Mutagenesis and Development of herbicide resistance in sorghum for protection against *Striga*

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Sorghum (*Sorghum bicolor*) is an important cereal crop in sub-Saharan Africa. The parasitic weed *Striga hermonthica* is a major biotic constraint to sorghum production. A novel technology where planting seeds are coated with herbicide to kill *Striga* that attach to the roots of the host has been shown to be effective in protecting the cereal crop from *Striga* damage. However, the host plant must have herbicide tolerance. This technology has not been tested in sorghum because there are no herbicide tolerant sorghum varieties available in Kenya and is, therefore, unavailable for subsistence farmers. One of the ways in which genetic variation can be enhanced and herbicide resistance developed is through chemical mutagenesis with ethyl methane sulfonate (EMS). The objectives of this project, therefore, were to: 1) identify sorghum production constraints through farmer PRA in order to determine breeding priorities.in two *Striga* endemic districts in western Kenya; 2) develop an EMS mutagenesis protocol for sorghum and to enhance the genetic variability of the crop using chemical mutagenesis; 3) evaluate EMS-derived sorghum mutants for improved agronomic performance; 4) develop acetolactate synthase (ALS) herbicide resistance in sorghum and to characterize the mode of inheritance of the trait; 5) determine the effect of herbicide coating of seed of herbicide tolerant sorghum on *Striga* infestation.

In order to determine breeding priorities and constraints in sorghum production and the likelihood of adoption of herbicide seed coating technology, a survey involving 213 farmers was conducted in two *Striga* endemic rural districts of Nyanza province in Kenya. Results indicated that local landraces like *Ochuti*, and *Nyakabala* were grown by more farmers (> 60%) than the improved varieties like *Seredo* and *Serena* (48%). Popularity of the landraces was linked to *Striga* tolerance, resistance to drought, bird damage and storage pests, yield stability and high satiety value. Major constraints to sorghum production were drought, *Striga* weed, storage pests, bird damage and poverty among the rural farmers. Important characteristics farmers wanted in new varieties were *Striga* and drought resistance, earliness, resistance to bird and weevil damage and good taste. *Striga* infestations in sorghum fields were > 70%. Cultural *Striga* control options were considered inadequate while inorganic fertilization and chemical control were considered effective but unaffordable. Farmers' willingness to pay a premium of over 30% for a *Striga* solution gave indication that herbicide seed coating if effective could be adopted by farmers.

As a prerequisite to development of herbicide resistance, a comparative study was carried out to determine optimum conditions for mutagenesis and to induce genetic variation in the sorghum. Two sorghum varieties were mutagenized using varying concentrations (0.1 to 1.5% v/v) of EMS and two exposure times (6h and 12h). In laboratory and greenhouse experiments, severe reduction of sorghum root and shoot lengths indicated effective mutagenesis. The LD_{50} based on shoot length reduction was 0.35% and 0.4% EMS for 6h for *Seredo* and *Kari/mtama-1*, respectively. The highest mutation frequency based on chlorophyll abnormalities was 56% for 0.3% EMS for 6h. In the M_2 generation, phenotypic variances for panicle characteristics were increased on treatment with EMS. However, significant effects of exposure time and variety indicated the necessity of genotype optimization for some traits.

In order to determine the significance of mutation breeding in sorghum, 78 mutant lines derived from EMS mutagenesis, their wild type progenitor (*Seredo*) and two local checks

(Kari/mtama-1 and Serena) were evaluated for agronomic performance in two locations in Kenya. There were significant ($P = 0.05$) effects among entries for grain yield, 1000-seed weight and visual scores for height uniformity, head exertion, head architecture and overall desirability. The highest yielding entry-mutant line "SB2M13" had a yield of 160% and 152% relative to the wild type (*Seredo*) and the best check *Kari/mtama-1*, respectively. Mutant line "tag27" had the highest 1000-seed weight which was 133% relative to the wild type. Seven mutant lines were rated superior to the wild type for panicle characteristics, head exertion and overall desirability. However, the majority of mutants were inferior to the wild type for most characteristics. Superior mutant lines may be developed into direct mutant varieties after multi-location trials or used as breeding material for sorghum improvement.

In order to develop acetolactate synthase (ALS) herbicide resistance in sorghum, over 50,000 seeds of *Seredo* were mutagenized with 0.3% EMS. Over four million M_2 plants were screened using 20g ha^{-1} of the ALS herbicide, sulfosulfuron. Five mutants (hb46 hb12, hb462, hb56 and hb8) survived the herbicide treatment and were confirmed to be tolerant. Mutant lines displayed differential herbicide tolerance, and the general order of tolerance after spray or seed coat application was hb46 > hb12 > hb462 ~ hb56 > hb8. The LD_{50} values for herbicide application as a spray, or seed coat, showed mutant lines to be up to 20 and 170 fold, respectively, more resistant than the wild type. Chi square analysis of data from herbicide screening of F_2 generation of mutant X wild type crosses indicated no difference from the Mendelian segregation of 1:2:1 indicating the herbicide tolerance was inherited as a single semi-dominant gene. Mutant X mutant crosses did not show allelism indicating that the tolerance in all five mutants could be a result of the same gene mutation.

To determine effect of herbicide seed coating on *Striga* infestation, the five herbicide tolerant mutant lines, hb46, hb12, hb462, hb56 and hb8 and the wild type progenitor *Seredo* were coated with varying concentrations (0.5-1.5% g ha^{-1}) of sulfosulfuron and planted in a *Striga* endemic field. There were significant ($P=0.05$) effects of herbicide concentration on *Striga* density, *Striga* flowering and seed set, and sorghum plant stand and biomass. All treatments with herbicide coated on sorghum seeds had lower *Striga* emergence. Coating sorghum seed with 1g ha^{-1} sulfosulfuron reduced *Striga* infestation, *Striga* flowering and *Striga* seed set by 47%, 52% and 77%, respectively, and was considered the most effective rate as it did not result in sorghum biomass reduction. Mutants displayed differential herbicide tolerance and *Striga* resistance. Combining seed coating with high herbicide tolerance and inherent *Striga* resistance would be most effective for *Striga* control.

Overall, the study showed that EMS mutagenesis is effective in inducing variation in sorghum for several traits including herbicide resistance. The mutants developed in this study will be important for sorghum breeding and for protection of sorghum against the *Striga* weed.

Declaration

I, declare that:

(i) The research reported in this thesis, except where otherwise indicated, is my original research.

(ii) This thesis has not been submitted for any degree or examination at any other university.

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Dedication

This work is dedicated to Ann Marie Ngendo and the memory of Nancy Wamaitha Kamundia and Joan Nyokabi Kamundia

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Abbreviations

EMS- Ethyl Methane Sulfonate

LD_{50}- lethal dose 50

ALS-Acetolactate synthase

CIMMYT- International Maize and Wheat Improvement Centre

ICRISAT-International Crops Research Institute for the Semi-Arid Tropics

FAO- Food and Agricultural Organization

GMO-Genetically Modified Organism

KARI-Kenya Agricultural Research Institute

WAP-Weeks after Planting

WAS-Weeks after Spraying

CAN-Calcium Ammonium Nitrate

DAP-Di-ammonium phosphate

HRC-Herbicide resistant crops

General introduction

The importance of sorghum in sub-Saharan Africa

Sorghum [*Sorghum bicolor* (L.) Moench.] is grown mainly in the semi-arid areas of the tropics and sub-tropics and ranks fifth in terms of importance among the world's cereals (Dogget, 1988). It is a major cereal within this region where it is utilized either as a food or feed-crop (Kenga et al., 2004). Although sorghum is ranked 5th in the world, in sub-Saharan Africa, it is ranked second to maize in supply of grain requirement (Mutisya, 2004). In Kenya, it is mostly grown in dry areas that are either too arid or generally unsuitable for maize production. Waxy leaves and an extensive root system make it ideally suited to the semi-arid regions making it superior to other cereals (Armah-Agyeman et al., 2002). Like maize, it is mainly eaten in a form of thick porridge or gruel called "ugali" and also used for brewing traditional beer.

The area under sorghum cultivation in Africa has generally shown an upward trend but the average yield trend is downwards (Dogget, 1988). Poor average yields are exemplified, for example in Kenya, where the total area under sorghum for the year 2007 was estimated to be 120,000ha, with maize and wheat, the only other cereals with higher acreage (FAOSTAT, 2008). However, the production statistics for the same year indicated Kenya produced on average 750kg ha^{-1} against an average of 1020kg ha^{-1} in Africa in general. Production in the same year in the USA was 4658Kg ha^{-1}. The dismal performance in Africa is as a result of a myriad of problems with *Striga*, drought and extreme poverty among farmers being some of the major constraints. The main challenge today, however, is to increase production among small-scale farmers with a limited resource base. Incentives will include better pricing of sorghum, proper marketing, good husbandry and adoption of improved varieties (Dogget, 1988). Generally, the increased demand of cereals within the sub-Saharan region means that sorghum is well positioned to satisfy the demand as the crop's potential has not been realized. However, realization of this increase will only occur with prudent and practical strategies, among them tackling the *Striga* menace which ranks as one of the most limiting factors for improved production of sorghum in the sub-tropics.

The parasitic "witchweed" *Striga*

Striga is a major biotic constraint to cereal production in sub-Saharan Africa and much of the semi–arid tropics (Sauerbon, 1991; Rich et al., 2004; Khan et al., 2005). Throughout much of sub-Saharan Africa, *Striga hermonthica* (Del.) Benth and *Striga. asiatica* (L.) Kuntze are known to be parasitic on maize (*Zea mays* L.), pearl millet (*Pennisetum spp*), finger millet (*Eleucine coracane*), sorghum [*Sorghum bicolor* (L.) Moench], and upland rice (*Oryza sativa)*, resulting in major losses in these crops (Kanampiu et al., 2003). It is estimated that 94% of all the area under cereal production within sub-Saharan Africa is cultivated with a host crop of *Striga* (Rodenburg, 2005). Within this region, sorghum is the most widely cultivated cereal crop with 25.5 million ha being grown which is 30.6% of the total area under cereal crop production (FAOSTAT, 2004). In Africa, *Striga* is estimated to infest 21 million ha causing an estimated yield loss of 4.1 million tons of grain per year (Sauerbon, 1991). Recent estimates however, paint a gloomier situation. According to Khan et al. (2005), *Striga* weeds infest up to 40% of arable land in sub-Saharan Africa, causing an annual crop loss of US$ 7 to 13 billion.

The *Striga* weed challenge in Africa

The genus *Striga* is in the family Scrophulariaceae and is composed of some 50 species, all of which are haloparasites of tropical cereals or legumes (Kiruki et al., 2006). The *Striga* weed presents a special challenge to food security as it inflicts most of the damage while it is still underground and therefore out of reach of most conventional control measures (Berner et al., 1997; Abayo et al., 1998; Rich et al., 2004). Once the *Striga* seeds have been stimulated to germinate they attach on to the roots of host plants and draw nutrients causing reduction in plant growth with yield reduction estimated to be between 2% and total yield loss (Abayo et al., 1998). The very intricate association of the life cycle of *Striga* to that of its host ensures that the parasite is well adapted, posing a major challenge in its control (Gurney et al., 2003a). Negative effects of the parasite include changes in the balance of plant growth regulators (Gurney et al., 1995) and addition of toxins into the host (Ejeta and Butler, 1993). Each *Striga* plant can produce up to 20,000-50,000 seeds, which lie dormant in the soil until a cereal host crop is planted (Khan et al., 2005).

Yield losses due to *Striga* are increased when plants are already in poor health because of drought and low soil fertility (Berner et al., 1994). This poses a major challenge as drought and low fertility are a common occurrence in much of sub-Saharan Africa. Many subsistence farmers end up abandoning farms once *Striga* infestation has reached unmanageable levels. While this was possible in the early days and in one way ensured yield improvement, it is not possible today due to population increase. Most of the cultivable land is being used and farmers must cultivate on existing farms under high *Striga* pressure which leads to dismally low or complete yield losses.

***Striga* in Kenya**

Striga hermonthica is widely distributed in western Kenya where it occurs at altitudes of between 1100m to 1600m above sea level (Odhiambo, 1998). It affects production of maize, sorghum, rice and sugar cane. *Striga asiatica* is more of a problem in the coastal region of the country. Yield losses associated with *S. hermonthica* within western Kenya can range from 20% in low infested areas to 100% where infestations are high (Odhiambo G.D., 1998). In western Kenya where *S. hermonthica* is particularly important, it is estimated that 76% of the land planted to maize and sorghum is infested causing an estimated $38 million in losses each year (Hassan et al., 1994).

Rationale and control options for *Striga*

Farmers are clearly in need of low-input solutions to the *Striga* problem for both the short and long terms (Rodenburg et al., 2005). Breeding for tolerance and resistance mechanisms is an option many consider as a viable solution to the *Striga* problem and sorghum is the only cereal host to the parasite in which some cultivars show partial resistance to infestations (Lane et al., 1996). Though complete resistance to infection by *Striga* species is not known to exist in cultivated cereals, wild relatives of sorghum could be a source of genes for tolerance and resistance (Gurney et al., 2002). Many interventions into breeding crops for tolerance or resistance to *Striga* have been undertaken (Ramaiah, 1987; Ejeta et al., 1991; Vogler et al., 1996; Haussmann et al., 2001; Gurney et al., 2002; Gurney et al., 2003b; Rich et al., 2004). Other interventions include cultural and field management options (Jacobsohn et al., 1980; Englesham et al., 1981; Ndung'u et al., 2000; Odhiambo and Ransom, 2000; Ransom, 2000; van Ast et al., 2005), use of herbicides (Abayo et al., 1998;

Kanampiu et al., 2002; Kanampiu et al., 2003), chemical means like the use of brine (Gworgwor et al., 2002) and use of transgenic crops (Joel et al., 1995).

Despite most of the above mentioned options having high potential to solve the *Striga* problem, no single option on its own has proven to be both sufficiently effective and durable as well as economically and practically applicable for low-input farming systems (Joel, 2000). None of the countless experiments set up to investigate *Striga* control approaches have had impact on farmers fields (Haussmann et al., 2000). Generally, *Striga* control has proved challenging, partly as a result of the intricate life cycle of the parasite with its host (Gurney et al., 2003). The ability of *Striga* to produce large amounts of seed translates into extremely high seed densities of *Striga* seed in the soil that are able to overwhelm even crops that have mechanisms conferring partial tolerance like reduced stimulant production (Kanampiu et al., 2003). Since most *Striga* damage occurs underground before the parasitic plant emerges, the parasite is out of reach of most control measures (Rich et al., 2004). Financial and practical constraints that limit the use of chemical forms of control, especially, in developing countries, are also seen to be a major stumbling block to *Striga* control (Gurney et al., 2003). These challenges of low purchasing power, lack of technical know-how and paucity of basic production information regarding *Striga* will continue to undermine the eradication of *Striga* in subsistence systems of Africa. While breeding for resistance may offer future solutions to *Striga*, currently, there are no sorghum varieties with complete resistance or tolerance. What the subsistence farmer needs is an option that is cheap, easy to manage, durable and economically feasible.

Herbicide seed coating of Herbicide resistant sorghum: A viable option to *Striga* management for the subsistence farmer

Striga is primarily a problem of small-scale subsistence farming systems with few options for external inputs and therefore, control options must of necessity be low-cost and practical (Rodenburg, 2005). For any control option to be accepted, cognizance of subsistence farming systems of manual cultivation, small and fragmented farms, multi-cropping and low input must be taken into consideration. The control option, therefore, has to be effective, inexpensive, require no complex application technique, fit into a complex cropping system and show a return on investment in the first season

(Berner et al., 1995; Berner et al., 1996). Seed treatment of crop seeds with herbicide has been advocated as a possible low cost solution to the *Striga* problem for subsistence farmers. The technology involves coating planting seed with herbicide so as to prevent *Striga* infestation on the growing plants. Kanampiu et al. (2003) have tested this model system of *Striga* control using commercial herbicide-resistant maize varieties coated with herbicide and found out that any *Striga* that attached to the roots of the host was killed by the herbicide. Seed treatments with two acetolactate synthase (ALS)-inhibiting herbicides, the sulfonyl-urea herbicide nicosulfuron and the imidazolinone herbicide, imazaquin have also been shown to control *Striga hermonthica* in maize (Berner et al., 1997). Joel (2000) has also shown herbicide resistant plants in combination with treatments with respective herbicides to be highly effective in controlling parasitic weeds. Use of Imazapyr on maize with ALS-target site resistance was found to increase harvest index by 17% and it was concluded that complete control was achievable at affordable cost by farmers in subsistence conditions (Abayo et al., 1998). Herbicide applications of 2-4 D and triclopyr have also been found to reduce *Striga* emergence in the field (Carskey et al., 1994). Imazapyr and pyrithiobac, used as seed coating on maize with ALS-target site resistance, were found to give season long protection against *Striga* in Kenya, Malawi, Tanzania and Zimbabwe, with resultant yield benefits of 3-4 fold (Vogler et al., 1996; Kanampiu et al., 2003).

Generally, selective herbicides have not been successful for African farmers because of their cost, and the technology required in their application (Berner et al., 1997). Herbicide seed coating precludes these problems. Seed coating of planting seeds ensures that the farmers need not engage in the technicalities of herbicide mixing. The very low amounts of herbicides required also make the technology very low cost and environmentally friendly (Kanampiu et al., 2003). Seed dressing for example, has been found to reduce the recommended rate of 0.6kg -1.7Kg ha^{-1} imazapyr by 20 fold (Kanampiu et al., 2003). The very little amounts of herbicide required also offers an opportunity for farmers, especially those farming sorghum to purchase the herbicide and coat it on the seeds they have saved in the previous season. However, although very low amounts of herbicide are used, herbicide concentration within the vicinity of the seed is very high thus necessitating a high level of herbicide resistance for the host crop (Bernasconi et al., 1995). Also, the herbicides were found to dissipate from the soil before the next planting season and intercropping is possible as long as the legume intercrop is at least 15cm from the treated maize seed (Kanampiu et al., 2002). Since the technology precludes the use of spray equipment, it is relatively economical to the farmer (Kanampiu et al., 2003). While the technology has been well documented and research is ongoing for maize, the same cannot be said of sorghum. So far, there is no record of herbicide tolerant sorghum being developed. Transgenic, herbicide tolerant sorghums have been put forward as a possible stop-gap and cost-effective control for *Striga* but these cultivars are not yet available (Joel, 2000).

Acetolactate synthase (ALS) Herbicide resistance

The herbicide of choice for development of herbicide resistance in this project was the ALS inhibiting herbicide sulfosulfuron. Herbicide resistance occurs as a result of heritable changes to biochemical processes that enable plant survival when treated with a herbicide (Preston and Mallory-Smith, 2000). Induction of genes conferring resistance to the ALS inhibiting herbicides has been achieved via mutagenesis in several crops including wheat (Newhouse et al., 1992; Pozniak and Hucl, 2004), rice (Sandhu et al., 2002), sunflower (Kolkman et al., 2004), sugerbeet (Wright et al., 1998), and maize (Anderson and Georgeson, 1989). However, ALS resistance has also been found to be naturally occurring (Bernasconi et al., 1995). Point mutations at the ALS site resulting from mutagen treatment are thought to confer herbicide resistance to previously susceptible crops (Preston and Mallory-Smith, 2000).

Mutagenesis for herbicide resistance development

Currently there are no sorghum herbicide resistant varieties available. One of the ways in which herbicide resistance can be developed in sorghum and other crops is via ethyl methane sulphonate (EMS) mutagenesis since mutagenesis is known to alter genes and produce heritable changes in organisms (Koornneef, 2002). There are no studies that have been done on herbicide tolerance development of sorghum via mutagenesis. However, this has been achieved in other cereals including wheat (Newhouse et al., 1992) and maize (Newhouse et al., 1991). Herbicide resistance development has also been successful in other crops including soybean, where chlorsulfuron resistant soybean mutants have been developed via seed mutagenesis (Sebastian and Chaleff, 1987). One of the major advantages of EMS is that it causes small nucleotide changes or point mutations within the genome as opposed to other mutagens which are responsible for deletion of large sections of the genome, thus causing major changes and disrupting most of the characteristics of the variety. With EMS treatment, only small changes are effected, and thus the general characteristics of the variety are maintained (Weil and Monde, 2007). In addition, EMS is generally easy to use and is easily available. Apart from mutagenesis, herbicide resistant crops have also been developed by means of genetic engineering as in the case of barley and tobacco (Le et al., 2005; Shimizu et al., 2008), but this method might not be appropriate to develop herbicide resistance in crops, especially, in sub-Saharan Africa, where GMO (genetically modified organisms) have generally not been accepted.

