI-GORIA, M., and M. VILLA. 1988. Pollen competitive ability in maize: Within population variability and response to selection. *Theoretical and Applied Genetics* 76:601-608.

PEDERSEN. J.F., MARX, D.B., and D.L. FUNNELL. 2003. Use of A3 cytoplasm to reduce risk of gene-flow through sorghum pollen. *Crop Science* 43:1506-1509.

QUIST, D., and I.H. CHAPELA. 2001. Transgenic DNA introgressed into traditional Maize landraces in Oaxaca, Mexico. *Nature* 414:541-543.

RAEMAKERS, R.H., and C.W. SMITH 2001. Risk assessment for transgenic sorghum in Africa: *crop-to-crop gen-flow in Sorghum bicolor (L.) Moench.*

RAYBOULD, A.F., and A.J. GRAY. 1993. Genetically modified crops and hybridization with wild relatives: a UK perspective. *Applied Ecology* **30:**199-219.

REDDY, B.V.S., RAMESH, S.S., and P.S. REDDY. 2006. Sorghum genetic resources, cytogenetics and improvement. Genetic resources, chromosome engineering and crop improvement. *Genetic Resources* 309-363.

REDDY, B.V.S., and H.C. SHARMA. 2007. Ethanol from sweet sorghum does not

compromise food security. *Science in Africa,* July 2007.

RIESEBERG, L.H., and J.F. WENDEL. 1993. *Introgression and its Consequences in Plants*. In: R. Harrison (ed.), Hybrid Zones and the Evolutionary Process. Oxford University Press: Oxford, UK.

RISSLER, J., and N.M. MELLO. 1996. *The Ecological Risks of Engineered Crops*. Mass MIT Press: Cambridge, UK.

RIESEBERG, L.H**.** 1997. Hybrid origins of plant species. *Annual Review Ecological System* 28:359-389.

RHYMER. J.M. and D. SIMBERLOFF.1996. Extinction by hybridization and introgression. *Annual Revolution Ecology System* 27:83-109.

ROSENOW, DT., and L.E. CLARK. 1995. Drought and lodging resistance for a quality sorghum crop. *Proceedings of the Annual Corn and Sorghum Industry Research Conference* 15:82-97.

SANGDUEN, J., and W.W. HANNA. 1984. Chromosome and fertility studies on reciprocal crosses between two species of auto-tetraploid sorghum, *Sorghum bicolor* (*L.) Moench and S. halepense* (L.) Pers. *Journal of Heredity* 75:293-296.

SCHIMIDT, M., and G. BOTHMA. 1993. Prevalence and drivers of seed and pollen-mediated gene-flow in sorghum. Implications for biosafety regulations and policy in Kenya. *African Journal Economical Conference* (Beijin, China). August 16-22. 2009**.**

SCHIMIDT, M., and G. BOTHMA. 2006. Risk assessment for transgenic sorghum in Africa. Crop-to-crop gene-flow in *Sorghum bicolor (L.) Moench. Crop Sci*ence

46:790-798.

SHARMA, H.C. 1993. Host-plant resistance to insects in sorghum and its role in integrated pest management. *Crop Protection* 12:11-34.

SHARMA, H.C., and B.A. FRANZMANN. 2001. Host-plant preference and oviposition responses of the sorghum midge, *Stenodiplosis sorghicola* towards wild relatives of sorghum. *Journal of Applied Entomology* 125:109-114.

SLATKIN, M. 1987. Gene-flow and the geographic structure of natural populations. *Science* 236:787-792.

SMALL, E. 1984. Hybridization in the domesticated-weed-wild complex. *Plant Biosystematics* 195-219.

SMARTT, J., and N.W. SIMMONS. 1995. Evolution of Crop Plants. Longman Scientific and Technical: Harlow 466-471.

SMITH, C.W., and R.A. FREDERIKSEN. 2000. Sorghum origin, history, technology, and production. 824.

SNOW, A.A., and P. MORAN. 1997. Commercialisation of transgenic plants: *Potential ecological risks*. *Bioscience* 47:86-96.

TAO, Y.Z., HARDY, A., DRENTH, J., HENZELL, R.G., FRANZMANN, B.A., and D.R. JORDAN. 2003. Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. *Theoretical and Applied Genetics* 107:116-122.

TAYLOR, J.R.N., and E. EMMAMBUX. 2008. *Traditional African Cereal Grains Overview*. University of Pretoria: Pretoria.

TESSO. T.I., KAPRAN, C., GRENIER, A., SNOW, A., SWEENEY, P., PEDERSEN,

J., MARX, D., BOTHMA, G., and G. EJETA. 2008. The potential for crop-to-wild gene-flow in Sorghum in Ethiopia and Niger: Geographic survey. *Crop Science* 48:1425-1431.

TORRES-CARDONAS, S. 1990**.** *Fertility Restoration to A,A, and A Cytoplasm Systems of Converted Sorghum Lines.* MP-1721. The Texas Agriculture Experiment Station, Texas A and M University, College Station: Texas.

VISARADA, K.B.R.S., and N.S. KISHORE. 2007. Improvement of Sorghum through transgenic technology. *ISB News Report March 2007:*1-3.

WHITTON, J., WOLF, D.E., ARIAS, D.M., SNOW, A.A., and L.H. RIESEBERG. 1997. The persistence of cultivar alleles in wild populations of sunflowers five Generations after hybridization. *Theoretical and Applied Genetics* 95:33-40.

WOLF, D.E., TAKEBAYASHI, N., and L.H. RIESEBERG. 2001. Predicting the risk of extinction through hybridization. *Conservation Biology* 15:1039-1053.

WRIGHT, S. 1931. Evolution in Mendelian populations. *Genetics* 16:97-159.

Zhao, Z.Y., CAI, T., TAGLIANI, L., WANG, N., PANG, H., RUDERT, M., SCHROEDER, S., HONDRED, D., SELTZER, J., and D. PIERCE. 2000. Agrobacterium-mediated sorghum transformation. *Plant Molecular Biology* 44:789-798.

ZHU, G., KRISHNAVENI, H., LIANG, H., and S. MUTHUKRISHNAN. 1998. Biolistic transformation. of sorghum using a rice chitinase gene. *Genetics Breeding* 52:243-252.

APPENDICES

Appendix 6.1 Analysis of variance for the number fertile panicles per plot among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals and eight arm directions from pollen source.

Number of fertile panicle					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	9.66	4.83	1159.00	0.01
Distance (D)	19	2132.00	112.20	26926.89	0.01
Arm Direction (AD)	7	1478.00	211.10	50670.71	0.01
V x D	38	104.20	2.74	657.95	0.01
V x AD	14	30.14	2.15	516.71	0.01
D x AD	133	922.20	6.93	1664.10	0.01
V x D X AD	266	416.00	1.56	375.36	0.01
Residual	480	2.00	0.00		
Total	959	5094.00			

Appendix 6.2 Analysis of variance for the number sterile panicles per plot among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals and eight arm directions from pollen source.

Number of sterile panicle					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	2.63	1.32	1264.32	0.01
Distance (D)	19	427.20	22.48	21584.84	0.01
Arm Direction (AD)	7	1525.00	217.86	209142.86	0.01
V x D	38	126.80	3.34	3203.37	0.01
V x AD	14	18.08	1.29	1239.77	0.01
D x AD	133	934.60	7.03	6745.98	0.01
V x D X AD	266	427.90	1.61	1544.30	0.01
Residual	480	0.50	0.00		
Total	959	3424.00			

Appendix 6.3 Analysis of variance for panicle length among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals and eight arm directions from pollen source.

Panicle length					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	5337.00	2668.00	2562000.00	0.01
Distance (D)	19	0.02	0.00	1.00	0.46
Arm Direction (AD)	7	0.01	0.00	1.00	0.43
V x D	38	0.04	0.00	1.00	0.47
V x AD	14	0.01	0.00	1.00	0.45
D x AD	133	0.14	0.00	1.00	0.49
V x D X AD	266	0.28	0.00	1.00	0.50
Residual	480	0.50	0.00		
Total	959	5338.00			

Appendix 6.4 Analysis of variance for number of seeds per panicle among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals and eight arm directions from pollen source.

Number of seeds per panicle					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	296998	148499	0.4	0.67
Distance (D)	19	6613860	348098	0.93	0.55
Arm Direction (AD)	7	4973063	710438	1.9	0.07
V x D	38	12917641	339938	0.91	0.63
V x AD	14	58036939	4145496	11.08	0.01
D x AD	133	46275683	347937	0.93	0.69
V x D X AD	266	103410164	388760	1.04	0.36
Residual	480	179636261	374242		
Total	959	412160610			

Appendix 6.5 Analysis of variance for seed yield among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals and eight arm directions from pollen source.

Seed yield					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	3633	1816.00	1744000.00	0.01
Distance (D)	19	30840	1623.00	1558000.00	0.01
Arm Direction (AD)	7	21490	3070.00	2947000.00	0.01
V x D	38	1903	50.07	48064.37	0.01
V x AD	14	589.8	42.13	40443.86	0.01
D x AD	133	13210	99.36	95381.03	0.01
V x D X AD	266	5631	21.17	20320.85	0.01
Residual	480	0.5	0.00		
Total	959	77300			

Appendix 6.6 Analysis of variance for thousand seed mass among three CMS sorghum varieties at Syferkuil when evaluated at 20 distance intervals and eight arm directions from pollen source.