Baseline study to identify farmer's constraints: Participatory Rural Appraisal (PRA)

A PRA was conducted to identify farmers' perceptions of key constraints and possible solutions for sorghum production. A key attribute of PRA is that farmers are able to develop their own "home-grown" solutions to problems in agricultural production. Participation of farmers in agricultural research is increasingly seen as a powerful methodology to increase the relevance of technologies developed to benefit farmers' communities (Mangione et al., 2006) and farmers have been used to solve problems related to overuse of pesticides, fertilizers, seed rates, and to manage nitrogen use in rice in China (Huan et al., 2004; Hu et al., 2007). In Africa, farmer knowledge has also come in handy in development of superior cassava varieties in Ghana (Manu-Aduening et al., 2006) and to manage fisheries in Kenya (Lwenya and Abila, 2003).

Striga constraints from the point of view of the researcher are well documented from the numerous research articles published. However, there is a general paucity of information detailing the *Striga* problem from the perspective of the farmer. Farmer input on breeding options of *Striga* resistant or tolerant material is also scarce. Since agricultural solutions are geared towards the alleviation of farmers' constraints, farmer perception of *Striga* control measures should be paramount. More often than not, researchers have come up with solutions developed in research stations that have not been effective in farmers' field conditions. A participatory rural appraisal was deemed important to indulge the farmers in various aspects of sorghum production and to generate discussion on the possible benefit of herbicide seed coating, so as to deviate from the all too often situation of 'lack of adoption' of technologies meant for the farmer.

Objectives and approach

The primary aim of the research outlined in this thesis was to develop herbicide resistance in sorghum so as to utilize the resistance in the technology of herbicide seed coating to protect sorghum from *Striga* infestation. An experiment was first conducted to develop a protocol for sorghum mutagenesis with ethyl methane sulphonate (EMS). Two sorghum varieties, *Kari/mtama-1* and *Seredo* were evaluated for effects of EMS mutagenesis on grain sorghum. Mutants derived from mutagenesis of sorghum were also evaluated for agronomically important farmer preferred characteristics. Selection of sulfonylurea herbicide resistant mutants was achieved by spraying mutants with the herbicide and selecting mutants that survived. A dose level evaluation of the herbicide resistant mutants was done in order to determine the levels of herbicide tolerance for the different mutants. A genetic study was also done to determine the mode of inheritance of the herbicide resistance trait. As a proof of concept that the technology of herbicide seed coating of herbicide resistant sorghum can protect sorghum from *Striga* infestation, seed of herbicide resistant mutants were coated with the sulfonylurea herbicide sulfosulfuron and planted in a *Striga* infested field to determine the effect of the technology on *Striga* infestation.

Outline of Thesis

Chapter one is a review of the literature. Conceptions, knowledge gaps, and opportunities are discussed and highlighted. Chapter two outlines the PRA process where farmers were interviewed so as to quantify *Striga* and other sorghum related constraints in western Kenya. Farmer preferences and reasons for growing different sorghum varieties were also discussed. Production constraints and their relationship to breeding are also highlighted. In the 3^{rd} chapter, the process of mutagenesis in sorghum is highlighted. A protocol on mutagenesis which can be useful as a general guide for sorghum mutagenesis is discussed. The concentration of herbicide causing 50% mortality or reduction in a growth parameter (LD_{50}) was determined for *Seredo* and *Kari/mtama-1*. While most mutants that derive from the process of mutagenesis are usually inferior to the parent material, the possibility of superior mutants does exist. Chapter 4 details an agronomic evaluation of EMS derived mutants from sorghum mutagenesis. The 5th chapter details the development of herbicide resistant sorghum mutants through seed mutagenesis using the chemical mutagen ethyl methane sulphonate (EMS). A genetic analysis study to decipher the mode of inheritance of the herbicide resistance trait is also discussed. The 6th chapter is on the technology of herbicide seed coating of herbicide resistant seed and its effect on *Striga* infestation on sorghum. Herbicide coated seed of herbicide resistant mutants were planted in a *Striga* endemic zone to determine if herbicide seed coating was effective in precluding *Striga* infestation. General discussion and overview (chapter 7) of the present study, including opportunities and the way forward are discussed.

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Chapter 1
Literature review

1.1 Introduction

Sorghum is an important crop within sub-Saharan Africa. One of the most limiting constraints to its production within this zone is the *Striga* weed and many options have been put forward for its control. The challenges posed by *Striga* are mainly associated with its biology and relation to its host. For example, *Striga* produces tens of thousands of seeds per plant. This biological aspect of the parasite renders the development of tolerant varieties challenging as these extremely large numbers of seeds are thought to be able to overwhelm even tolerant varieties (Kanampiu et al., 2003). Also, the well synchronized life cycle of the parasite to that of the host ensures the parasite survives even under extreme conditions. Generally, control options developed to remedy the *Striga* problem have not had the desired impact in subsistence farming systems in Africa. Major interventions to the *Striga* problem, their shortcomings and the opportunities available through exploitation of herbicide resistance with special reference to sorghum are explored.

1.2 Sorghum breeding

Africa is thought to be the origin of sorghum (Dogget, 1988). However, most of the development of sorghum has not taken place here but in areas of the developed world. The United States for example is a pioneer in genetic improvement of temperate sorghum and research today is actively pursued by both public organizations and private companies (Chantereau et al., 2001). Major strides have taken place over the years in sorghum breeding. For example, traditional sorghum varieties were tall and made combining almost impossible, but the current commercial hybrids have dwarfing genes and stand only 0.5m to 1.5m tall (Armah-Agyeman et al., 2002). Development of sorghum within the developed world has been significant. In the USA for example, despite sorghum having only been recently introduced into the Americas in the nineteenth century, breeding has resulted in superior high yielding varieties with new hybrids yielding over 4000kg ha^{-1} under dryland conditions while yields under irrigation are well over 8000kg ha^{-1} (Chantereau et al., 2001). However, in Africa, sorghum breeding has not been so phenomenal mainly because breeding priorities are different. While the developed world has concentrated on hybrid development, the breeding in Africa is mainly concentrated on traditional local varieties (Chantereau et al., 2001). In addition, the lack of commercial sorghum farming in Africa has relegated sorghum to second class, with little funding for its improvement. However, research by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is contributing to the improvement of sorghums for the semi-arid zones (Chantereau et al., 2001).

Breeding objectives depend on the use of the intended variety. In the Americas and Europe, breeding of sorghum is geared towards high yield for production of animal feed and this is largely achieved through use of hybrids while in the case of tropical agriculture, breeding for yield stability takes precedence to high yield (Chantereau et al., 2001). Nonetheless, the development of adapted hybrid varieties of sorghum in some of the areas of Africa is seen as a primary means available for increasing yield production (Devries and Toenniessen, 2001). High food demand due to rapid population growth, combined with changes in agro-ecological conditions necessitate the breeding of new varieties that combine adaptation, to the agro-ecological constraints like drought, pests and diseases, and appropriateness to specific end-use (Uptmoor et al., 2003). As mentioned above, one of the most important constraints to sorghum production today, and that deserves immediate attention is infestation by the *Striga* weed.

1.3 *Striga* Weed: a major challenge for sorghum production in Africa

Striga asiatica and *S. hermonthica* are recognised as the largest biological constraints to sorghum production in sub-Saharan Africa (Ejeta and Butler, 1993; Gurney et al., 2002). The *Striga* problem in Africa is mainly related to the increase in human population (Berner et al., 1995). *Striga* control has become more of a problem because the traditional cropping systems of fallowing, rotations and intercropping, which kept *Striga* at manageable levels, can no longer be practiced because of increased population pressure (Lagoke et al., 1991). Also, increased cropping of highly susceptible maize has substituted more tolerant land races of sorghum and millet (Gworgwor et al., 2002). This has exacerbated the *Striga* problem as these “improved” high yielding maize varieties did not evolve with *Striga*, and hence they have no resistance to *Striga*. This has led to a drastic increase in *Striga* seed density in many of the zones infested by the weed (Berner et al., 1995).

1.4 *Striga* biology

The understanding of metabolic and developmental aspects of root parasites is essential in order to develop effective durable control measures (Joel, 2000). Control options geared towards *Striga* have proved challenging especially for resistance breeding due to the intricate association between the parasite and its host (Gurney et al., 2003). Different levels of parasitic associations between the sorghum host and the parasite, like germination, stimulation, haustorial initiation and vascular penetration, however, offer opportunities for genetic resistance development in sorghum for *Striga* (Rich et al., 2004). Since *Striga* is heavily dependent on the host for its survival (Haussmann et al., 2000a), an understanding of this association is important so as to identity points of intervention to break the bond between the parasite and it's host. The life cycle of *Striga*, from germination to seed production, takes from 90 days to 120 days (Chantereau and R. Nicou, 1994). All the important stages within the life cycle of *Striga* offer specific challenges to development of any control option and especially for resistance breeding.

1.4.1 *Striga* seed

Striga seeds are very tiny, 0.3mm long and 0.15mm wide (Haussmann et al., 2000b). The seeds are produced in vast quantities with production estimated at 58,000 plant^{-1} for *S. asiatica* and 200,000 plant^{-1} for *S. hermonthica* (Parker and Riches, 1993). *Striga* seeds can remain viable in the soil for many years and estimates vary from six months to 20 years according to climatic conditions (Ayensu et al., 1984; Dorr, 1997). These are some of the reasons why many of the soils in *Striga* endemic areas of sub-Saharan Africa have extra-high *Striga* seed densities. These high *Striga* seed densities pose extremely dire consequences for breeding for resistance since even those varieties with tolerance mechanisms like low *Striga* stimulation can get overwhelmed by the large seed numbers rendering them ineffective. Also, physiological strains are likely to be encountered due to the large seed numbers in the soil. These strains will potentially overcome the resistance of some varieties.

1.4.2 *Striga* seed germination

Striga seed requires an after ripening period and, therefore, cannot germinate at the end of the rainy season in which it is produced (Ayensu et al., 1984). *Striga* Seeds also require conditioning and stimulation by chemical compounds exuded by the host before they can germinate and parasitize a host plant successfully (Ejeta et al., 1992). Seeds generally, remain dormant in the soil until chemical signals (Hydroquinones), released from the roots of the potential hosts initiate seed germination (Hauck et al., 1992). Some of the interventions to *Striga* control involve breeding for varieties with low *Striga* germination stimulants. Low production of compounds that *Striga* requires as stimulants for germination is one of the better understood mechanisms of resistance to *Striga* in sorghum and may offer opportunities for development of *Striga* resistance (Vogler et al., 1996, Rich et al., 2004). However, as mentioned above, even low production of *Striga* germination stimulants can prove ineffective in the face of high *Striga* seed densities in the soil.

1.4.3 *Striga* attachment

After stimulation of germination, the radicle of the seed grows towards the host root through chemicals released by the host (Ayensu et al., 1984). Upon contact, the tip of the radicle transforms itself into a haustorium, apparently due to a chemical secretion from the host root known as the haustorial initiation factor (Ramiah et al., 1983). The radicle of the seedling secretes enzymes that assist its penetration into the host root (Kuijt, 1991). Once established, the haustorium forms a morphological bridge between the host and parasite (Ejeta et al., 1992). The haustorium generally sets up the parasitic association and nutrients then flow from the host plant to the parasite (Ramiah et al., 1983). The important aspect of this association is that it occurs subterranean. By the time the *Striga* emerges, most of the damage to the plant has already occurred (Abayo et al., 1998; Kanampiu et al., 2003). Many of the control options have failed to make impact since they only tackle the weed when it has emerged. While the process of parasitic attachment is the cause of debilitating consequences for the plant, it can however, be used to the advantage of the host plant if chemicals, for example herbicides, which kill the parasite are administered from the host to the weed which is the case for herbicide seed coating. The fact that initial attachment is precluded or delayed and yield damage reduced makes the technology especially appropriate to subsistence farmers.

1.4.4 Host-parasite interaction

Once established, the parasite becomes a metabolic sink for the carbohydrates produced in the host thus depriving the host of some of its photosynthates (Ramaiah et al., 1983). The *Striga* seedling then grows parasitically underground for approximately 4-6 weeks, during which period it wholly depends on the host for assimilates and water, causing severe damage to the host (Pieterse and Pesch, 1983). Therefore, *Striga* control options like herbicide seed coating, that prevent initial establishment before the parasite does any damage would be more attractive to farmers as the benefits of the technology are immediately seen.

1.5 *Striga* Control

Striga seed banks in many of the areas in sub-Saharan Africa where *Striga* is endemic are extremely high and the primary objective of many research programmes is to reduce the *Striga* load by initiating suicidal *Striga* germination using chemicals (Worsham, 1987) and killing seeds by fumigation. However, most of these *Striga* control measures, including nitrogen fertilization, have proved too costly and beyond the means of poor subsistence farmers (Vogler et al., 1996; Mohammed et al., 2003) with few methods having impact today in farmers' fields (Haussmann et al., 2000a). In order to be adopted, *Striga* control practices must improve crop yield per unit area, maintain soil fertility and be acceptable to farmers even in the absence of *Striga* infestation (Berner et al., 1996). Therefore, management of the weed needs an integrated approach that includes host plant resistance, cultural practices and chemical treatments (Gworgwor et al., 2002). Any control option must at least integrate one of the control options from the major categories of: (1) reduction of the soil seed bank; (2) limitation of *Striga* seed production; and (3) reduction/prevention of *Striga* seed dissemination to uninfested fields

(Haussmann et al., 2000a). Some of the control options advocated for subsistence farming systems are presented and their shortcomings are discussed.

1.5.1 Manual hand weeding

Manual hand weeding of *Striga* before flowering has been shown to reduce *Striga* infestation (Dogget, 1988; Carskey et al., 1994; Ransom, 2000). However, this method of *Striga* control is labour intensive and requires constant monitoring so as to stop replenishment of *Striga* seed in the soil. Generally, Hand weeding, whose effect is only evident after many seasons, has very limited effect on managing *Striga* infestations (Pierce et al., 2003).

1.5.2 Crop rotation with "trap crops" and fallowing

Crop rotation with non-host species "trap-crops" and leaving the farms fallow have been used by farmers to improve soil fertility and to reduce the rate of *Striga* seed bank build up in the soil (Gethi, 2003). "Trap crops" or false host crops stimulate germination of *Striga* or root parasites without themselves being parasitized (Visser and Beck, 1987; Ndung'u et al., 2000). Major trap crops recommended for use against *Striga* include cotton (*Gossypium hirsutum* L.), cowpea (*Vigna unguiculata*_Walp.), soybean (*Glycine max* L.) groundnut (*Arachis hypogeae* L.) and sunflower (*Helianthus annus* L.) (Ariga, 1996). While this system has proved effective, it is impractical in areas where land is already scarce (Gethi, 2003) as rotation periods required for effective control are not achievable in most of the areas where *Striga* is a menace.

1.5.3 Intercropping

Intercrop cultivars that produce *Striga* germination stimulant without allowing for attachment can play an integral role in an integrated *Striga* control package. The fodder legumes *stylosanthes*, *mucuna* and *desmodium* as intercrops were found to reduce *Striga* infestations and to improve maize yields (Ndung'u et al., 2000). *Desmodium* as an intercrop in maize is used in a highly effective *Striga* and stem borer control technology known as "push and pull" (Khan et al., 2005). Farmers have come to appreciate intercropping as it ensures growing of much needed cereals and legumes and at the same time achieve some level of productivity (Gethi, 2003). Benefits from mixed cropping in reducing *Striga* incidence are well demonstrated (Salle et al., 1987; Carson, 1988). Nitrogen fixed and released by some of intercrops like cowpea or fodder legumes (Englesham et al., 1981; Ndung'u et al., 2000) is thought to contribute to *Striga* suppression since the amount of available nitrogen apparently affects *Striga* density (Pieterse and Verkleij, 1991). However, the benefits from intecropping are only realized after several seasons, making the option only marginally effective.

1.5.4 Soil fertility management

The use of farmyard manure in improving grain yields on *Striga* infested soil has long been known (Ayensu et al., 1984) and nitrogen fertilizers are strongly recommended in integrated control of *Striga* as it is thought they decrease *Striga* infestation (Pieterse and Verkleij, 1991). Use of composted manure with inorganic nitrogen was found to decrease *S. hermonthica* incidence in western Kenya (Odhiambo and Ransom, 2000). However, despite the many advantages of using manure in the control of *Striga*, it is not readily available to most of the farmers in *Striga* endemic areas and the amounts required for effective control are not usually attainable since most farmers keep only a few animals. Inorganic fertilisers are unaffordable for most of the small-holder farmers and even when they can afford to buy, the availability and distribution is not guaranteed (Ariga, 1996).

1.5.5 Host plant resistance

Host plant resistance is probably the most feasible and potentially durable method for control of *Striga* (Ejeta and Butler, 1993; Haussmann et al., 2000a; Mohammed et al., 2003). *Striga*-resistant sorghums could form a major component of integrated *Striga* control approaches if resistance is incorporated into adapted and productive cultivars (Haussmann et al., 2000a). The development and use of genotypes that can withstand *Striga* parasitism holds the greatest promise in *Striga* control for subsistence agriculture (Kiruki et al., 2006). However, major challenges abound in developing *Striga* resistant varieties. The complex biological relationship between *Striga* and its host has made resistance breeding progress very slow (Ejeta et al., 1992; Haussmann et al., 2000a). Due to the erratic nature of *Striga* seed banks in the soil, evaluating resistance in the field is difficult (Haussmann et al., 2000a). Known sorghum sources of resistance to *Striga* have also been found to be frequently low yielding with poor agronomic background (Ramaiah, 1987) while presence of *Striga* biotypes is also thought to be responsible for breakdown of resistance in crops like sorghum where resistance has been identified (Ramaiah, 1987; Gethi, 2003). However, years of conventional breeding and variety testing have not produced crop varieties that have shown stable resistance in heavily infested fields (Kanampiu et al., 2003).

1.6 Herbicide seed coating: A new approach to *Striga* control for the subsistence farmer

Any technology that is able to preclude *Striga* attachment, before the parasite does damage to the crop will be the most suitable as benefits from using the technology are instantly visible. The technology must of necessity be cheap and easy to manage when it is intended for the subsistence farmer. A new technology developed by CIMMYT (International Maize and Wheat Improvement Centre) has been found to accommodate these requirements. The technology uses imazapyr resistant (IR) maize coated with the herbicide imazapyr to give season long control of *Striga* in maize (Kanampiu et al., 2003). One of the important aspects of this strategy of control for *Striga* is that the yield benefits from using the technology are evident in the season that it is used as germinating *Striga* is killed by the herbicide. Also, the technology is cheap and does not require complicated application equipment as the herbicide is coated on the seed. However, the maize crop must be resistant to the herbicide or it gets killed by the high concentration of herbicide around the germinating seed. In developing the herbicide seed coating technology, CIMMYT used a maize line which already had a mutation conferring acetolactate synthase (ALS) resistance to the imidazolinone herbicides (Newhouse et al., 1991). Currently, this is the only cereal where the technology has been tried and good progress is being made. Herbicide resistant sorghum has so far not been developed. However, opportunities exist to develop the resistance in sorghum and thus apply the technology of herbicide seed coating to protect sorghum from *Striga* infestation.

1.7 Creating genetic variation in sorghum

Most of the breeding techniques that have been employed for sorghum improvement are mainly those that relate to self pollinating crops as sorghum is considered autogamous (Chantereau et al., 2001) as its outcrossing rate is very low (Dogget, 1988). According to Chantereau et al. (2001), there are basically four ways of generating variability in sorghum; 1) Germplasm surveys: where new genes are sought and introgressed into the adapted cultivars. An example is the search for new sources of *Striga hermonthica* resistance genes in wild relatives of sorghum (Gurney et al., 2003); 2) Crosses between complementary varieties, where already established varieties are crossed and selections made for traits of interest; 3) Development of composites where different sorghum lines with different origins are used to form a group with diverse characteristics after which the base population is recombined and recurrent selection methods used to increase favourable genes. However, the costs associated with this process are usually very heavy; and 4) mutagenesis which involves modification of the genes within the genome of sorghum.

In certain instances a lack of variability for certain traits has prompted the use of biotechnology. A case in point is the lack of genetic variation in the gene pool for nutritional quality which necessitated the use of mutation breeding to develop genes for high lysine content in sorghum (Axtell et al., 1979). While genetic transformation may also offer a solution for lack of variability in sorghum, the technology has not been widely accepted in Africa prompting the use of other means to induce much needed genetic variation.

1.8 Mutagenesis

Mutagenesis alters the genetic makeup of plants by interference and modification of genes (Koornneef, 2002). Mutants with new alleles and genes are created which enhances genetic variation (Koornneef, 2002; Singh and Kole, 2005). Production of heritable changes is an important aspect of many breeding programmes and breeders use mutations to produce these changes (Neuffer et al., 1997). It is well established that mutation breeding has made significant contribution to plant improvement (Larkin, 1998). For example, the non-edible oil from linseed flax, *Linum usitatissimum,* was turned into an edible oilseed oil (linola) and a new industry in potential through induced mutations of the fatty acid biosynthesis pathway (Larkin, 1998). A lot of work on mutagenesis has been accomplished with many mutants of agronomic importance recorded as well reviewed by Natarajan (2005). More than 2000 mutant plant varieties have been released for cultivation, and faced none of the regulatory restrictions imposed on genetically modified material (Waugh et al., 2006). Generally, mutation breeding has remained popular for the last 70 years because it is simple, cheap to perform and applicable to all plant species (Siddiqui and Khan, 1999).

Use of mutation breeding is especially useful today to create genetic variation in crops where the genetic variability is limited. For example, mutation breeding using EMS has been found effective in generating much needed variation for certain traits where the genetic variation was lacking (Yadav, 1987; Singh and Kole, 2005). In *capsicum*, mutation studies have also shown that EMS mutagenesis increases the variation in many characters including leaf area, days to flowering, days to fruiting, and plant height. Such variation is important to breed for desirable characters (Jabeen and Mirza, 2002). In maize, the most efficient means of producing gene mutations has been found to be chemical mutagenesis and a rational protocol of chemical mutagenesis in this crop is well presented by Neuffer et al. (1997). Many agronomically important mutations affecting plant and grain characters have been identified, including alteration of grain color, stem rust resistance, and earliness in wheat (Chopra, 2005). In oats, isolation and characterisation of novel starch mutants has also been achieved (Verhoeven et al., 2004). The success of mutation breeding in ornamentals and horticultural crops in India is impressive with 46 mutants commercially released (Chopra, 2005).

1.9 Mutagenic Agents

Mutagenesis can be achieved by use of chemical, physical and biological means (Koornneef, 2002). Both physical and chemical mutagens are known to act in different ways to cause DNA lesions (Chopra, 2005). They are known to induce a high frequency of mutations at random locations across the genome (Waugh et al., 2006). Among the chemical mutagens are the vegetable oils, alkylating agents including EMS, Butyl Methane sulphonate, (BMS) and arsenic (Natarajan, 2005). The most widely used physical mutagens are the ionising agents such as gamma and X-rays (Koornneef, 2002). Many crops including wheat, rice, maize, barley and *Brassicas* were mainly treated with radioactive isotopes (32P, ^{35}S), X-rays or fast neutrons (Natarajan, 2005). Initial studies on induced mutations performed on a variety of species including wheat, barley, rice, tobacco, maize, Brassica, fruit crops and vegetables, were carried out for both physical and chemical mutagens and were directed towards finding an optimum combination of the mutagen and dose (Chopra, 2005).

In many mutation breeding programmes for seed propagated crops, the starting material for mutagenesis is usually seed (van Harten, 1998; Koornneef, 2002). However, other material can also be used including whole plants, ex-plants like leaves or shoots, and gametes (pollen or egg cells) (van Harten, 1998). Plant parts are usually treated for the case of vegetatively propagated plants (Koornneef, 2002). While seed has been shown to be most widely used in many self-pollinating crops, this is not the case for maize where seed is not recommended for mutagenesis because the mature maize kernel has separate germ line cells and since mutations are single cell occurrences, a recessive mutant produced in this seed will only be seen in the M_3 generation (Neuffer et al., 1997; van Harten, 1998; Koornneef, 2002). Seed was chosen as the starting material for sorghum mutagenesis as it was easier to handle and protocols of seed mutagenesis were easily accessible.

Usually before embarking on a mutation breeding programme, it is important that mutagen dose optimization experiments are conducted. This is because the dose and exposure time to the mutagen are important in determining the frequency and types of mutations. Optimization of dose, frequency of mutations and induction of genetic variability studies have been done in many crops including rice (Seetharami Reddi, 1984), grain sorghum (Seetharami Reddi and Prabhakar, 1983), chickpea (Shah et al., 2006), mungbean (Singh and Kole, 2005; Singh et al., 2005), common bean (Svetleva, 2004) and oats (Verhoeven et al., 2004).

In sorghum, mutagenesis is recognised as one of the approaches that can be used to create genetic variability (Chantereau et al., 2001) though there is paucity of information on mutagenesis in sorghum. Brataudeau and Traore (1990) have shown that EMS has the potential to induce favourable mutations in sorghum with important mutants for drought and earliness being realized. Mutagens used in sorghum range from chemical agents such as ethyl methane sulfonate (EMS) to physical agents like gamma rays. The most popular agents used for sorghum are physical agents (Chantereau et al., 2001).

1.10 Development of herbicide resistance

Mutagenesis of seed or microspores of pollen followed by selection under herbicide selective pressure has been utilized widely to develop crop resistance to herbicides (Mulwa and Mwanza, 2006). Chemical mutagenesis using EMS was used to develop herbicide resistant mutants with ALS resistance in common wheat (Pozniak and Hucl, 2004). Mutant selection from mutagenized seed has also been used to generate soybean mutants with increased tolerance to the sulfonylurea herbicides (Sebastian and Chaleff, 1987). Maize lines resistant to imidazolinone herbicides have been developed by pollen mutagenesis (Greaves et al., 1993; Shaner et al., 1996). Canola expressing a highly herbicide-resistant ALS to sulfonylurea and trazolopyrimidine herbicides has also been developed (Blackshaw et al., 1994).

Another way of developing herbicide resistance is plant tissue culture (Mulwa and Mwanza, 2006). The identification of crop-selective sulfonylurea herbicides has mostly been accomplished by screening thousands of chemical analogs for the desired properties (Saari and Mauvais, 1996). Maize lines used in the herbicide seed coating technology (Kanampiu et al., 2003) were developed by *in vitro* selection and plant regeneration (Newhouse et al., 1991). In cell culture, certain *in vitro* conditions are able to induce heritable changes, called somaclonal variations, making it possible to select for various desirable traits including herbicide resistance (Maliga, 1978). Maize lines with resistance to the imidazolinone herbicide have been selected from embryogenic maize callus cultures (Anderson and Georgeson, 1989).

Genetic plant transformation is also used to develop herbicide resistance in plants and it has gained popularity in recent years. Plant genetic transformation is the science of direct gene transfer and integration, from one plant to another or from a micro-organism to a plant, to create plants with altered genetic make-ups to achieve specific crop production goals (Mulwa and Mwanza, 2006). Plant transformations using mutant ALS genes from the bacterium *A. thaliana* and tobacco has been undertaken for crops like canola, chicory, flax and rice (Saari and Mauvais, 1996) to develop herbicide resistance. A major disadvantage of genetic transformations is that GMO (genetically modified organisms) have not gained acceptance in Africa. This was one of the major reasons for using EMS mutagenesis for herbicide development in this project.

1.11 Acetolactate synthase (ALS) inhibiting herbicides

The herbicide chosen in this project is a sulfonylurea which is in the group of herbicides collectively called ALS inhibitors, as they inhibit the ALS pathway in plants. The herbicides are known to have wide selectivity and are effective at low rates (Preston and Mallory-Smith, 2000). These qualities combined with low mammalian toxicity and season long control of weeds have resulted in wide acceptance (Saari et al., 1994). Their mode of action is inhibition of the Acetolactate synthase enzyme which is involved in the synthesis of branched chain amino acids (leucine, isoleucine, and valine), (Saari and Mauvais, 1996; Preston and Mallory-Smith, 2000; Peterson et al., 2001). This inhibition severely or fatally disrupts metabolism in herbicide-susceptible genotypes (Shaner et al., 1996). The ALS pathway is absent in animals thus making ALS herbicides relatively non-toxic to animals (Newhouse et al., 1991). Two groups of herbicides are known to inhibit the ALS pathway, - the sulfonylureas and the imidazolinones (Christopher et al., 1992). The herbicides are readily absorbed by the roots and the foliage and translocated in both the phloem and the xylem to the site of action at the growing points (Peterson et al., 2001). Usually, most of the crops with resistance to the ALS herbicides have target site resistance (Gressel, 1992; Preston and Mallory-Smith, 2000). Target site resistance occurs by alteration or modification of proteins targeted by the herbicide, thus impairing the ability of the herbicide to bind or interact (Preston and Mallory-Smith, 2000). Target site resistance in the crop is important for the seed coating technology as it allows for the systemic translocation of unmetabolized herbicide to the attached parasite (Gressel, 1992). Injury symptoms of ALS herbicides, which are only apparent several days after application include stunting, interveinal chlorosis, chlorotic banding on grass leaves, red leaf venation, purpling, root pruning and gradual death (Peterson et al., 2001). This will be important in evaluation of any herbicide resistant mutants that will be generated in this project.

1.12 Developing sulfonylurea herbicide resistance

Sulfonylurea resistance has been introduced into crops mainly by selection of mutant cells or seeds on sulfonylurea impregnated media and by introduction of genes encoding insensitive forms of ALS through transformation (Saari and Mauvais, 1996). Callus or suspension cultures are the starting material for selection of most resistant lines for sulfonylurea resistance (Saari and Mauvais, 1996), as in the case of sugarbeet (Wright et al., 1998), Brassica (Swanson et al., 1988) and maize (Newhouse et al., 1991). However, mutagenised seed have also been used for selection of resistant lines (Newhouse et al., 1992; Pozniak and Hucl, 2004). In most cases, observed resistance has mainly been due to selection of crop lines having an ALS that is less sensitive to inhibition by the sulfonylurea herbicide (Saari and Mauvais, 1996).

1.13 Evaluating herbicide resistance

There exists several ways of evaluating herbicide resistance including determination of enzymic sensitivities, metabolism rates, and mutations conferring resistance. But the surest way of confirming resistance is obtained by the application of herbicides to the whole plant (Saari et al., 1994). A useful measurement for comparing resistant organisms is the resistant factor, which is a ratio of the response, organismic or enzymic, of resistant to susceptible isolates for example, the herbicide rate required to inhibit a growth parameter, e.g. LD_{50} (lethal dose 50 -dose with a survival rate of 50%), as measured by changes in fresh weight, dry weight, or other means (Saari et al., 1994). The technology of herbicide seed coating requires the plant to have a high level of resistance to the herbicide. Proper evaluation of herbicide resistant mutants in this project will be necessary to identify mutants with high resistance levels.

1.14 Inheritance of sulfonylurea herbicide resistance

Resistance to ALS has mostly been found to be conferred by a dominant- or partially-dominant gene in many crops (Saari et al., 1994). In maize, the resistance to the imidazolinones was found to be semi-dominant (Newhouse et al., 1991), in wheat, resistance in five lines developed was found to be semi-dominant and dominant for one of the lines (Newhouse et al., 1992; Pozniak and Hucl, 2004), while in Brassica, resistance to chlorsulfuron was consistent with a semi-dominant mode of inheritance (Swanson et al., 1988). Knowledge of the inheritance of herbicide resistance in this study is important for breeding of the trait.

1.15 How herbicide seed coating works

Herbicide coated seed is produced by treating the seed with a slurry containing herbicide and binder followed by drying. As the maize crop grows, *Striga* plants stimulated to germinate by the maize host attach onto the maize roots but are killed by the herbicide as it is systemically translocated in the plant. *Striga* attaching to the roots of the herbicide resistant maize plants where the planting seed was coated with traces of pyrithiobac and imazapyr died before emerging from the soil (Kanampiu et al., 2003). The technology makes it possible for packaging of herbicide coated seed thus making it attractive to the small-scale farmer as there is no application technique required. Another important aspect of the technology is that it gives season long protection against *Striga* infestation. Imazapyr (Abayo et al., 1998) and pyrithiobac (Gurney et al., 2003) herbicides were found to have sufficient residual activity for season long control but to dissipate from the soil prior to the next season, making it safe to plant a crop with no herbicide resistance in the next season (Kanampiu et al., 2003). The technology also allows for intercropping as long as the intercrop plants are not within a minimum distance of 15cm from the maize crop (Kanampiu et al., 2003). However, it remains to be seen if farmers can adhere to the 15cm distance, taking into consideration that many farmers plant very haphazardly, hardly taking note of the distance between the maize/sorghum crop and the intercrop. Farmer education campaigns will be necessary in order to get the full benefit of the technology.

Normally, very little amounts of herbicide are needed for the seed treatments to be effective against *Striga*. In the case of Imazapyr which is used as a general herbicide at the rate of 0.6-1.7 kg ha^{-1} (Arhens, 1994), the seed application technology lowers this rate more than 20-fold, and precludes the need for spray equipment thus rendering the technology relatively economical to the farmer (Gurney et al., 2003). One of the reasons why many control options have not worked for subsistence farming is the cost needed to apply the technology. Ethylene application and fumigation for example, have been found to be highly effective for *Striga* control but not in Africa where they are considered too expensive.

A major limitation in the development of the technology in other cereals apart from maize is that there are no herbicide resistant varieties. Currently, there are no sorghum or millet herbicide-tolerant varieties. The possibility to quickly generate transgenic herbicide-tolerant varieties in these crops does, however, exist and development of transgenic herbicide-tolerant sorghum is seen as a possible immediate, cost-effective control of *Striga* by herbicides (Haussmann et al., 2000a). However, there are still major concerns on development of transgenic crops especially in Africa. The only other opportunity would be to develop ALS resistance using means other than transformation. On the global scene, very little movement has taken place in the development of herbicide resistant minor crops mainly because of the cost of herbicide registration and associated potential risks which makes herbicide manufacturers have little interest in marketing herbicides for minor crops (Duke, 1996). This project set out to explore ways of developing herbicide resistance in sorghum through sorghum mutagenesis, thus avoiding transformation. The sulfonylurea herbicide of choice, monitor is cheap and easily available making it attractive as the added increase in price would be minimal while offering the benefits of *Striga* control.

1.16 Conclusions from review of the literature

Literature review established that:

1. Sorghum is an important crop within sub-Saharan Africa.
2. *Striga* is one of the most limiting factors to sorghum production. Losses of between 10% – 100% can be incurred according to the level of infestation.
3. *Striga* Control measures for small scale subsistence farmers have not been effective owing mainly to the complexity of the host and parasite interaction and the high general fitness of the parasite. Also, the development of the various options has not involved farmers.
4. A cheap, cost effective and attractive control option is required for *Striga* in sorghum.

5. A novel technology for *Striga* control would be herbicide seed coating of herbicide resistant seed where *Striga* is killed on attachment to the host by translocated herbicide.
6. There are no herbicide resistant varieties in sorghum that are necessary for application of the technology.
7. A viable option would be to generate herbicide resistant non-GMO sorghum using EMS mutagenesis.
8. Mutation induction is variety specific and each variety has an optimum mutagen dose and exposure time, therefore, requiring an optimization of mutagenesis before embarking on a mutagenesis breeding program.
9. The technology of herbicide coating of seed of herbicide resistant varieties can be useful in protecting sorghum against *Striga* infestation.

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Chapter 2

An appraisal of the significance and impact of *Striga* and other production constraints in sorghum in Nyanza province of Kenya

ABSTRACT

Striga, among other factors, is a major constraint in the rural sorghum growing districts of Kenya. As a basis for determining sorghum breeding priorities and effects of *Striga* weed in Western Kenya, a survey involving 213 small-scale farmers was conducted in two districts in Nyanza province. Participatory rural appraisal (PRA) tools, such as focus group discussions and open ended questionnaires, were employed to identify sorghum varieties grown by farmers, reasons for their preferences, and *Striga* management methods. Results showed that the local varieties "*Ochuti*", and "*Nyakabala*" were more popular than improved varieties "*Serena*" and "*Seredo*". Reasons for choosing the different varieties varied from resistance to *Striga*, drought, and bird damage, reliability and adaptability, early maturity, taste and high satiety value. In both districts,over 60% of farmers identified *Striga* as one of the most constraining factors to sorghum production, with most reporting over 70% infestation in their fields. Significant negative impact of *Striga* on sorghum productivity was revealed by farmers' willingness to pay a premium price of between 20% and 30% for a *Striga* resistant variety. Options employed by farmers to manage *Striga* included use of manure, intercropping, hand weeding and use of tolerant varieties. However, farmers thought use of chemicals and nitrogen fertilizers as more effective but unaffordable. The other constraints identified by farmers included drought, bird damage, low soil fertility, unavailability of seed and long distances to seed and commodity markets. The attributes that farmers preferred in new varieties included tolerance to *Striga* and drought, resistance to bird damage and storage pests, earliness and good taste. Implications of these results for breeding and food security are discussed.

Keywords: *Striga*, PRA, landraces, sorghum.

2.1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a major cereal in the semi-arid regions of the world where it is an important food and feed crop (Kenga et al., 2004). It is ranked fifth after wheat, rice, maize and barley in terms of production among the world's cereals (Dogget, 1988) and plays a fundamental role in food security in many of the marginalized areas in Africa. In Kenya, sorghum is mainly grown in medium and low altitude areas (Enserink, 1995). Some of the main attractions of sorghum include its drought tolerance, and ability to yield under low input and relatively infertile conditions which are prevalent in most semi-arid areas in Kenya. Most of the sorghum grown in Kenya, as in most of sub-Saharan Africa is mainly utilized for subsistence purposes. However, in the western part of the country, where conditions are favourable for other high income crops like maize, sorghum production is still widely practiced. One of the reasons could be *Striga* which makes maize production risky in this part of Kenya.

The *Striga* species are noxious parasitic weeds that cause considerable damage in the semi-arid tropics (Rich et al., 2004). In sub–Saharan Africa where the weed is most prevalent and its effects more felt, large areas of land have been made non-productive owing to devastation from this parasite. *Striga* has its greatest impact on subsistence farming systems (Gurney et al., 1995). In Kenya, it infests large areas of western Kenya and the coastal province. Despite its relative importance as a major cause of low production, existing *Striga* control methods have not given conclusive and consistent feasible results for farmers. This is due to the high fecundity of the parasite and the mismatch between technologies and farmers' socio-economic conditions (Kiruki et al., 2006).

The lack of collaboration between farmers and researchers may be one of the major reasons for the low adoption "syndrome" of technologies in many parts of the developing world. In the case of *Striga*, many control options suggested by researchers including cultural methods like hand weeding, trap cropping and nitrogen fertilization, breeding options like improved sorghum varieties with low *Striga* stimulant production, and physical or mechanical options like deep ploughing and solarization, have had very little impact in farmers fields. Many researchers have indicated that no single control on its own can be effective, and an integrated approach is more relevant, especially, for subsistence systems. However, the diversity of farming systems in Africa demands that integrated *Striga* control strategies be tailored to local needs like ecological zone, ethnic group, population density, food preference, and market acceptability (Haussmann et al., 2000a). In this regard, farmer input during design of any *Striga* control option is paramount. However, the situation in most of the developing world is that control options lack end-user input leading to low adoption because farmers' characteristics were not considered during the development of the control strategy. Use of participatory approaches to breeding has been recommended as a way of increasing adoption of technologies (Haussmann et al., 2000a).