Thousand seed mass					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	636.0083	318.0042	11306.81	0.01
Distance (D)	19	0.51146	0.02692	0.96	0.51
Arm Direction (AD)	7	0.35729	0.05104	1.81	0.08
V x D	38	1.11667	0.02939	1.04	0.40
V x AD	14	0.05833	0.00417	0.15	1.00
D x AD	133	3.58021	0.02692	0.96	0.61
V x D X AD	266	7.81667	0.02939	1.04	0.34
Residual	480	13.5	0.02813		
Total	959	662.949			

Appendix 6.7 Analysis of variance for the number fertile panicles per plot among three CMS sorghum varieties at Roedtan farm when evaluated at 20 distance intervals and eight arm directions from pollen source.

Number of fertile panicle					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	9.20	4.60	736.17	0.01
Distance (D)	19	7329.00	385.70	61716.84	0.01
Arm Direction (AD)	7	2209.00	315.60	50488.00	0.01
V x D	38	70.13	1.85	295.29	0.01
V x AD	14	34.28	2.45	391.79	0.01
D x AD	133	1280.00	9.62	1539.28	0.01
V x D X AD	266	579.70	2.18	348.70	0.01
Residual	480	3.00	0.01		
Total	959	11510.00			

Appendix 6.8 Analysis of variance for the number sterile panicles per plot among three CMS sorghum varieties at Roedtan when evaluated at 20 distance intervals and eight arm directions from pollen source.

Number of sterile panicle					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	2.85	1.43	1369.44	0.01
Distance (D)	19	169.80	8.94	8579.37	0.01
Arm Direction (AD)	7	339.20	48.46	46518.86	0.01
V x D	38	153.40	4.04	3875.37	0.01
V x AD	14	36.46	2.60	2500.11	0.01
D x AD	133	1204.00	9.05	8690.53	0.01
V x D X AD	266	587.00	2.21	2118.50	0.01
Residual	480	0.50	0.00		
Total	959	2485.00			

Appendix 6.9 Analysis of variance for panicle length among three CMS sorghum varieties at Roedtan when evaluated at 20 distance intervals and eight arm directions from pollen source.

Panicle length					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	5313.41	2656.70	41136.06	0.01
Distance (D)	19	1.14	0.06	0.93	0.55
Arm Direction (AD)	7	1.05	0.15	2.32	0.05
V x D	38	2.51	0.07	1.02	0.44
V x AD	14	0.53	0.04	0.58	0.88
D x AD	133	7.99	0.06	0.93	0.69
V x D X AD	266	17.56	0.07	1.02	0.42
Residual	480	31.00	0.06		
Total	959	5375.18			

Appendix 6.10 Analysis of variance for number of seeds per panicle among three CMS sorghum varieties at Roedtan when evaluated at 20 distance intervals and eight arm directions from pollen source.

Number of seeds per panicle					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	58947170.00	29473585.00	972.47	0.01
Distance (D)	19	771918.00	40627.00	1.34	0.15
Arm Direction (AD)	7	37024.00	5289.00	0.17	0.99
V x D	38	2071354.00	54509.00	1.80	0.00
V x AD	14	72516.00	5180.00	0.17	1.00
D x AD	133	3292498.00	24756.00	0.82	0.92
V x D X AD	266	7801062.00	29327.00	0.97	0.62
Residual	480	14547814.00	30308.00		
Total	959	87541355.00			

Appendix 6.11 Analysis of variance for seed yield among three CMS sorghum varieties at Roedtan when evaluated at 20 distance intervals and eight arm directions from pollen source.

Seed yield					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	453.20	226.60	21750000	0.01
Distance (D)	19	10800.00	568.40	54570000	0.01
Arm Direction (AD)	7	3426.00	489.50	46990000	0.01
V x D	38	9312.00	245.00	23520000	0.01
V x AD	14	3392.00	242.30	23260000	0.01
D x AD	133	37260.00	280.20	26900000	0.01
V x D X AD	266	64990.00	244.30	23460000	0.01
Residual	480	0.01	0.00		
Total	959	129600.00			

Appendix 6.12 Analysis of variance for thousand seed mass among three CMS sorghum varieties at Roedtan when evaluated at 20 distance intervals and eight arm directions from pollen source.

Thousand seed mass					
Source of variation	d.f.	s.s.	m.s.	v.r.	P ≤
Variety (V)	2	634.02	317.01	33814.33	0.01
Distance (D)	19	0.18	0.01	1.00	0.46
Arm Direction (AD)	7	0.07	0.01	1.00	0.43
V x D	38	0.36	0.01	1.00	0.47
V x AD	14	0.13	0.01	1.00	0.45
D x AD	133	1.25	0.01	1.00	0.49
V x D X AD	266	2.49	0.01	1.00	0.50
Residual	480	4.50	0.01		
Total	959	642.99			