Participatory approaches in developing countries are critically necessary because on-farm conditions here are strikingly different from on-station conditions, while the reverse is true for developed countries (Manu-Aduening et al., 2006). For example, it is widely recognized that conventional plant breeding has been more beneficial to farmers in high-potential environments or those who can modify their environments to suit new cultivars, than to the poorest farmers who cannot afford to modify their environment through application of additional inputs and cannot risk the replacement of their traditional, well known and reliable varieties (Ceccarelli and Grando, 2007). Farmer participatory approaches like participatory breeding, participatory variety selection (PVS) and others seek to remedy the situation with adoption being on the main agenda of these approaches. Participatory variety selection (PVS) facilitates rapid spread and adoption of acceptable varieties (Doward et al., 2006). The involvement of farmers in the early stages of cassava improvement in Ghana facilitated early release- an important factor in cost-effectiveness (Manu-Aduening et al., 2006) and high adoption. Participatory approaches are also important in conservation of elite germplasm. Farmers' knowledge in combination with molecular markers has, for example, been utilized for identification of superior germplasm in terms of both agro-morphological properties and food quality traits and recommended for the conservation of sorghum in Benin (Kayodé et al., 2006). Other participatory approaches involve farmers directly in the process of variety testing and improvement at a much earlier stage than conventional plant breeding (Doward et al., 2006).

2.2 Objectives of the study

There is a lack of information on the significance and impact of *Striga* and other related constraints on sorghum production from a farmer's point of view. The objective of this study, therefore, was to appraise the significance and impact of *Striga* on the rural population from two *Striga* endemic zones and to get farmers perspectives of *Striga* and other related problems in agricultural production with special reference to sorghum production. In addition, information was sought on the most important traits which farmers wanted in new sorghum varieties, and what should be improved in existing ones.

2.3 Materials and Methods

2.3.1 Characteristics of study area

The study was conducted in the western Part of Kenya in Nyanza Province (Figure 2.1). Two districts were included in this study, that is, Bondo (0°14'19"N 34°16'10"E) and Kisumu **(**-0°5'23"N 34°45'0"E**).** Bondo is at an altitude of 1226m while Kisumu is 1131m above sea level. Population density for Bondo and Kisumu are 242 and 549 persons per km^2, respectively (Table 2.1). The main ethnic group in the two districts is the Luo community whose main occupation is fishing and small-scale agriculture. The average annual rainfall for Kisumu is 1100-1500mm in two well defined rain seasons. The district has several agro-ecological zones but the most dominant one is the LM3 (Lower Midland cotton zone) (Table 2.2) with medium to long term cropping season followed by a short or very short one. The rainfall in Bondo is bimodal with the long rains coming between March and June and the short rains between September and November. Most of Bondo is also within the LM3 agro-ecological zone (Table 2.2). The cropping season in Bondo is short to very short. Annual rainfall increases from 800mm near the lakeshore to 2000mm further away from the lake.

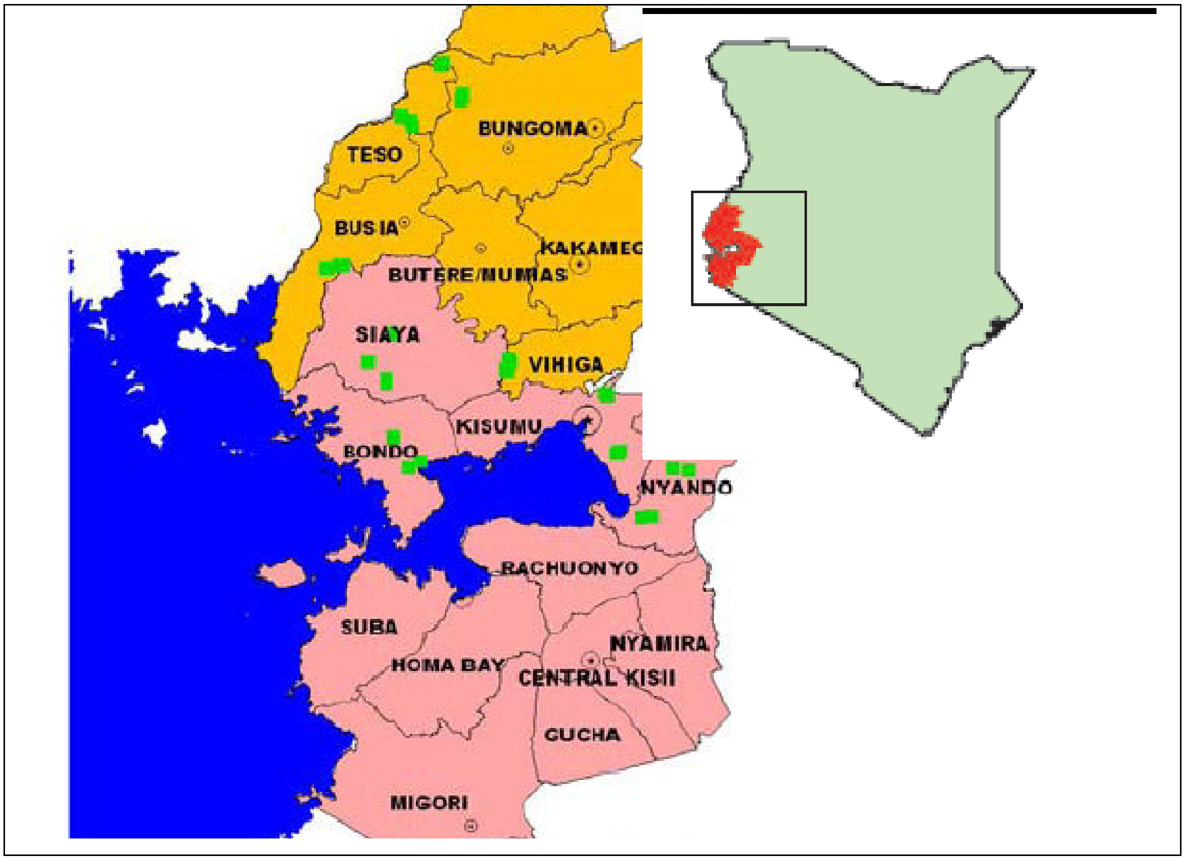

Figure 2.1: Map of Western Kenya showing Kisumu, Bondo and surrounding districts. The inset is a map of Kenya showing the Province where the study was conducted.

Table 2.1: Population demographics of Bondo and Kisumu districts of Kenya

District	Number of divisions	Population	Area km^2	Population Density (km^{-1})
Bondo	4	238,780	987	242
Kisumu	4	504,359	919	549

Table 2.2: Important Agro-ecological zones for Bondo and Kisumu districts in Kenya

Agro-ecological zone	Description	Altitude above sea level (m)	Annual temperature (°C)	Annual av. Rainfall (mm)
UM 1	Coffee tea zone	1500-1600	20.5-19.9	1600-1800
UM2	Main coffee zone	1450-1700	21.1-19.3	1400-1600
UM3	Marginal coffee zone	1500-1600	20.8-20.2	1000-1600
LM1	L. Midland sugar zone	1300-1500	21.7-20.5	1600-1800
LM2	Marginal sugar zone	1200-1500	22.3-20.9	1200-1600
LM3	L. Midland cotton zone	1140-1450	22.7-21.0	1000-1400
LM4	Marginal cotton zone	1135-1200	32.1-22.3	1000-1100

Adapted from (Jaetzold and Schmidt, 1982)

2.3.2 Study sites and Conduct of PRA

The PRA study was conducted in two villages in each of the two districts (Table 2.3). The two districts were chosen because *Striga* is endemic within the whole region and a major constraint to agricultural production. In each of the villages, approximately forty to sixty farmers were involved in the study. A multi-faceted PRA involving structured questionnaires and group discussions were utilized. PRA methods were used to engage the farmers in far ranging aspects of sorghum farming. Scoring, ranking and diagramming techniques were used in order to generate information about the community and on sorghum production. In the group discussions, reference points ranged from the different types of sorghum the farmers grow, their preferences for the different varieties grown and the production constraints they face. Issues on the general farming systems practiced in each of the communities were also discussed. Discussions were conducted using, but not limited to, a checklist, thus ensuring capture of a wider picture of the farming situation in the community.

Table 2.3: PRA sites and dates of PRA conduct

Village	District	Sub-location	location	No. of farmers	Dates for PRA
Akala market	Bondo	Got Ramogi	Central Yimbo	45	7/09/2006
Seme	Bondo	Rageg'ni	East Uyoma	63	08/09/2006
Kombewa	Kisumu	Seme	Asembo	48	09/09/2006
Wathorego	Kisumu	Wathorego	Kibos	57	10/09/2006

2.3.3 PRA team and data analysis

The PRA team comprised a socio-economist, plant breeder and technicians from the ministry of Agriculture who were conversant with the local Luo language. After each PRA exercise, the PRA team took time to analyse the data and document the findings. This not only helped lessen the load of paper work but also ensured that information was not lost. The findings from previous sessions were used to either re-structure or improve or plan on the pending sessions. Data was analysed using SPSS (Statistical Package for Social Science) version 15 (SPSS, 2005).

2.4 Results

2.4.1 Sorghum varieties grown by the farmers

The main sorghum varieties grown in the two districts included both the improved varieties *Serena* and *Seredo*, and landraces *Ochuti*, *Nyakabala* and *Andiwo* (Table 2.4). *Ochuti*, a local landrace was the most popular being grown by over 70% of farmers in the two districts. *Nyakabala*, a local landrace, was also grown by over 50% of farmers in both districts. The improved varieties, *Serena* and *Seredo* were grown by 43.3% and 54.8% of farmers in Kisumu and 38% and 58% of farmers in Bondo, respectively. Jowi Jamuomo and *Andiwo* were some of the other local landraces grown by the farmers. Gadum, also an improved variety was not grown by any of the farmers in Kisumu and only 2% of the farmers grew it in Bondo district. However, most farmers grew more than one sorghum variety.

Table 2.4: Farmers growing different sorghum varieties/landraces in Bondo and Kisumu districts

Sorghum variety/landrace	% farmers growing the variety	
	Kisumu	Bondo
Serena (Released)	43.3	38.8
Seredo (Released)	54.8	58.0
Ochuti (Landrace)	78.1	89.8
Andiwo (Landrace)	34.4	30.6
Gadum (Released)	0.0	2.0
Jowi jamuomo (Landrace)	37.1	41.5
Nyakabala (Landrace)	62.5	53.1
Other varieties	35.5	61.2

2.4.2 Criteria for choosing a sorghum Variety

Farmers in the two districts indicated tolerance to *Striga* as one of the important criteria they used to choose the sorghum varieties they grew (Table 2.5). On average, over 60% of farmers in the two districts indicated ability to yield better under *Striga* as an important criterion for choosing a sorghum variety. High yield was also an important criterion for them as was early maturity and tolerance to drought. A higher percentage of men than women thought yield was important as a criterion for choosing the sorghum varieties. However, most of the women (71%) thought that early maturity was an important criterion while only 52% of the men considered it as important. Approximately 60% of men and 43% of women indicated that pest tolerance, especially, to the larger grain borer, was an important criterion for choosing a sorghum variety. Less than 10% of both women and men thought that grain colour was important, while less than 3% of both males and females thought that the type of soil was an important criterion to consider when planting sorghum. Good taste and high satiety value were considered as important criteria with over 50% of both men and women indicating them to be important when they choose a variety to grow. High satiety in this context was used to refer to those varieties that kept one feeling “full” for a long period. Farmers indicated that, a single meal of these high satiety varieties kept hunger away the entire day. Less than 10% of farmers of both sexes indicated disease tolerance and tolerance to other weeds apart from *Striga* as important. Over 60% of individuals of both sexes considered drought tolerance as an important sorghum variety selection criterion. On average, over 60% of farmers in both districts considered early maturity, bird resistance and tolerance to *Striga* as important criteria for preference of certain sorghum varieties.

Table 2.5: Criteria for choosing sorghum varieties in Bondo and Kisumu districts*

Selection Criteria	Percentage		
	Men	Women	Average
High yield	57.1	51.4	54.3
High market value	26.8	18.9	22.9
Drought tolerance	60.1	65.9	63
Grain Colour	7.3	5.4	6.4
Early maturity	52.4	71.1	61.8
Bird resistance	61.4	66.2	63.8
Soil type	2.4	2.7	2.6
Tolerance to other weeds	4.8	2.7	3.3
Pest tolerance	59.8	43.5	51.7
Taste/satiety	54.5	59.8	57.2
Tolerance to disease	4.9	8.1	6.5
Tolerance to *Striga*	63.6	62.2	62.9
Others	29.3	22.2	25.8

NB: * Combined data for Bondo and Kisumu districts

2.4.3 Attributes of sorghum varieties in the region

Table 2.6 shows the various sorghum varieties and landraces ranking by farmers. Generally, the released varieties scored highly in terms of yield but were poor with regard to tolerance to *Striga* and drought, and yield stability. On the other hand, the landraces, *Ochuti* and *Nyakabala* were rated highly for drought and yield stability. Landraces were also rated highly for pest tolerance, especially, birds. Improved varieties were rated highly for good taste but not for satiety value while the opposite was true for the landraces. Farmers indicated that some of their varieties had better taste, referring to less bitterness. In terms of market value, the released varieties were rated better than the landraces. Whereas the landraces were rated moderately for taste, they were rated highly for satiety value. The landraces fared moderately in most of the attributes, while the improved varieties were rated highly in some of the attributes but poor in others which were just as important.

Table 2.6: Combined mean scores for Bondo and Kisumu districts for various attributes for different varieties/landraces

Selection Criteria	variety					
	Serena	*Seredo*	*Ochuti*	*Nyakabala*	*Andiwo*	*Gadam*
Yield	4	4	3	3	3	3
Taste	4	4	3	3	2	4
Satiety value	3	3	4	4	3	3
Tolerance to *Striga*	1	1	5	4	4	2
Seed size	3	3	2	2	3	4
Seed colour	3	3	3	4	4	3
Tolerance to pests/birds	1	1	4	3	4	2
Market value	4	4	3	2	2	4
Yield reliability	1	1	5	4	3	2
Drought tolerance	2	3	5	4	4	2

Score: 1-very bad, 2-bad, 3-moderate, 4-good, 5-very good

2.4.4 Relative importance of sorghum criteria based on gender

Table 2.7 shows the results of the relative importance of different sorghum selection criteria based on gender. More men than women considered yield as an important criteria, though over 50% of the women still considered yield to be important. Seed colour as a trait was considered important by more women than men. For both men and women, over 50% ranked *Striga* tolerance as an important criterion. Over 80% of women considered taste as an important or moderately important criterion in choosing their sorghum varieties. However, just 50% of the men would choose the sorghum variety based on taste. Fifty percent of women thought resistance to disease was an important criterion while only 25% of the men thought it was moderately important. More women than men considered early maturity as important. Type of soil was not very important and only 25% and 33% of men and women, respectively, considered it important.

Table 2.7: Relative importance of various sorghum criteria as rated by both women and men from Bondo and Kisumu districts in Kenya.

Sorghum criteria	Men			Women		
	Important	Moderate importance	Not important	Important	Moderate importance	Not important
Yield	64.3	33.3	2.4	53.3	26	20
Color	30	63.6	6.4	85.7	14.3	0
Tolerance to *Striga*	54.2	20.4	25.4	63.4	20.4	16.2
Taste/Satiety value	38.9	16.7	44.4	25	58.3	16.7
Early maturity	54.3	12.4	33.3	64.5	7.4	29.1
Soil type	25	0	75	33.3	0	66.7
Bird resistance	16.7	75	33.3	57.1	14.3	28.6
Tolerance to other weeds	0	25	75	20	0	80
Tolerance to pests	11.1	66.7	22.2	50	40	10
Seed size	17.7	24.8	100	25	25	50
Resistance to disease	0	25	75	50	0	50
Other criteria	33.3	11.1	55.6	45.5	18.2	36.4

NB: Combined data for the two districts

2.4.5 *Striga* control options

Figure 2.2 shows the different control options employed by farmers and the percentage of farmers using them. Most farmers indicated they used crop rotation, hoe weeding, manure application and use to tolerant varieties. Less than 10% of farmers indicated they used Nitrogenous fertilisers to control *Striga*, while less than 5% indicated they used some form of chemical for *Striga* control.

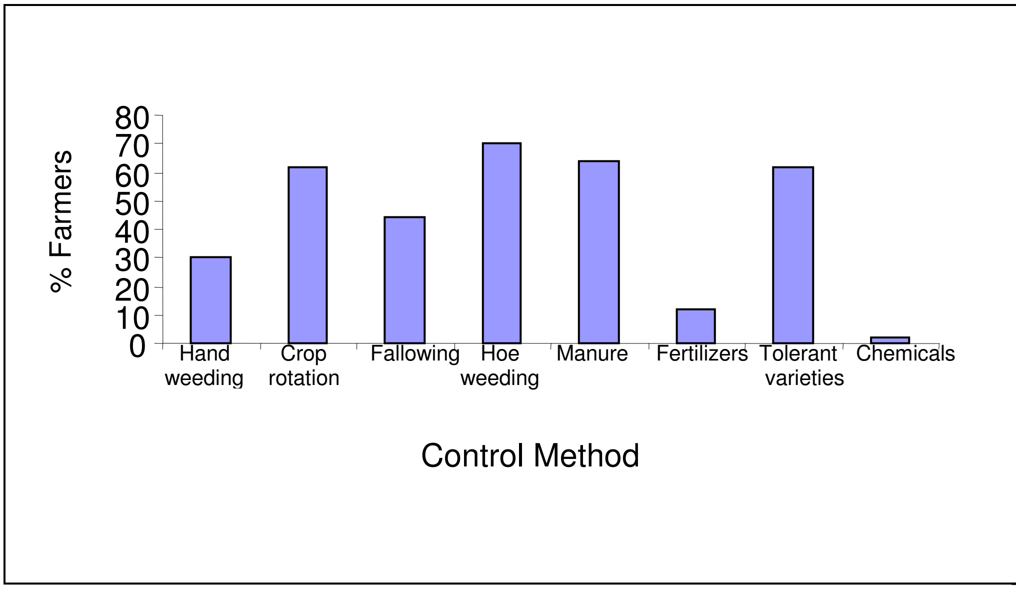

Figure 2.2. Combined data for Bondo and Kisumu districts for *Striga* control options used by farmers.

2.4.6 Relative importance of different *Striga* control options on different crops

Table 2.8 shows a matrix score for the various control practices and their relative importance on different crops. Farmers were asked to relate the level of importance of a control option to the different crops that they grow. For example, if they grew maize, sorghum and beans and they were using manure as a *Striga* control option, they were asked which of the three crops they would lay much emphasis on to control *Striga* using manure as control option. The matrix score indicated that more emphasis for most *Striga* control options was placed in the production of maize than either sorghum or finger millet. For example, for fertilizer application, the farmers indicated that they were ready to apply the control measure to the maximum for maize, but would only be very slightly willing to apply the same control measure in sorghum or finger millet.

Table 2.8 Matrix ranking of the various *Striga* control practices the farmers use on different crops in Kisumu and Bondo districts. XXXXX-maximum importance of the control measure. X=minimum importance

	Crop			
Striga control practice	Maize	Sorghum	Finger millet	Other
Manure application	**XXXXX**	**XX**	**XX**	**XX**
Normal weeding	**XXXXX**	**XXX**	**XXX**	**XX**
Hand pulling	**XXXXX**	**XX**	**X**	
Rotation	**XXXXX**	**XXX**	**XX**	**X**
Fertilizer Application	**XXXXX**	**X**	**X**	**XX**

2.4.7 Farmer perceptions on efficacy of control options

Figure 2.3 shows the farmers' perceptions of the control options currently in use in wathorego village of Kisumu district. Farmers were asked to indicate the control option they thought would be most effective if it were readily available to them. Generally, most farmers indicated that fertilization, and use of tolerant varieties and chemicals would be highly effective. Less than 50% of farmers interviewed indicated that cultural control methods like weeding, fallowing and crop rotation would be effective.

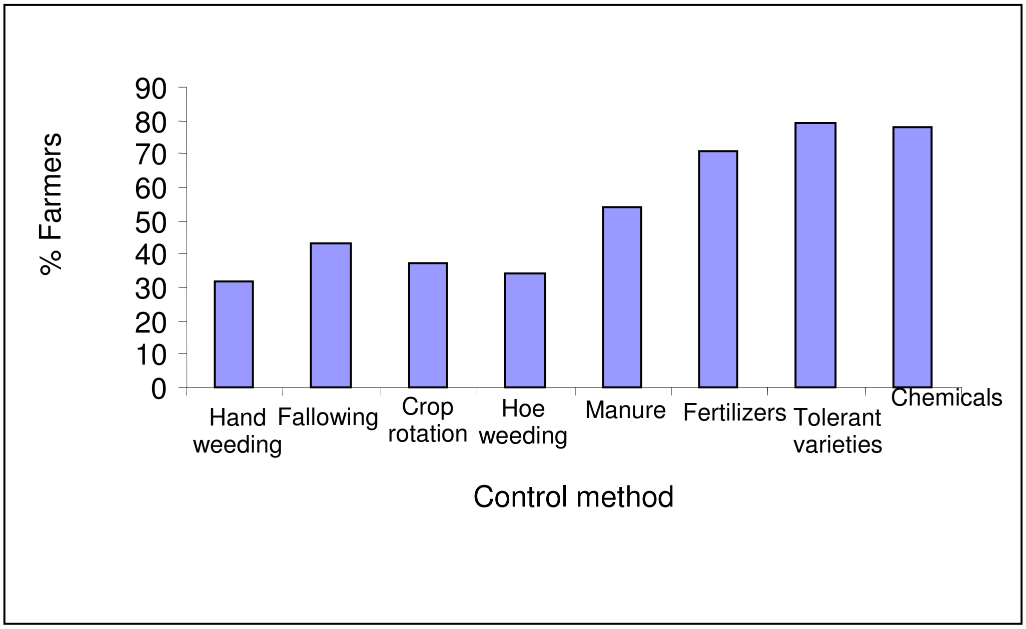

Figure 2.3: Farmers perceptions of effective control options in Wathorego village of Kisumu district. Bars represent percentage of farmers who thought a particular control option would be effective.

2.4.8 Expected price of *Striga* resistant seed

A survey was done for the two districts to get information on the prices the farmers were prepared to pay for a packet of *Striga* resistant sorghum seed if it was available. Over 90% of all the farmers in the two districts were prepared to pay for a *Striga* resistant variety at a price that was 10% above the current one (Table 2.9). In both districts over 30% of farmers were prepared to pay at 20% above the current price of a packet of sorghum seed. Thirteen percent of farmers in Kisumu were prepared to pay 30% over the current price whereas only 5% of the farmers in Bondo were prepared to pay 30% above the current price of a packet of sorghum seed.

Table 2.9: Expected price of a *Striga* resistant variety in Bondo and Kisumu districts

District	Percentage of farmers prepared to pay for *Striga* resistant variety @			
	Current price (Ksh. 180)	10% above current price (Ksh. 198)	20% above current price (Ksh. 216)	30% above current price (Ksh. 234)
Bondo	10	60	30	5
Kisumu	6	46	37	13

2.4.9 Susceptibility of sorghum varieties to *Striga*

Table 2.10 shows the susceptibility of some of the sorghum varieties grown in Kombewe village of Kisumu district and their production under high and no/low *Striga* infestation. The improved varieties *Serena* and *Seredo* were rated as very susceptible to *Striga* as were the local landraces *Ochuti* and Jowi Jamuomo. *Andiwo*, also a local landrace was rated as susceptible. There was no variety the farmers rated as not susceptible. The variety *Jowi jamuomo* was rated as the best, while improved varieties were rated to have the least potential when grown under high *Striga* infestation. Under no/low *Striga* infestations the farmers rated the two improved varieties better than the local varieties.

Table 2.10: Chart showing the susceptibility and production potential for some of the sorghum varieties grown in Kombewe village of Kisumu district

Sorghum variety/landrace	Susceptibility to *Striga*	Production potential under high *Striga* infestation	Production potential under no/low *Striga* infestation
Serena	**x x x**	low	Very good
Seredo	**x x x**	low	Very good
Ochuti	**x x x**	good	good
Jowi jamuomo	**x x x**	Very good	good
Andiwo	**x x**	moderate	moderate

Very susceptible=**x x x**   Susceptible = **x x**   Tolerant= **x**

2.4.10 Sorghum area infested with *Striga*

Table 2.11 shows the percentage area under sorghum that farmers indicated was infested with *Striga*, including associated yield loss, in the four villages. In all the villages, more than 60% of the land under sorghum was infested with *Striga*. Kombewe in Kisumu had the highest infestation with farmers indicating infestation levels of over 90% of the land under sorghum. The losses due to *Striga* were between 540kg and 900kg per acre in all the villages surveyed in the two districts.

Table 2.11: Area under sorghum production with *Striga* infestation and *Striga* related yield loss in Bondo and Kisumu district

Village	District	% of sorghum area infested by *Striga*	Loss due to *Striga* infestation-kg/acre
Akala market	Bondo	70	720
Seme	Bondo	80	540
Wathorego	Kisumu	60	720
Kombewe	Kisumu	90	630

2.4.11 Other production constraints

Table 2.12 shows the average distances to suppliers for various agricultural inputs and services providers. The average distance to a commercial seed supplier was 15km and 17km for Kisumu and Bondo districts, respectively. The minimum distance to a commercial seed supplier was 1.5km and 1km for Kisumu and Bondo, respectively. However, the maximum distance to a commercial seed supplier was 100km and 50km for Kisumu and Bondo, respectively. The average distance to any other seed supplier was approximately 6km for both Kisumu and Bondo. The distance to the closest fertilizer and pesticide supplier for both Kisumu and Bondo was around 6km. The distance to the closest agricultural services ranged from 7.5km and 5km for both Bondo and Kisumu.

Table 2.12: Distances to various commodity and service providers in Bondo and Kisumu districts

District		Distance to commercial seed supplier	Distance to other seed supplier	Distance to closest fertilizer and pesticide supplier	Distance to closest agricultural services
Bondo	Mean	17	6.5	6	7.4
	N	43	49	49	22
	Std. Deviation	10.7	10.0	9.4	10.5
	Minimum	1	1	1	0.5
	Maximum	50	50	50	45
Kisumu	Mean	15.4	6.8	6.3	4.9
	N	27	30	29	21
	Std. Deviation	19.8	7.0	6.5	5.5
	Minimum	1.5	1	1	1
	Maximum	100	35	35	20
Total	Mean	16.4	6.6	6.1	6.1
	N	70	79	78	43
	Std. Deviation	14.8	8.9	8.4	8.4
	Minimum	1	1	1	0.5
	Maximum	100	50	50	45

NB: N=Number of Farmers interviewed. Distances are in kilometres.

2.4.12 Recycling of seed in Bondo and Kisumu districts

Ninety percent and 74% of the farmers in Bondo and Kisumu districts, respectively, indicated that they recycled seed at least for one year (Table 2.13). However, less than 8% of farmers indicated recycling seed for two years. Less farmers in Bondo than in Kisumu indicated they recycled seed for more than two years.

Table 2.13: Farmers recycling sorghum seed in Bondo and Kisumu districts.

Recycle	Bondo	Kisumu
1 year recycle	90	74.1
2 year recycle	7.5	7.4
More than 2 years	2.5	18.5

2.5 Discussion

The study provides information on sorghum production, *Striga* and other related constraints for the two districts of Bondo and Kisumu in western Kenya. The most important criteria for choosing sorghum varieties included *Striga* resistance, yield stability and resistance to bird damage. More farmers grew the local landraces and evidently farmers were not using the improved varieties like *Serena* and *Seredo* for a variety of reasons which included:

- The improved varieties did not have *Striga* resistance or tolerance, while the local varieties at least were able to yield under heavy *Striga* infestations.
- Improved varieties were not adaptable or reliable. They would perform well in one season and then poorly in the next.
- Improved varieties were prone to pests especially bird damage and larger grain borer.
- Improved varieties yielded poorly under adverse conditions like drought.

Drought tolerance, *Striga* resistance/tolerance and yield stability were among the criteria that farmers indicated they used to select varieties to grow. While the local varieties were superior for most of the above criteria, farmers were also aware of the low yielding capacity of most of their local varieties in comparison with the improved varieties when environmental characteristics like drought and *Striga* infestation were minimal.

2.5.1 *Striga* resistance

Farmers' own varieties like *Ochuti* and *Nyakabala* were more popular than the introduced cultivars *Serena*, *Seredo* and *Gadam*. Farmers indicated that introduced varieties did not yield as much as the local varieties under high *Striga* infestation. Most of the areas within the districts had extra heavy *Striga* infestation levels and most farmers indicated that more than 60% of the area under sorghum on their farms had *Striga* (Table 2.11).The superiority of the local varieties was evident in the ratings of the different varieties under heavy or low/no *Striga* infestations (Table 2.10) where the improved varieties fared poorly under high *Striga* infestations but were rated highly under low/no *Striga* infestations. Farmers' were well aware of the superiority of *Ochuti* and *Jowi jamuomo* over the improved varieties under heavy *Striga* infestation levels.

Currently, much of the work on *Striga* has concentrated on developing sorghum varieties with resistance or tolerance to *Striga* (Ejeta et al., 1992; Gurney et al., 2002; Gurney et al., 2003). To date, there has not been any *Striga* resistant variety that has been identified in the cereals (Gurney et al., 2002). However, there are encouraging results in breeding and resistance/tolerance genes may be found in farmers' local germplasm. In the work of Gworgor, (2002), two local varieties *Idon Makoha* and *Ex Dapchi* have been found to yield higher than the improved varieties *ICSV 1002* and *ICSV 1007*, despite supporting higher numbers of *Striga,* and are seen as important for breeding for *Striga* resistance and high grain yield. Other studies (Haussmann et al., 2000a; Gurney et al., 2002) are looking for resistance genes in wild relatives of sorghum and farmers' local germplasm. Generally, the importance of farmers' local varieties as a source of *Striga* resistance genes cannot be overemphasized and the input of farmers in identifying local germplasm with resistance or tolerance will be invaluable. In Kenya, ongoing work at ICRISAT is looking for resistance and tolerance genes in the local landrace *Ochuti* for transfer into high yielding hybrids and improved varieties.

2.5.2 Yield stability and adaptability

Farmers rated the improved varieties as unreliable while the landraces were rated highly on yield stability especially under drought conditions and *Striga* infestation. One of the reasons that improved varieties are not reliable is because they are not adaptable to the variable climatic conditions in many areas within Africa. Mostly, sorghum is popular because most farmers rely on its adaptability to the unpredictable drought and other stress factors (Haussmann et al., 2000b). Priority for breeding should focus more on developing more adaptable sorghum varieties with yield stability in these highly unpredictable environments. Most of the local sorghum landraces may be considered to be adaptable as indicated by their yield stability especially under environmental stresses like drought or biological ones like *Striga*. Incorporation of these characteristics into improved varieties or hybrids would make them more popular with farmers.

2.5.3 Drought tolerance

Farmers rated the improved varieties lowly for tolerance to drought. One of the breeding priorities in Kenya is the development of sorghum varieties with drought tolerance.

Kenya Agricultural Research Institute (KARI) scientists are breeding for early maturing varieties which are able to escape drought conditions. Varieties like *Kari/mtama-1*, *Kari/mtama-3*, IS76#23, *Gadam el hamam*, *Serena* and *Seredo* are considered early maturing and used for drought prone areas. While drought escape is a good aspect, drought tolerance genes still need to be identified for incorporation into varieties currently being offered to the farmers. The current global trend of climate change and global warming necessitate the development of varieties able to withstand drought and other adverse conditions. Farmers rated their materials like *Ochuti* and *Andiwo* as good under drought situations, and these may be a starting point in sourcing for drought resistance genes.

2.5.4 Bird resistance and weevil resistance

Improved varieties were rated lowly for resistance to bird damage compared to the landraces. Farmers indicated that bird damage was more severe in the improved varieties than in the local ones and this was a major reason why they were not growing the improved varieties. Generally, a concerted effort is necessary to tackle the bird menace in the East African region so as to determine the migration routes and brooding characteristics of the birds for effective control. The ease of migrations between countries means that predictions of major influxes of quelea into agricultural areas have proved difficult to make, especially in regions where the rainfall patterns are complex and variable, such as in East Africa (Elliott, 2008). Methods being investigated include mechanical as well as chemical options where chemical repellants are sprayed directly onto the sorghum grain (Garanito et al., 2000). Bird resistance (the use of biochemical or morphological genetic traits in a crop to protect ripening seeds or grain from bird damage) remains a promising tool under certain situations and a lot of research has focussed on sorghum (Bullard, 1988). In this regard, the farmers’ local germplasm may be the place to look for resistance genes that could be incorporated into the improved cultivars. Use of hybrid maize resistant to bird damage has also been tried and studies indicate that varietal resistance could be a promising approach to bird damage (Russell et al., 1984).

2.5.5 Yield

Farmers rated the improved varieties high in terms of yield, and their landraces low, under low/no *Striga* conditions, and hybrid varieties would seem a good bet for improving yields for the farmers. While farmers were well aware of superiority of improved varieties in terms of yield especially when under low/or no *Striga* conditions (Table 2.10) they could not abandon their reliable cultivars for high yielding improved varieties that would give them good yield in one season and poor yield in the next. The socio-economic circumstances of small-scale farmers in Africa make it difficult for them to try new approaches that might put their grain production at risk or that require purchased inputs (Ransom, 2000). Still, hybrids have shown great superiority in terms of yield over local cultivars and hybrid breeding for target semi-arid areas in Kenya is seen as a promising approach (Haussmann et al., 2000b). The Kenya agricultural research institute (KARI) in collaboration with other international institutes like ICRISAT and ACCI (African Centre for Crop Improvement) are working to develop hybrid sorghum for Kenya and there are indications that hybrids can be relatively easily produced in Kenya with yield increments of up to 50% (Karari et al., 2005). However, it is indicative that unless the hybrid varieties developed have other characteristics that the farmers desire (taste, resistance to *Striga*, etc.) the adoption rate would be minimal. It should also not be forgotten that hybrid production and successful marketing require, among other things, skilled labour, well developed seed industry and infrastructure, and a stable financial base (Haussmann et al., 2000b). Also, whereas in many countries like the USA, Argentina and Brazil, the introduction of hybrids has revolutionized agriculture, sorghum and maize cultivars introduced in Africa do not have resistance to *Striga* though the introduction of genes for resistance into these hybrids has already been initiated at Purdue University with some promising results (Ejeta and Butler, 1993). Hybrids with desired farmer characteristics of taste, *Striga* resistance, and resistance to bird damage would solve the problem of low yield found with the local varieties.

2.5.6 Taste and satiety

Farmers indicated good taste and high satiety value as criteria they want in their varieties. They were aware that local varieties had high satiety value which meant that particular sorghum varieties, on consumption, kept one feeling satisfied for a long period. Taste of some varieties is considered inappropriate and farmers' knowledge on good tasting sorghum is crucial. Palatability of sorghum is considered a hindering factor and breeding for more palatable sorghums with less tannin content is ongoing (National Research Council, 1996). However, the challenge for breeding is to improve on the digestibility of the brown or tannin sorghums which means reduction in the amount of tannins on the testa of the sorghum seed. However, the lowering of the levels of condensed tannins presents a problem to breeders because these same tannins are what make these brown sorghums resistant to bird and mold damage (Rooney and Pflugfelder, 1986).

2.5.7 Early maturity

Early maturity was also an important criterion that farmers desire in their varieties. Generally, early maturing varieties were considered able to grow fast before onset of the dry season and also because the farmers indicated that they had less *Striga* infestation levels. In Kenya several improved early maturing sorghum cultivars like Kari mtama-1, ICSV, *Serena* and *Seredo* have been recommended for the dry areas. Early maturing varieties may also reduce the amount of damage inflicted upon by the *Striga* weed as there is less time that the crop is in the ground. Most farmers' varieties are usually late and the incorporation of genes for earliness would ensure they were able to avoid drought.

2.5.8 Other constraints to adoption of improved varieties

The long distances to suppliers of agricultural inputs and commodity services may be a contributing factor to the minimal use of the improved varieties and it is possible farmers have not been exposed to most of the improved varieties. Some farmers indicated that the distances to commercial seed merchants were too far (up to 100km) and they were unable to get seed. Also, agricultural extension was not active thus exacerbating the situation. From the discussions, many of the participants indicated that the agricultural extension network was not very functional although it did exist. Many of the farmers (over 70% in both the two districts) indicated that they recycled seed at least for one year (Table 2.13). Mostly farmers will recycle seed due to lack of finances to buy new or improved seed and thus poverty is a major contributory factor in the lack of adoption of improved varieties. However, the lack of improved seed due to the long distances to commodity markets could also lead to increased recycling of previous season's seed. Lack of extension services may also contribute to the recycling of seed as the farmers are not aware of the benefits of hybrid seed.

2.5.9 Role of gender in sorghum production

Group discussions on the roles of different gender in sorghum production indicated that women were more engaged in activities revolving around sorghum production as compared to the men. Differences in the criteria men and women deemed important in the sorghum varieties they grew were evident (Table 2.7). These differences were most likely a reflection of the different roles of men and women within the society. For example, more women than men thought that early maturity was an important criterion for choosing the sorghum variety. This may be as a result of the fact that in most cases the women are the ones who tend to the crop from planting to harvesting and the benefits of early maturity are well known to them. In a PRA exercise to identify needs of the farmers for rice in Ghana, women identified taste, aroma, ease of threshing, and good milling without par boiling as important traits for rice and this was a reflection of the activities women undertook both in the household and commercially (Doward et al., 2006). Also, in a study to get farmers' perceptions on rice varieties in Mali, women were found to dominate farming of subsistence crops and thus the authors suggested that scientists should engage women more to get their preferences for these crops (Efisue et al., 2008).

2.5.10 *Striga* control

Various control options were employed by the farmers to combat *Striga* and these included mainly the cultural options of weeding, crop rotation, using animal manure and using tolerant varieties (Figure 2.2). The use of fertilisers and chemicals was very minimal. In any case, the returns on the crops that farmers plant, may not be able to afford them the capital to buy inorganic fertilisers or chemicals for *Striga* control. Some farmers indicated they had tried out herbicides but only to a small extent. Since these control options require both time and money, it was evident that farmers were only prepared to use the different options on the crops on which they placed a high premium (Table 2.8). Thus, most farmers carried out most of the control practices on maize as they placed a high premium on maize. Sorghum and millets were more or less neglected in terms of employing the different control practices.

Farmers were also asked to indicate the control options they thought would be effective if they were readily available. More often than not, the farmers' choice of a control option was determined by the financial constraint the option imposed upon the farmers and not necessarily because of its efficacy. Farmers' perceptions were that use of chemicals and inorganic fertilizer application would be most effective. *Striga* severity has been known to be associated with low soil fertility and farmers were aware that *Striga* infestation levels were more severe in areas of the farm with low soil fertility. However, a myriad of cultural control methods have been advocated for in many studies (Berner et al., 1995; Ransom, 2000) have failed to make impact in the field. In the work of van Ast et al. (2005), they found that cultural control options of deep planting, use of transplants and shallow tillage were effective in the laboratory experiment but no yield benefit was found when the same practices were done in the field. Most farmers indicated that the only option they thought could get rid of *Striga* was a chemical able to kill it, as they had tried all other methods without much success.

2.5.11 Expected price of a *Striga* resistant variety

The problem of *Striga* as a limiting factor in sorghum production was exemplified by the willingness of farmers to pay a premium for a *Striga*-resistant variety (Table 2.9). De Groote et al. (2005) estimated a huge potential market for herbicide coated maize seed (a novel *Striga* control strategy) of 64, 000 tons annually and an estimated $ 129 million for western Kenya. Generally, farmers will be prepared to invest in a technology that they think will be beneficial to them. Most small-scale farmers are so constrained financially as not to be in a position to even buy seed and the fact that a majority of them were prepared to pay over 10% for a *Striga* resistant variety was indicative of the severity of the *Striga* menace. Bondo for example, is one of the poorest districts in Kenya with poverty being very high in the region.

2.5.12 Area under sorghum infested by *Striga*

Striga infestation on sorghum in all the villages was found to be very high with some villages indicating that over 80% of the area under sorghum was infested. Losses due to

Striga were substantial with farmers indicating high losses. These losses ranged from 630kg to 720kg per acre. Taking into consideration that on average the farmers only get less than 900kg per acre, these losses were considered very high. In some cases the farmers indicated that they lost everything especially when the situation was exacerbated by bad weather.

2.5.13 Implications for sorghum breeding

Farmer participatory breeding has become popular in recent years in both international and national research systems (Sall et al., 2000). This study has established that improved varieties of sorghum have not had the intended impact with farmers preferring their local landraces to the improved ones like *Serena* and *Seredo*. The improved varieties lacked some of the characteristics like drought tolerance, *Striga* resistance and reliability and adaptability that farmers indicated were important in a variety. The challenge for breeding is to incorporate some of the characteristics in the farmers' varieties into the improved varieties. However, this can only happen with the involvement of farmers before embarking on developing control options. More engagement of farmers is necessary if progress is to be attained in developing varieties which have desirable farmer characteristics like high yield, *Striga* resistance and wide adaptability. Participatory techniques can play a major role. *Striga* control is of paramount importance for the rural communities of these two districts. However, *Striga* control should not be dealt with in isolation as it is just one of the problems affecting cereal production in Africa and problems affecting the entire production system need to be considered when designing a research and extension program (Ransom, 2000). Research on other characteristics desired by farmers should go hand in hand with developing a *Striga* resistant variety that is adoptable.

2.6 Conclusions

Important conclusions from the study are the following:

- Farmers' varieties were more popular than improved varieties because of *Striga* and drought tolerance, yield stability especially under adverse conditions and resistance to storage pests and bird and damage.

- Breeding priorities for improvement of sorghum in the two districts should, therefore, encompass farmers' preferences which include *Striga* resistance, drought tolerance, good grain quality, and resistance to bird, mold and storage pests damage.
- Whereas there is an urgent need for a comprehensive *Striga* control option, it should not be looked at in isolation but should encompass other production constraints like drought and poor grain quality.

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Chapter 3

Genetic variability enhancement, dose and exposure time reaction of sorghum on mutagenesis with ethyl methane sulphonate (EMS)

Abstract

A comparative study was initiated to determine the optimum conditions for sorghum mutagenesis with EMS, and to induce genetic variation in two popular sorghum lines in Kenya. Replicated experiments in completely randomized designs were conducted. Pre-soaked (12h) sorghum seeds were treated with 0.1, 0.3, 0.5, 0.8, and 1% v/v EMS for 6h and 12h. Root lengths were recorded for seeds germinated in petri-dishes in the laboratory while shoot lengths were recorded for plants grown in troughs in the greenhouse. An additional EMS concentration of 1.5% v/v was tested in the field, and observations on chlorophyll mutations induction and plant traits were made on the $M_{2:3}$ generation. Severe reduction in germination, root and shoot lengths which was inversely proportional to increase in EMS concentration was observed. The LD_{50} based on shoot length reduction was 0.4% and 0.35% EMS for *Kari/mtama-1* and *Seredo* respectively. In the field experiment, 0.3% EMS concentration had the highest chlorophyll mutation frequency. Increasing EMS concentration resulted in more than 6-fold decrease in plant emergence, flowering and seed set. Phenotypic variances for panicle characteristics were increased on treatment with EMS. Increasing EMS dose concentration increased the formation of semi-compact and loose/open heads. Varietal and exposure time differences were observed for seed germination, plant emergence and head morphology, indicating the necessity of genotype optimization for concentration and exposure time for some traits. Overall, the study indicated that EMS mutagenesis was effective in inducing large variation that could be exploited in breeding new sorghum lines.

Key words: Sorghum, EMS, mutagenesis, LD_{50}

3.1 Introduction

Sorghum is one of the most important crops in Kenya, but yield of this crop over the years has been dismally low. Desirable characteristics like drought and *Striga* resistance, high yield and yield stability, which are important in the variable environments in Kenya, are missing in farmers' varieties. One of the reasons could be that full genetic potential of the species has not been harnessed since the crop has only recently been domesticated (Dillon et al., 2007), or there is a lack of natural genetic variability for some of these important traits in monogamous sorghum (Sarma, 1998). Also, some of the traits are quantitatively inherited meaning they are hard to transfer into high yielding cultivars or hybrids (Tao et al., 2003). An ingenious way in which breeders can generate much needed genetic variation in these instances is by artificial induction of mutations through mutagenesis (Seetharami-Reddi, 1984; Sarma, 1998; Sasi et al., 2005; Waugh et al., 2006; Weil and Monde, 2007).

There is little evidence of improvement of sorghum via mutation induction which has relied more on contemporary methods of improving self pollinated crops like pedigree and recurrent selection, backcrossing, and hybridization (Chantereau et al., 2001). Sorghum mutation induction for breeding purposes has only appealed to few researchers among them Sharma and Sharma, (1981) and Seetharami-Reddi, (1984). As an example of how much sorghum has been neglected in the field of mutation breeding, the number of mutant cultivars released in India by 2005, were 39 for rice, 13 for barley and only three for sorghum (Chopra, 2005). In Africa, apart from the work of Bretaudeau and Traore (1990), and Kiruki et al., (2006), very few studies have been done on mutagenesis in sorghum. A better understanding of variables determining mutagenesis in sorghum offers an opportunity to exploit the process for the improvement of sorghum in Kenya.

Mutagenesis alters the genetic makeup of plants by changing nucleotide sequences in the DNA (Weil and Monde, 2007), which leads to the alteration of genes and eventual change in plant phenotype (Koornneef, 2002). Mutation induction has usually utilized physical or chemical mutagens (Koornneef, 2002; Henikoff and Comai, 2003). Physical mutagens include X-rays and ultraviolet rays (Saari and Mauvais, 1996), or radioactive isotopes like 32P and 35S (Sree-Ramulu, 1970a; Chopra, 2005; Natarajan, 2005).

Chemical mutagens include ethyl methane sulfonate (EMS), hydroxyl amine (HA), methyl methane-sulphonate among others (Sree-Ramulu, 1970a; Chopra, 2005). Different types of mutations are known to be induced within the genome. Deletion mutations for example involve the removal of base pairs within the DNA, while insertion mutations involve the addition of base pairs to the DNA. Others include single pair changes like transitions and transversions (Weil and Monde, 2007). However, some of the important mutation changes for crops are point mutations, which are small nucleotide changes involving the substitution of one nucleotide for another on DNA (Henikoff and Comai, 2003; Weil and Monde, 2007).

An important feature of point mutations is that they cause small changes within the DNA as opposed to other forms of mutations known to remove large sections of DNA and effecting large changes in the organism. Since only small changes occur in the organism, these point mutations can be utilized in the improvement of specific agronomic traits like yield and disease resistance while maintaining the overall characteristics of the cultivar (Weil and Monde, 2007). The main approach for mutagenesis today is to improve on specific traits in which a variety may not be superior (Ahloowalia et al., 2004). Ethyl methane sulfonate (EMS) is among the most popular chemical mutagens (Koornneef, 2002) because it is known to cause a high density of point mutations within the DNA (Henikoff and Comai, 2003). The popularity of EMS is shown by the many studies where it has been used including in capsicum (Jabeen and Mirza, 2002), sugarbeet (Hohmann et al., 2005), maize (Neuffer et al., 1997) and even sorghum (Sree-Ramulu, 1970a; Sree-Ramulu, 1970b).

Seed is normally the material of choice for treatment with EMS though other plant material including pollen or explants can be used. Explants can be mature or immature embryos or adventitious shoots or shoots that have origin from single cells (van Harten, 1998). Plant parts are usually treated in the case of vegetatively propagated plants (Koornneef, 2002). The choice of material for mutagenesis depends on the crop species. Use of seed for mutagenesis in maize for example, has been shown to be inefficient as the mature maize kernel has separate germ line cells, and since mutations are single cell occurrences, a recessive mutant produced in this seed will only be seen in the M_3 generation (Neuffer et al., 1997; van Harten, 1998; Koornneef, 2002).

Consideration of the varieties to use is also important as the choice may determine what kind of progress will be attained in a breeding programme. Usually, starting material should be of the best cultivars available and improvement should be focused on one or two traits (van Harten, 1998).

An important factor determining the sensitivity of material to mutagenesis is the dose of the mutagen (Sree-Ramulu, 1970b). A good starting point for determining the most effective dosage is to use the lethal dose (LD) criteria especially in situations where there is no prior information on mutagenesis of the chosen crop (Singh and Kole, 2005). The LD_{50} can be determined from any growth parameter, including shoot, petiole or root length, or even germination. Hohmann et al. (2005) used germination to determine the LD_{50} for sugarbeet while Singh and Kole (2005) calculated the LD_{50} and LD_{100} for mungbean based on germination and presence of normal seedlings. Often, the reduction of germination is used to measure the effectiveness of the mutagenic treatment (Koornneef, 2002). The LD_{50} is also a good comparison of the effectiveness of different mutagenic treatments (Hohmann et al., 2005).

Commencement of a mutation breeding programme requires optimization not only of mutagen dose and exposure time to mutagen, but also to other conditions that result in high frequency of mutations, thus maximising the efficiency of mutagenic treatment (Koornneef, 2002, Singh and Kole, 2005). Usually, chlorophyll mutations are used as they are easy to detect. However, other mutagenic effects like the number of dwarfs or any other phenotypic deformity can be used. There is a lack of in-depth phenotypic and genetic studies on mutagenesis of sorghum with EMS. This study was initiated in order to develop a mutagenesis protocol for sorghum and to enhance phenotypic variation of two important sorghum varieties.

3.2 Research objectives

The main objective of this study was to determine the response of two sorghum varieties to treatment with varying concentrations of EMS so as to develop an EMS protocol for efficient mutagenesis of sorghum. The second objective was to increase the genetic diversity of sorghum for various traits and develop a mutant population for use as a source of new pure breeding lines.

3.3 Research hypothesis

The following hypothesis was tested:

- The effect of mutagenic treatment and amount of genetic variation in sorghum is independent of the variety, the mutagen dose and the exposure time to the mutagen.

3.4 Materials and Methods

3.4.1 Experimental site

The laboratory and green house experiments were conducted at the Kenya Agricultural Research Institute (KARI) biotechnology laboratory at Katumani Research Institute in Machakos, Kenya. The field experiments were conducted at the field station at the Kiboko sub-centre in Makueni district in Kenya.

3.4.2 Plant materials

Seeds for mutagenesis were from two local varieties of sorghum commonly grown by farmers in Kenya. The two varieties, *Kari/mtama-1* and *Seredo* are locally derived varieties developed by the Kenya Agricultural Research Institute (KARI). *Kari/mtama-1* has white grains and was released by KARI in 1994, while *Seredo* is a brown seeded sorghum. Both the two varieties are recommended for the dry semi-arid zones of Kenya as they are considered to be early maturing.

3.4.3 Chemicals

Ethyl methane sulfonate (EMS, sigma, formula weight 124.16, and density 1.206, 1 M = 108.5 ml/l) was sourced from Sigma chemical company India. Sodium hydroxide (NaOH) and Sodium thiosulphate ($Na_2S_2O_3.5H_2O$), important for neutralizing the highly carcinogenic EMS, were purchased from a local laboratory chemicals store.

3.4.4 Mutagenesis

Seeds of the two local sorghum varieties were subjected to varying concentrations of EMS. Mutagenesis was performed according to the procedure set out by Koornneef (2002). The nomenclature for the different generations are as follows: Mutagenized seed (M_1 seed) giving rise to M_1 plants bearing M_2 seeds ($M_{1:2}$ plants) giving rise to M_2 plants giving rise to M_3 seed ($M_{2:3}$). The treatment parameters for the laboratory and greenhouse experiments were five concentrations of EMS (0.1, 0.3, 0.5, 0.8, and 1.0% v/v) and two durations of exposure (6h and 12h) for the two varieties resulting in a total of 24 treatments including the controls. The controls were seed treated with distilled water instead of the EMS. For the field experiment, the same varieties were used and the same exposure times but the concentrations of EMS were 0.3, 0.5, 0.8, 1.0 and 1.5% v/v resulting in 24 treatments including the controls. Seeds were first soaked in water for 12h and then dried. For each of the treatments, 400 seeds were counted and put into test tubes. Fifty ml solution of EMS was then added to completely immerse seeds in mutagen. Test tubes were covered in silver foil and placed on a shaker for the appropriate exposure time of 6h or 12h. Seeds were then thoroughly washed with sodium hydroxide (NaOH) and sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$) and then washed under water for approximately three hours to eliminate the mutagen and make them safe for handling.

3.4.5 Experimental design and management

3.4.5.1 Laboratory experiment

One hundred mutagen treated seeds for each of the treatments were taken and placed on filter paper in a petri-dish. A few drops of distilled water were added and the petri-dishes were placed in a growth chamber set at a temperature of $27^{O}C$. For the control, seeds were treated in the same way but instead of EMS, distilled water (H_2O) was used. This gave a total of 24 treatments including the controls. The experiment was set out as a completely randomized design with three replications. After germination, the radical lengths for all the seeds were recorded after three days. Radical lengths were measured from the point of emergence to the tip of the radicle. Since EMS is highly carcinogenic, it's inhibitory effect on such growth parameters as the root or shoot length is usually an indication that it is effective in the particular crop of choice.

3.4.5.2 Greenhouse experiment

Another batch (100 mutagen treated seeds per treatment) was planted in trays containing a growth media of sandy loam soil from the Katumani Research Station field combined with animal manure at a ratio of 1:1. As in the laboratory experiment, there were a total of 24 treatments including the controls. Mutagenised seeds and the respective controls were placed in the planting trays at a depth of 1 cm and covered with the same soil media. The planting trays were irrigated using a watering can every two days. Fourteen days after planting, shoot lengths, measured as the length from the base of the plant to the tip of the flag leaf were recorded.

3.4.5.3 Field experiment

For the field experiment, another batch of mutagenized seed (M_1) and controls were planted in the field to raise M_1 plants. The M_1 plants were selfed at harvest. At maturity, M_1 panicles were harvested at random and panicles with the same treatment bulked. The bulked seed was planted in plots to raise M_2 plants. Plot size was 2.4m x 3.2m with the distance between plants at 0.3m and that between rows at 0.4m. Seeds were planted two in a hole giving a plant population of 210,937 plants ha^{-1}. Standard planting procedure using a hoe was done. Insecticide was applied to protect the seeds from insect damage and fertilizer was applied at the rate of 50kg ha^{-1} DAP (Di-ammonium phosphate) at planting and 50kg ha^{-1} CAN (Calcium ammonium nitrate) 45 days after planting. The experiment was set out in a randomized complete block design with three replications.

Two weeks after planting, data on plant emergence and the number of plants with chlorophyll mutations were recorded. Chlorophyll mutation frequency was expressed as a percentage ratio of number of plants with chlorophyll deformities per the total number of M_2 plants that were recorded. Data was recorded for number of plants that had flowered 11 weeks after planting. At harvest the number of plants with seed was also recorded to determine the effect of mutagenic treatment on seed set. Ten random plants were chosen at harvest and data scored for panicle length, width and whole panicle and panicle seed weight. Another 10 plants were chosen and scored for the type of head morphology (compact, semi-compact or open/loose panicle).

3.4.5.4 Data analysis

Data was subjected to general analysis of variance (ANOVA) using Genstat. The statistical model was a fixed model based on a randomized complete block design (RCBD) analysed as a fixed model.

$$y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

Where:

y_{ij} = the observation in the ith treatment of the jth block

μ = *the general mean*

α_i = the effect of the i^{th} treatement

β_j = the effect of the j^{th} block on the mean of y_{ij}

ϵ_{ij} = the experimental error

Means were separated using Fisher's Protected LSD test at α = 0.05 significance level. Linear regression analyses were also performed. All the treatments in the laboratory, greenhouse and field were replicated three times. Data on root length reduction was pooled for the two varieties as there were no significant (P = 0.05) differences between the two varieties The LD_{50} was calculated using generalized non-linear probit analysis (exponential decay) as the EMS rate that reduced the shoot length by 50%. Variances were calculated for panicle lengths, widths, weight and grain weight from the ten plants that were randomly chosen. For head morphology, the percentage of open, semi-compact and compact head types was calculated from the 10 randomly chosen panicles.

3.5 RESULTS

3.5.1 EMS dose response of sorghum seedlings (M1 generation)

Laboratory results from the treatment of sorghum seed with EMS indicated significant (P = 0.05) reduction of root length (Table 3.1 and 3.2) with increasing mutagen dose. However, the interactions between variety, concentration and time were not significant (P=0.05). The mean root length measured three days after treatment application was 3.76 mm and 0.39 mm for the control and 1.0% EMS, respectively. For shoot length reduction in the greenhouse, EMS concentration showed significant difference

($P < 0.001$) (Table 3.2). However, variety, exposure time and interaction effects did not show significant differences ($P > 0.05$). Figure 3.1 shows the effect of EMS concentration on shoot length reduction. Generally, increased shoot length reduction with increasing EMS concentration with 1% EMS concentration causing a reduction of approximately a 90% for both *Seredo* and *Kari/mtama-1*. The prediction for LD_{50}, (concentration of EMS causing 50% reduction), from probit analysis of values for reduction of shoot length was 0.35% and 0.40% v/v EMS for *Seredo* and *Kari/mtama-1*, respectively (Table 3.3).

Table 3.1: Radicle lengths in control and EMS treated seeds of sorghum under laboratory conditions

Dose of EMS (% v/v)	Radical length (mm)
0.1	3.81
0.3	2.70
0.5	1.78
0.8	0.96
1.0	0.39
0.0 (Control)	3.76
L.S.D$_{(0.05)}$	0.284
C .V. (%)	18

Table 3.2: Mean squares for plant radical length and shoot height of sorghum in the laboratory and greenhouse, respectively, on mutagenesis with EMS

Source of variation	df	Mean squares	
		Radicle length	Plant shoot height
Replication	2	4.3	109.4
Concentration	5	208.0**	10521.3**
Time	1	20.4	534.0
Variety	1	14.5	789.1
Variety X Concentration	5	12.8	631.0
Variety X Time	1	9.2	150.0
Concentration X Time	5	15.2	194.9
Variety X Concentration X Time	5	8.4	76.1
Error	46	1.1	165.2

**- Data is significant at P<0.001.

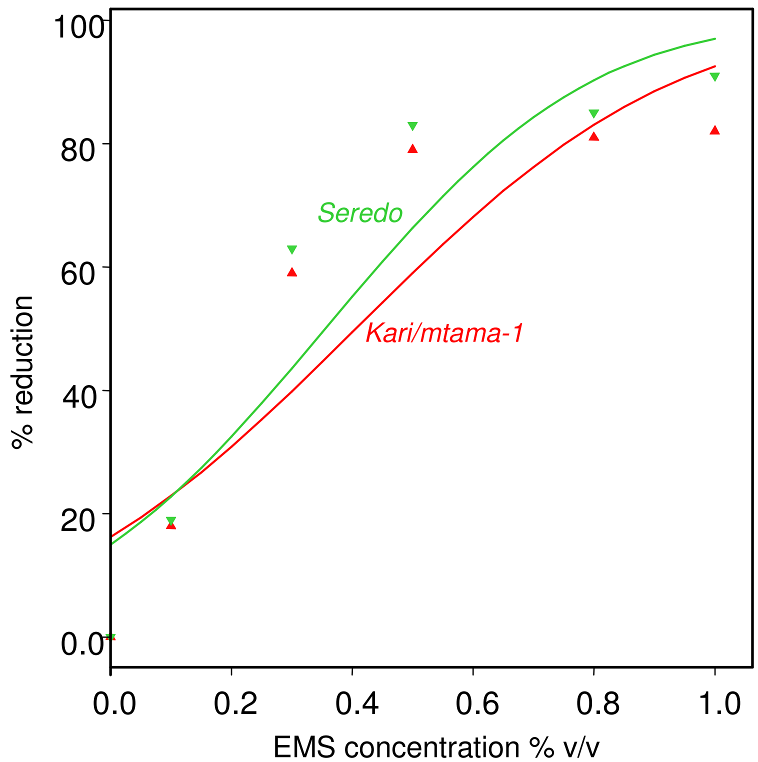

Figure 3.1 Probit analysis graph for effect of EMS concentration on shoot length for *Kari/mtama-1* and *Seredo*

Table 3.3: LD_{50} values based on plant shoot height reduction for *Kari/mtama-1* and *Seredo*

Variety	LD_{50}#	Standard error
Seredo	0.35	0.024
Kari/mtama-1	0.40	0.038

#LD_{50} was calculated as the concentration of EMS (% v/v) that reduced shoot length by 50%

3.5.2 Plant emergence in the field

The effect of EMS concentration was highly significant ($P < 0.01$) for plant emergence in the field for M_2 plants (Table 3.4). However, the effect of variety and time of exposure to the mutagen were not significant ($P > 0.05$). Percentage emergence at the two lower concentrations (0.3% and 0.5%) was 60.9% and 57% respectively and was not significantly ($P > 0.05$) different from the control which was 70.6% (Table 3.5). However, plant emergence at 0.3% EMS (60.9%) was significantly different ($P = 0.05$) from the emergence at 1% and 1.5% EMS which was 45.2 and 19.3%, respectively. The biggest reduction in plant emergence was in the treatments with the highest EMS concentration, where the emergence was only 19.3% of seeds planted, and significantly different from all the other EMS concentrations.

3.5.3 Frequency of chlorophyll mutations in M2 plants

The effect of concentration of EMS on chlorophyll mutation induction was highly significant ($P < 0.01$) (Table 3.4). Some of the chlorophyll mutations easily identifiable included albinos (Figure 3.2), xantha (yellow to yellowish white), chlorina (yellowish green) and xanthovirdis (green with white apex). Mutation frequency was calculated as the ratio of the number of plants with the different mutations to the total number of plants recorded. The highest mutation frequency (56%) was recorded for treatments where EMS at a concentration of 0.3% v/v was used (Table 3.5). Increasing the EMS concentration after this resulted in decline in the frequency of chlorophyll mutations. At a concentration of 0.3% EMS, the frequency of chlorophyll mutations was 56.3%, while at 1.5% EMS concentration, the frequency reduced to 24.2%.

Figure 3.2: Albino chlorophyll mutation in the field at Kiboko, Makueni district, Kenya

3.5.4 Flowering Plants in M2 generation

The effect of Ethyl methane sulphonate concentrations exhibited significant differences ($P < 0.001$) on the number of flowering plants (Table 3.4). Variety and the time of exposure did not show significant differences (P > 0.05). However, the variety X time interaction was significant. In the control treatments where EMS was not used, 85% of the plants had flowered at 11 weeks after planting, while all EMS treatments had less than 47% of plants that had flowered. All the treatments with EMS were significantly (P = 0.05) different from the control but not different from each other (Table 3.5). Flowering was also affected by the time of exposure for the two varieties (Table 3.6). For both the varieties, flowering was decreased with an increase in the exposure time to the mutagen.

3.5.5 Seed set of M_2 plants

The effect of concentration of EMS was highly significant (P < 0.001) for seed set (Table 3.4). Variety X time interaction effects were significant (P = 0.05). The lowest seed set was recorded at the highest EMS concentration while the highest was for the control treatment (Table 3.5). However, there was no significant (P > 0.05) difference between the effect of EMS at 0.3% v/v with the control and also between 1.0% and 1.5% v/v EMS. All other treatments were significantly (P = 0.05) different. Variety X time interaction effects for plants with seed were significant (P=0.05) (Table 3.4). Increase in the exposure time decreased the number of plants that set seed for both *Kari/mtama-1* and *Seredo* (Table 3.6). In treatments where the exposure time to EMS was 6h, 50.4% of plants had seed, while in the treatments for 12h EMS exposure time, only 37.3% of plants set seed.

Table 3.4: Mean squares for different morphological traits of sorghum on (EMS) treatment

Source of variation	df	Mean squares			
		Plant emergence	Plants with chlorophyll mutations	Plants with flowers	Plants with seed
Replication	2	2259.9	49.4	542.9	51.0
Variety	1	357.5	205.5	289.3	8.5
Concentration	5	3730.5**	3422.9**	4314.5**	12083.8**
Time	1	389.8	29.2	77.2	283.2
Variety X Concentration	5	113.5	238.3	100.0	218.9
Variety X Time	1	251.7	424	890.3*	1381.5*
Concentration X Time	5	382.1	407.7	437.4	370.3
Variety X Concentration X Time	5	66.9	159.3	299.9	677.1
Error	46	256.3	556.9	179.7	243.1

*, **- Data is significant at P<0.05 and P<0.001, respectively.

Table 3.5: Means for the different morphological characteristics of sorghum on EMS treatment

EMS Concentration	Plant emergence (%)	Plants with chlorophyll mutations (%)	Plants with flowers (%)	Plants with seed (%)
0.0 (Control)	70.6	0.0	85.1	76.3
0.3	60.9	56.3	41.7	66.5
0.5	57.0	41.8	45.1	44.7
0.8	49.4	37.1	46.8	18.9
1.0	45.2	32.8	45.8	4.7
1.5	19.3	24.2	41.0	2.3
$L.S.D_{(0.05)}$	8.57	11.0	9.63	12.81

Table 3.6: Effect of time of exposure to mutagen on yield of chlorophyll mutations and seed production

Exposure times	Flowering plants		Plants with seed	
	variety		variety	
	Kari/mtama-1	*Seredo*	*Kari/mtama-1*	*Seredo*
6h	54.2	58.2	50.4	49.6
12h	19.2	21.1	37.3	40.2
$LSD_{(0.05)}$	8.33	6.72	7.24	8.04

3.5.6 Phenotypic variation in M_2 generation

Maximum panicle head weight variance for *Kari/mtama-1* and *Seredo* was observed at the highest concentration of 1.5% EMS (Figure 3.3), while the minimum variance for panicle head weight for *Kari/mtama-1* and *Seredo* was observed at 0.8% and 0.5% EMS concentration, respectively. Maximum panicle seed weight variance was observed at 1.5% EMS for both the two varieties while the minimum panicle seed weight variance was observed at 1.0% and 0.8% EMS for *Kari/mtama-1* and *Seredo*, respectively Maximum head width variation for both *Kari/mtama-1* and *Seredo* was observed at the highest concentration of EMS (1.5% v/v) (Figure 3.4). The lowest variation for panicle head width for *Kari/mtama-1* was observed at the 1% EMS concentration and in the control group for *Seredo*. The maximum panicle head length variation for *Kari/mtama-1* and *Seredo* was at 0.5% and 0.8% EMS for *Kari/mtama-1* and *Seredo*, respectively. The lowest panicle head length variation was observed for the controls for both varieties.

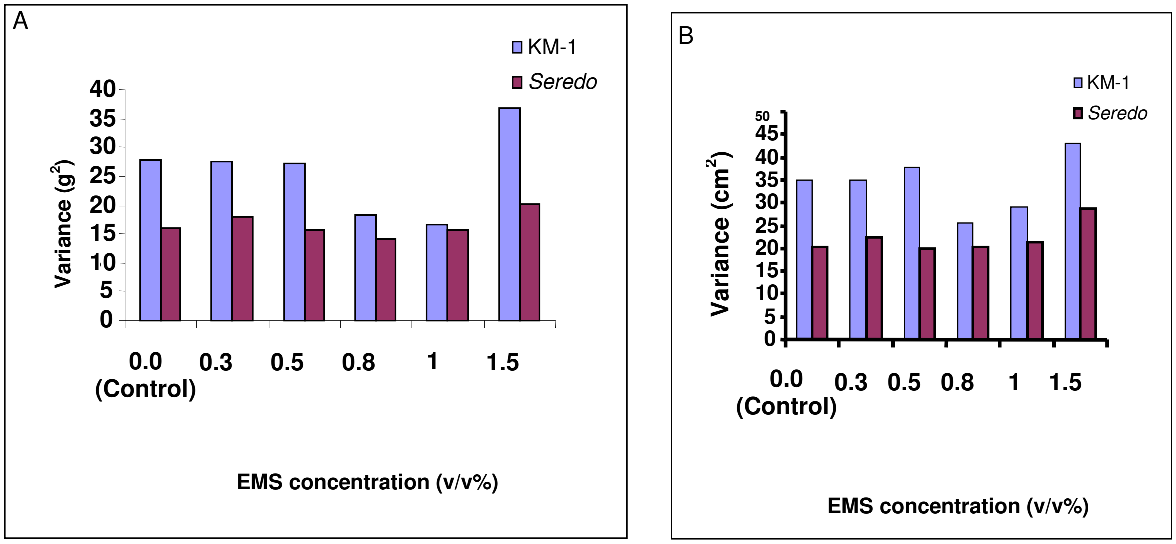

Figure 3.3 Variances for panicle seed (A) and Panicle head weight (B) for *Kari/mtama-1* and *Seredo* on treatment with different EMS concentrations. Variance was calculated from measurements of 10 plants. Note: KM-1=*Kari/mtama-1*

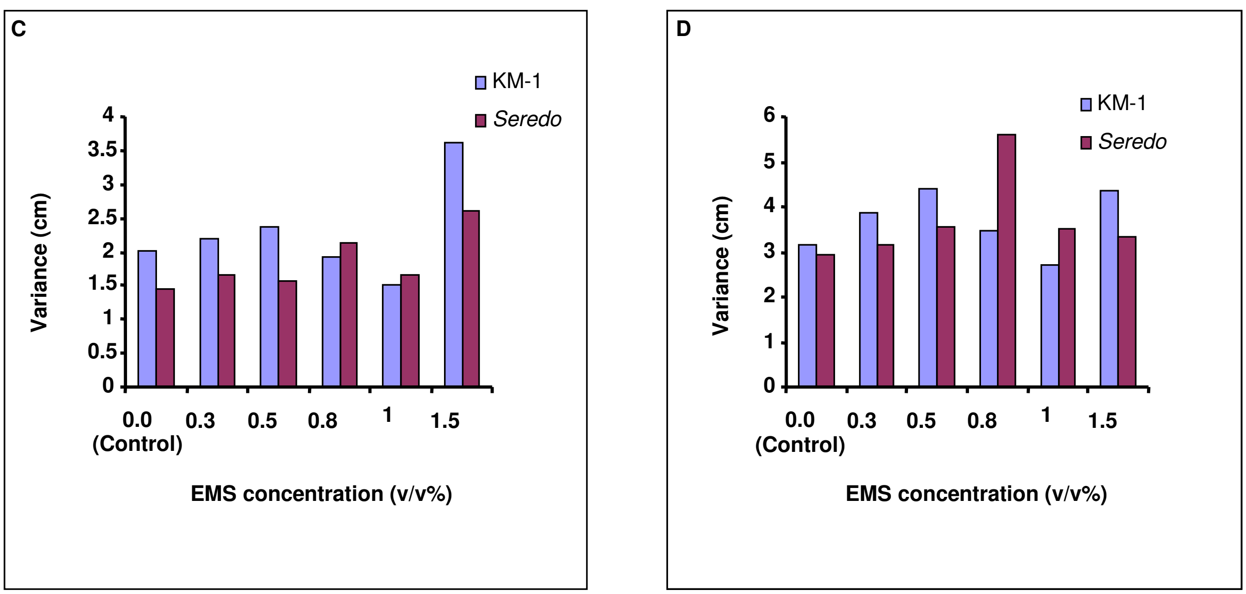

Figure 3.4 Variances for panicle head width (C) and length (D) on treatment with different EMS concentrations. Variance was calculated from the measurements of 10 plants. Note: KM-1=*Kari/mtama-1*

3.5.7 Effect of EMS Mutagenesis on formation of sorghum head types

Effect of mutagen concentration was highly significant ($P < 0.001$) in the formation of open types and significant ($P = 0.05$) for formation of semi-compact head types (Table3. 7). Variety was also significant ($P = 0.05$) for formation of both open and semi-compact head types. Generally, increase in the concentration of EMS also increased the percentage of open head types (Figure 3.5 and 3.6). For any given concentration, a higher percentage of plants with open head types were recorded for *Kari/mtama-1* than for *Seredo* (Figure 3.6). For example at a concentration of 1.5% EMS over 50% of the head types for *Kari/mtama-1* were open, while approximately 37% of the head types were open for the *Seredo.* Over 35% of the head types for *Seredo* were open at the highest concentration of 1.5% EMS and only approximately 5% of the head types were open for the control.

Table 3.7: ANOVA for effect of mutagen treatment on frequency of sorghum head types

Source of variation	d.f.	Mean squares	
		Head type	
		Open	Semi compact
EMS concentration	5	1936.3**	1325.4*
Exposure time	1	34.9	870.6
Variety	1	994.7*	1046.3*
Concentration * time	5	289.3	309.4
Concentration * variety	5	135.1	461.4
Time * variety	1	512.4	38.4
Concentration * time * variety	5	172.1	629.1
Error	46	2233.6	312.5

*, **, Data is significant at P≤0.05 and P≤0.001, respectively.

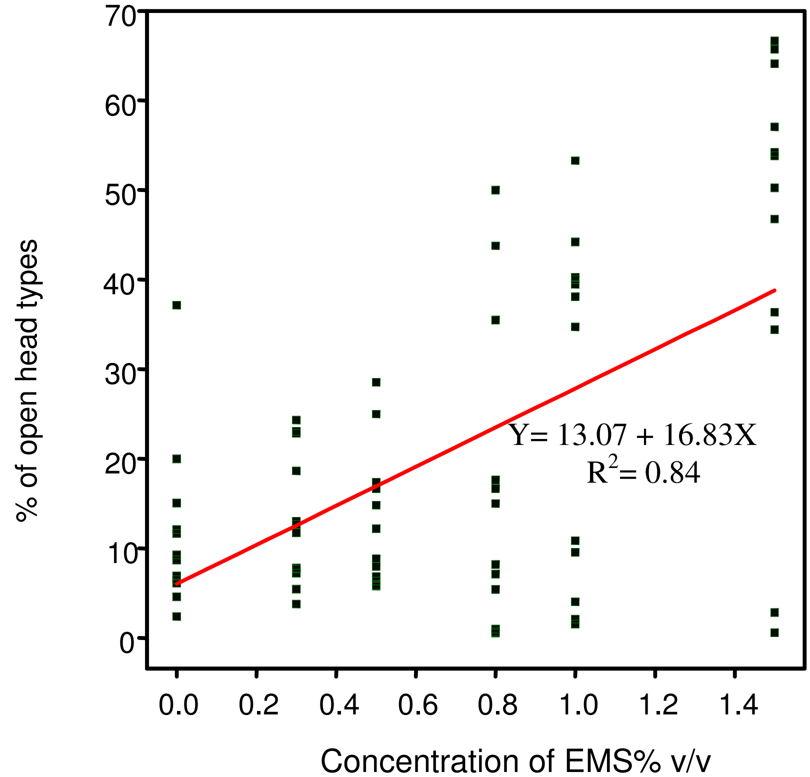

Figure 3.5: The effect of EMS concentration on the formation of loose/open head types in sorghum

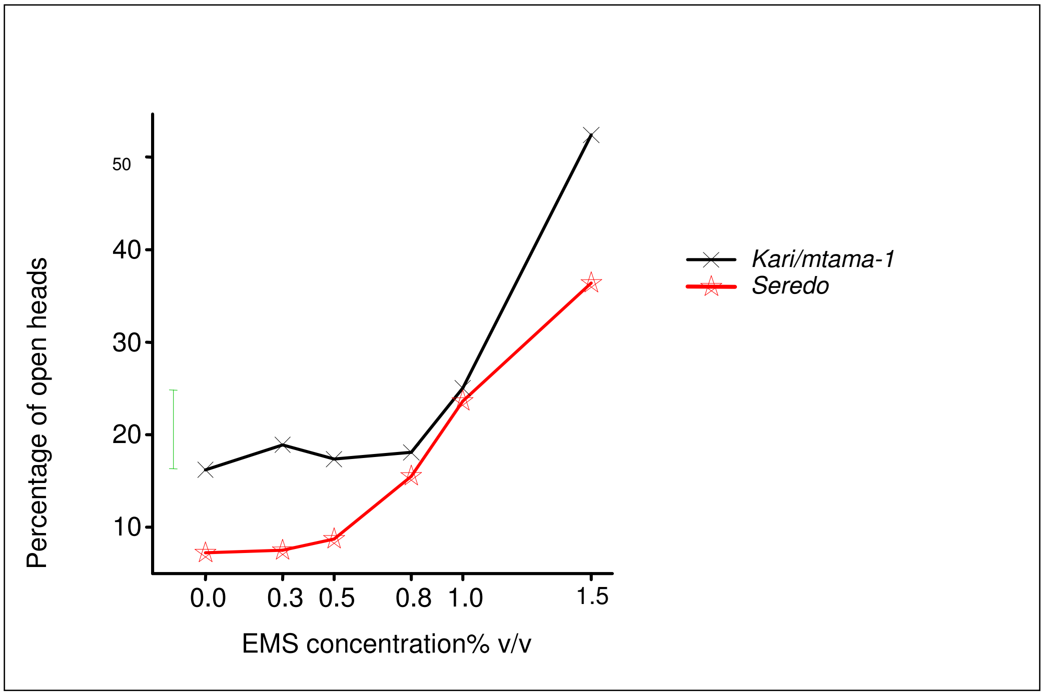

Figure 3.6: Effect of concentration of EMS on the formation of open head types for *Kari/mtama-1* and *Seredo* sorghum varieties

3.6 DISCUSSION

In both the laboratory and the greenhouse experiments EMS treatments reduced radicle and shoot lengths. This indicated that EMS was effective in mutagenizing the two sorghum varieties. (Koornneef, 2002).Although studies have shown that EMS mutagenesis is effective in sorghum (Sree-Ramulu, 1970b) there are also studies that indicate that different varieties have different resistances to mutagenesis (Sree Ramulu, 1970a). *Seredo* and *Kari/mtama-1* behaved similarly on mutagenesis. Effective mutagenesis has also been reported in other crops (Yadav, 1987; Natarajan, 2005; Singh and Kole, 2005). The drastic reduction was verification that the mutagen used had good potency.

3.6.1 EMS dose response

In this study, LD_{50} values were calculated from the reduction of the shoot length on treatment with EMS, though any other growth parameter can be used as well (Hohmann et al., 2005). The LD_{50} calculated from probit analysis was 0.35% and 0.4% of EMS for *Seredo* and *Kari/mtama-1*, respectively. Effect of exposure time was not significant for plant shoot length. These LD_{50} values were not very different from those attained by Sree-Ramulu (1970b) who found that the LD_{50} based on seedling height for three different sorghum varieties varied between 0.1 and 0.25% EMS. Varietal differences were evident, with *Kari/mtama-1* having a higher LD_{50} than *Seredo*. Sree-Ramulu (1970b) also found there were varietal differences in the LD_{50} of three sorghum varieties and attributed it to differential resistance to mutagenic treatment. In sugar beet, LD_{50} was obtained after treatment with 1% EMS for 1h (Hohmann et al., 2005), while in mungbean, LD_{50} based on germination was found to be 0.66% EMS. While the LD_{50} is usually a good point to start mutagenesis experiments, it is not always the treatment criterion that works to generate the highest frequency of mutations. Therefore, the need to balance lethal dose and mutation frequency necessitates optimization for each species (Hohmann et al., 2005). In the current study, the LD_{50} for *Kari/mtama-1* and *Seredo* were at concentrations ranging between 0.35% and 0.4% EMS and the highest mutation frequency was attained at an EMS concentration of 0.3% which was not very different from the mutagen concentration for LD_{50}. This was an indication that LD_{50} would also be effective in generating high frequency of mutations for these two sorghum varieties.

3.6.2 Field emergence

An increase in the concentration of the mutagen was accompanied by a corresponding decrease in the percentage of plants that emerged in the field for the two varieties. Mutagen toxicity resulting in lethality was most probably the cause of the reduced emergence. However, the highest mutation frequency was achieved at the 0.3% EMS concentration (Table 3.5) where the percentage germination was 60%. Therefore, there would be no added benefit of using a higher dose level than 0.3% EMS for these two sorghum varieties.

3.6.3 Frequency of chlorophyll mutations in M_2 plants

The highest frequency of chlorophyll mutations was recorded at the concentration of 0.3% EMS. After this, there was a gradual decrease in the frequency of chlorophyll mutations with increasing EMS concentration. The frequency of chlorophyll mutations has been used in many crops as criteria for determining the effectiveness of mutagenic treatment with the mutagenic conditions producing the highest chlorophyll mutations being used for mutagenesis. Usually an increase in the chlorophyll mutations is observed with an increase in the concentration of EMS. For example, an EMS concentration of 0.5% was found to produce more mutations than 0.25% EMS in broad bean (*Vicia faba* L.) (Vishnoi and Gupta, 1980). Other studies on sorghum have also shown a dose dependent relationship between mutagen dose and chlorophyll mutations (Sree-Ramulu, 1970b; Seetharami-Reddi and Prabhakar, 1983). Pre-soaking of seed for 16h and a concentration of 0.001M sodium azide concentration was found to induce the highest number of chlorophyll mutations in sorghum (Seetharami-Reddi and Prabhakar, 1983). In the current study, mutation frequency was highest at a concentration of 0.3% EMS, which was followed by a gradual decline in the yield of these mutations. Sree Ramulu (1970b) reported a mutation saturation effect on treatment of sorghum seed with EMS. It is possible that mutation saturation for *Kari/mtama-1* and *Seredo* occurs at 0.3% EMS and additional increases of the mutagen dose will not increase the mutation frequency. Variety and time of exposure were not significant in determining the frequency of chlorophyll mutations. However, Sree-Ramulu (1970a) reported varietal differences in the chlorophyll mutation induction. A possible reason could be genotypes used in the two studies and indicates the need for genotypic optimization in mutagenesis experiments. Mutagenesis has generally been shown to be very sensitive (Koornneef, 2002) with specific mutagen conditions like temperature and the potency of the mutagen affecting the type and frequency of mutations (Koornneef, 2002). While genotype effects have been shown to be significant factors in reaction to mutagens (Seetharami-Reddi, 1984; Singh and Kole, 2005), there is a possibility that it may not be important for certain characteristics. In this case, there would seem to be a critical interaction between the genotype, mutagen concentration, time of exposure and even specific genotype characteristics. This means that apart from optimizing the mutagenic conditions for the genotype, it may also be important to optimize dose for specific characteristics.

3.6.4 Flowering and seed set in the M_2 generation

The effect of EMS concentration was significant for flowering and seed set with a higher seed set being recorded for the lower EMS concentrations. This study confirms the work of Sree-Ramulu (1970b) who found that fertility in sorghum also decreased with increase of mutagen dose. Increased sterility has also been shown to occur with increased mutagenic treatment with EMS in finger millet (Aradhya and Madhavamenon, 1979). The interaction between variety and exposure time was found to be significant. In both the two varieties, there was decreased flowering with an increase in the time of exposure to EMS. At 6h exposure time, the percentage of plants that were found to flower was 54.2% and 58.2% for *Kari/mtama-1* and *Seredo*, respectively. While at 12h exposure time, 19.2% and 21.1% of plants flowered for *Kari/mtama-1* and *Seredo*, respectively. The results indicate that sensitivity to mutagenic treatment for certain traits may be higher than for other traits. However, overall seed set was very low for the 1.5% and 1.0% EMS concentrations and would not be recommended especially in experiments where a large number of plants are required for selection. In this study, there were plants that set seed even at the highest concentration of 1.5% which is in discrepancy with the results of Sree-Ramulu (1970b) who indicated that total sterility of sorghum was recorded at concentrations of 0.2 and 0.3% EMS. These differences may arise as a result of different genotypes or due to different races of sorghum. Generally, the upside of increasing mutation dose is that more mutations can be achieved (Koornneef, 2002) as confirmed in the current study. The downside is that problems with fertility increase concurrently with EMS concentration. Therefore, a balance is crucial in establishing a suitable dose that on the one hand is effective in inducing mutations and on the other, does not result in low fertility levels which will drastically reduce the number of plants to be screened.

3.6.5 Phenotypic variation of panicle characteristics

Maximum variation in the width, length, weight and seed weight of the sorghum panicle, were observed at different dose levels of EMS. For example, the highest phenotypic variance for panicle head length was at 0.8% EMS for *Seredo* while for *Kari/mtama-1* it was at 0.5% EMS. This difference between the two sorghum varieties may be a result of differences in mutagenic resistance. However, a far more important point that emerges is that it may be necessary to optimize mutagenic conditions for different morphological characters that need to be improved. In context, therefore, if the interest is in increasing variance for panicle length in a breeding programme, then an optimization is necessary to identify the mutagenic conditions that induce the highest variation for the particular phenotype. Jabeen and Mirza (2002) were able to increase variance of traits including leaf area, leaf number, plant height and days to flowering in capsicum with EMS treatment. Like in the current study, they also found that the highest variances for different phenotypic characters were induced by different mutagen doses although there was a general trend of increasing variance with increasing EMS dose for most of the characters studied. In chickpea, valuable genetic variance has also been induced for important traits (Shah et al., 2006). Mutagenic treatment was also shown to increase the variance of several characters like pod length, 100 seed weight, number of clusters per plant, and seed yield per plant in green gram (*Vigna radiata*) (Sarma, 1998). In sorghum, studies on phenotypic variance induction are few although Bretaudeau and Traore (1990) have reported on increased variance on mutagenesis with gamma rays, with promising mutants for earliness, drought, plant height and other important characteristics being achieved for sorghum in West Africa.

3.6.6 Effect on head morphology

The effect of mutagen concentration was significant in altering the head morphology of the two varieties. Generally, an increase in the EMS concentration increased the ratio of open headed and semi-compact head types. This means that EMS mutagenesis can be used to improve head morphology of sorghum varieties where a more open head type characteristic is needed. Bretaudeau and Traore (1990) have also isolated open headed mutants developed via gamma irradiation of two West African sorghum varieties *Gadiaba* and *CSM 388*. Generally, open headed types of sorghum are more preferred as opposed to the closed or compact heads due to the close relationship of moldy heads and insect damage with closed/compact head types. Many of the Kenyan local varieties like *Ochuti* and *Nyakabala* have compact head types and mutagenesis would be effective in changing the head morphology of these varieties to more open head types. Generally, not much work has been done on the mutation induction in sorghum and much less in the recent past.

3.7 Conclusions

This study has established that:

- Ethyl methane sulphonate (EMS) is an effective mutagen for the two sorghum varieties, *Seredo* and *Kari/mtama-1*.
- The LD_{50}, values based on shoot length reduction for *Kari/mtama-1* and *Seredo* are 0.4% and 0.35% EMS, respectively, while the highest mutation frequency for both varieties was achieved at 0.3% EMS concentration for between 6h-12h.
- Mutagenesis with EMS was effective in generating phenotypic variation in important morphological characteristics like panicle seed weight, total panicle weight, panicle length and also head architecture like open and semi-compact head types.

This is probably the first time that a comprehensive study has been done on mutagenesis and genetic variation induction on sorghum. The protocol developed here can be useful as a general guide for EMS mutagenesis in sorghum. The mutants developed in this project will be useful for sorghum breeding.

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Chapter 4

Preliminary Evaluation of Ethyl Methane Sulphonate (EMS) Mutation Derived Sorghum Lines for Agronomic Performance in Kenya

Abstract

Conventional breeding approaches have not resulted in new varieties that are superior to the widely grown old variety "*Seredo*". Mutation breeding can be used to enhance genetic variation in some important characteristics for sorghum improvement. Seventy eight EMS-derived mutants, their wild type progenitor *Seredo* and two check varieties were evaluated for agronomic performance in 9x9 simple lattice designs, at two sites in Kenya, during 2008. There were significant differences ($P < 0.05$) among entries for grain yield and 1000-seed weight. Most of the mutants performed worse than *Seredo* for most characteristics, an indication of the deleterious nature of mutagenesis. However, the mutant line "SB2M13" had the highest yield which was 160% and 152% relative to *Seredo* and the best check, *Kari/mtama-1*, respectively. Nine mutant lines displayed high grain yield of between 100% and 147% relative to *Seredo*. Mutant line "tag27" showed the highest 1000-seed weight of 133% relative to *Seredo*. There were also significant differences ($P < 0.05$) among entries for head architecture, exertion, height uniformity and desirability scores. Six of the top 10 yielding mutant lines were superior to *Seredo* for these traits. The entries were also significantly different for the days to 50% flowering and 42 mutants flowered earlier than *Seredo*. Entries were significantly different ($P < 0.05$) for *Striga* infestation but most had higher infestation than *Seredo*. The realized gains in grain yield, maturity dates, head architecture and desirability scores in some mutants could be considered to be significant for sorghum breeding and improvement in Kenya.

Keywords: EMS, *Seredo*, *Kari/mtama-1*, mutant lines,

4.1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) in Kenya is mainly grown in the drought prone zones of Eastern, Nyanza and Coast provinces, and ranks as the 2nd most important cereal after maize. The main sorghum varieties grown in the country are improved ones like, *Seredo*, *Kari/mtama-1* and *Serena*, and farmers' own landraces like, *Ochuti*, *Nyakabala* among a myriad of others. Some of the major constraints to sorghum production in the country include low yield potential, *Striga* (Haussmann et al., 2000b; Kanampiu et al., 2003; Kiruki et al., 2006), diseases and poor economic status of the farmers. Although there are improved varieties released in Kenya, a participatory survey gave strong indication that most of the improved varieties accessible to farmers did not meet farmers selection criteria (see Chapter 2).

In Western Kenya, where the *Striga* weed is one of the greatest constraints to cereal farming systems, introduced sorghum varieties lack *Striga* resistance or tolerance which is a criterion farmers consider important in a sorghum variety. Farmers have, therefore, not taken up the improved varieties and have had to rely on low yielding local varieties which they consider *Striga* tolerant and reliable. Also, many of the varieties lack drought resistance which is essential in the unpredictable environments of African farming. With increasing food demand, it means that more land has to be put into cultivation of sorghum to satisfy the ever growing demands to feed the families in these subsistence farming systems. However, population pressure does not allow for the increase in land under sorghum. High yielding cultivars are required to satisfy the demand of the farmers and to provide surplus grain on the same acreage. This puts pressure on the sorghum improvement programme in Kenya to find new approaches to break the yield ceiling while accumulating or retaining alleles for the desired traits at the same time.

An area that has not been utilized in the search for high yielding sorghum varieties is mutation breeding. The development of mutants with diverse characteristics is essential for sorghum varieties intended for tropical agriculture. For example, while pure lines, with fixed genes, have benefited the developed world in development of hybrids and yield improvement, the same cannot be said for the developing world where breeding has mostly concentrated on development for adaptation and yield stability at the expense of yield (Chantereau et al., 2001). African cultures are diverse, with varying needs among communities. These different criteria necessitate breeding of sorghum with varied characteristics to suit the different cultures. Quality in sorghum for example, is a very important criterion for African farmers as they use the grain for malting beer and for porridge preparation. Conventional breeding for these traits sometimes has been difficult owing to the fact that variation for some of the traits does not exist and also because some of the characteristics are quantitatively inherited making introgression difficult. Transferring some of these traits would also lose the uniqueness, be it yield or disease resistance, of the variety (van Harten, 1998). Also, the fact that improvement of plant quality characteristics is associated with depressed agronomic characteristics (Pedersen et al., 2005) makes improvement for some of these traits even more challenging.

Mutation breeding has been shown to improve the genetic variation of West African landraces (Bretaudeau and Traore, 1990). It has been demonstrated that EMS mutagenesis can be used to generate the much needed genetic variation, and that EMS mutagenesis causes point mutations which may be beneficial in changing the plant phenotype on specific important characteristics like drought and *Striga* resistance, and head architecture (see Chapter 3). Change of one or two characteristics that limit the potential of an already advanced cultivar is the goal of mutation breeding today (Ahloowalia et al., 2004). Point mutations or single nucleotide changes in the DNA which are known to alter one or a few genes affecting one or two characteristics (Weil and Monde, 2007) could be used to alter specific traits of interest like yield, drought, and head morphology for improvement of grain quality in sorghum.

Isolation of mutants of agronomic and economic significance has been a goal of major mutation breeding worldwide (Chopra, 2005) resulting in release of approximately 2270 mutant varieties (Ahloowalia et al., 2004; FAOSTAT, 2008). Most of these mutants have been released as direct mutants, while a small percentage is through crossing of mutants with other varieties (Ahloowalia et al., 2004). The contribution that these mutants have had on yield and quality of crops is immense. In India, for example, mutation breeding was used to alter the un-preferred red colour of Mexican wheat grains renowned for ushering in the green revolution (Chopra, 2005). The economic impact of these mutation derived cultivars includes improvements in yield and quality, reduction in use of chemicals, drought tolerance and many other traits (Ahloowalia et al., 2004). For sorghum however, only 15 mutant varieties have been released (FAOSTAT, 2008). The situation is worse in Africa where apart from the work of Bretaudeau and Traore (1990), very little is being done for sorghum mutation breeding.

Global climate change and greenhouse effects have today put sorghum on the pedestal for improvement because of the increased demand for biofuels. Sorghum is one of the crops targeted for improvement of biofuel production and one of the areas researchers are looking at to provide the much needed genes for enhancement of biofuel production in sorghum are mutants generated through mutagenesis (Vermerris et al., 2007). Despite the general deleterious nature of mutation breeding (Chopra, 2005) it has continued and will continue to be widely used and more so for some of those characteristics that the natural variation does not exist, and in situations where backcrossing of a characteristic may be too laborious (van Harten, 1998). In particular, farmers, as evidenced by numerous studies in the literature carried out in Kenya and the results of the participatory study reported in this thesis (see Chapter 2), have indicated grain yield, *Striga* resistance and good head architecture are among the important traits they would like in their new varieties. It appears breeders have not been able to find adequate genetic variation to improve sorghum for these traits, especially in Kenya. This prompted the use of mutation breeding to generate such variation, and to identify some useful mutants which combine these traits. The prospective mutant lines are envisaged to be used as source material to breed new or to improve existing varieties in Kenya and elsewhere in sub-Saharan Africa where they will be best adapted.

4.2 Research Objectives

The specific objective of this study was to evaluate EMS-derived sorghum mutants for grain yield, 1000-seed weight, *Striga* resistance and other secondary traits preferred by farmers in Kenya.

4.3 Research Hypothesis

Mutation breeding results in mutants which are higher yielding and with better head architecture and resistance to *Striga* than the wild-type and other popular sorghum varieties grown by farmers in Kenya.

4.4 Materials and methods

4.4.1 Mutant lines

Mutants were derived from EMS mutagenesis of the variety *Seredo*, which is an improved variety developed by the Kenya Agricultural Research Institute (KARI). Over 40,000 seeds of *Seredo* were mutagenized using 0.3% v/v EMS. This EMS concentration had been recommended from the previous study in this thesis (Chapter 3) on sorghum mutagenesis as the dose level with highest frequency of mutations. Mutagenized M_1 seeds were planted to give rise to M_1 plants bearing M_2 seed. From a population of about 20,000 plants that survived, 200 M_1 plants were tagged, selfed and seed for each plant kept separately forming 200 seed lots. Selection was based on best looking plants that showed minimal stunting and other mutagenic treatment related plant deformities. In the next season, the M_2 seed lots were planted in different rows forming 200 M_2 families. One hundred of the best M_2 family lines were selected, seed (M_3) harvested and seed for each of the mutant lines bulked separately. The 78 M_3 mutant lines with sufficient seed for planting in trials were used for the agronomic evaluation. The M_3 lines were fairly uniform giving indication that many of the genes were fixed.

4.4.2 Experimental design, trial management and data collection

Seventy eight mutant lines, the wild type *Seredo* and two checks, *Kari/mtama-1* and *Serena* were evaluated at the Kiboko research field station in Makueni district (37°20'E 1°38'S) of Kenya during January to June 2008. The trial was repeated at Kibos in Kisumu district (-0°5'23"N 34°45'0"E), in the western part of Kenya, a *Striga* endemic zone, during May to August 2008. Both experiments were laid out as 9x9 simple lattice designs. The statistical Model was:

$$y_{ijk} = \mu + t_i + \beta_k + r_j + \epsilon_{ijk}$$

Where:

Y_{ijk}= is the observation made on the ith treatment in the kth block in the jth replication

μ = general mean

t_i = the effect of the ith treatment.

β_k = the effect of the kth block

r_j = the effect of the Jth replication

ϵ_{ijk}= the experimental error

At Kiboko, plants were planted two in a hole in single row plots of 2.8m X 0.5m. The distance between holes was 0.4m with 16 plants per plot giving a plant population of 114,285 plants ha^{-1}. In Kibos, seeds were planted in 2.0m X 0.7m single row plots. The distance between the holes was 0.2m giving a plant population of 157,143 plants ha^{-1} at two seeds per hole. Trials were planted by hand at both sites, and fertilizer was applied at the recommended rate of 50Kg N ha^{-1} and 128kg P_20_5 ha^{-1} at planting in the form of di-ammonium phosphate (18-46-0). Calcium ammonium nitrate (CAN) was also applied four weeks after planting at the rate of 120kg ha^{-1}. At Kibos, artificial *Striga* seed infestation was done to enhance the *Striga* seed density in the soil. *Striga* seeds were mixed with fine sand and the sand/*Striga* mixture added in each planting hole to ensure approximately 2000 *Striga* seeds per hole and the seeds mixed thoroughly into the top 15cm of the soil. Standard crop procedures for weeding and crop protection against shoot fly and sorghum midge were followed at both sites. Irrigation water was applied to supplement rainfall at both sites. At Kiboko, hoe weeding was done three times while at Kibos, the first two weedings were done using a hoe and then hand weeding was done where all weeds were removed except *Striga*. At Kiboko, visual ratings were done for plant height uniformity, head exertion, head architecture and desirability 12 weeks after planting (WAP) as follows:

Plant height uniformity:

1= not uniform;

3= averagely uniform;

5= uniform.

Head Exertion:

1=poor head exertion;

3=average head exertion;

5=good head exertion.

Head architecture:

1=compact panicle head type;

3=semi-compact head type;

5=open/loose panicle head type.

Overall desirability:

1=not desirable,

3=averagely desirable;

5=desirable.

All the scores for the different traits were visual ratings, whereby each of the mutant line or variety was observed and scored for the different characteristics. For general desirability the score was for overall visual appeal of the mutant line or variety. At harvest, measurements were taken for yield and 1000-seed weight. At Kibos, *Striga* counts were taken 25cm on each side of the row from the sixth WAP until the 16th WAP. Days to 50% flowering were also taken. There was no yield data collected at Kisumu as the crop was planted off-season leading to exceptionally high sorghum midge infestation; hence very few panicles managed to set seed. All quantitative data was analysed using REML programme in Genstat version 11.

4.5 RESULTS

4.5.1 Grain Yield and 1000-seed weight

Yield and 1000-seed weight differences between means of the treatments were highly significant (P < 0.01) (Table 4.1). The distribution of the mean yields of the mutants and the checks are shown in Figure 4.1. Most of the mutants yielded worse than *Kari/mtama-1* and seredo, while approximately half of the mutants yielded worse than Serena. Tag27 and SB2M13 were the poorest and highest yielding mutant lines, respectively. The mean yields for the top and bottom 10 mutant lines and for the checks are shown in Table 4.2. Mutant line SB2M13 with a yield of 6618.8kg ha^{-1} was 160% and 152% relative to the parent *Seredo* and the best check *Kari/mtama-1*. This yield was 171% relative to the mean of the checks. Eight of the top ten mutant lines had yields of between 100 to 160% relative to the wild type *Seredo* (Table 4.2). Five mutant lines had relative yields of over 100% compared to the best check. However, the majority of the mutant lines yielded below the control genotypes. The yield of the bottom 10 mutants ranged between 15% and 44% of the yield of the wild type *Seredo* and the best check *Kari/mtama-1*. All were significantly (P = 0.05) lower than the wild type *Seredo*. The mutant line tag27 had the lowest yield of 684.8kg ha^{-1}, but this mutant line was ranked first for 1000-seed weight (Table 4.3). However, the mutant line SB3M13 with the highest yield also recorded a low 1000-seed weight and was ranked number 76 for this trait. *Kari/mtama-1*, one of the checks was ranked second highest for 1000-seed weight. Both the highest ranked mutant and *Kari/mtama-1* had significantly higher 1000-seed weight than *Seredo* and the other mutant lines. There were six other mutants among the top highest yielding mutant lines with significantly higher seed weight than *Seredo*. The wild type *Seredo* ranked number 41 for seed weight while the other standard check *Serena*, was ranked number 40.

Table 4.1: Mean squares from analysis of variance for yield and1000-seed weight at kiboko

Source of variation	df	Yield	1000 grain weight
Replication	1	4559111	7.6
Reps*Blocks	16	6932645	11.9
Treatment	80	3249223**	8.0**
Residual	64	1518920	3.2

** Significant at P < 0.001

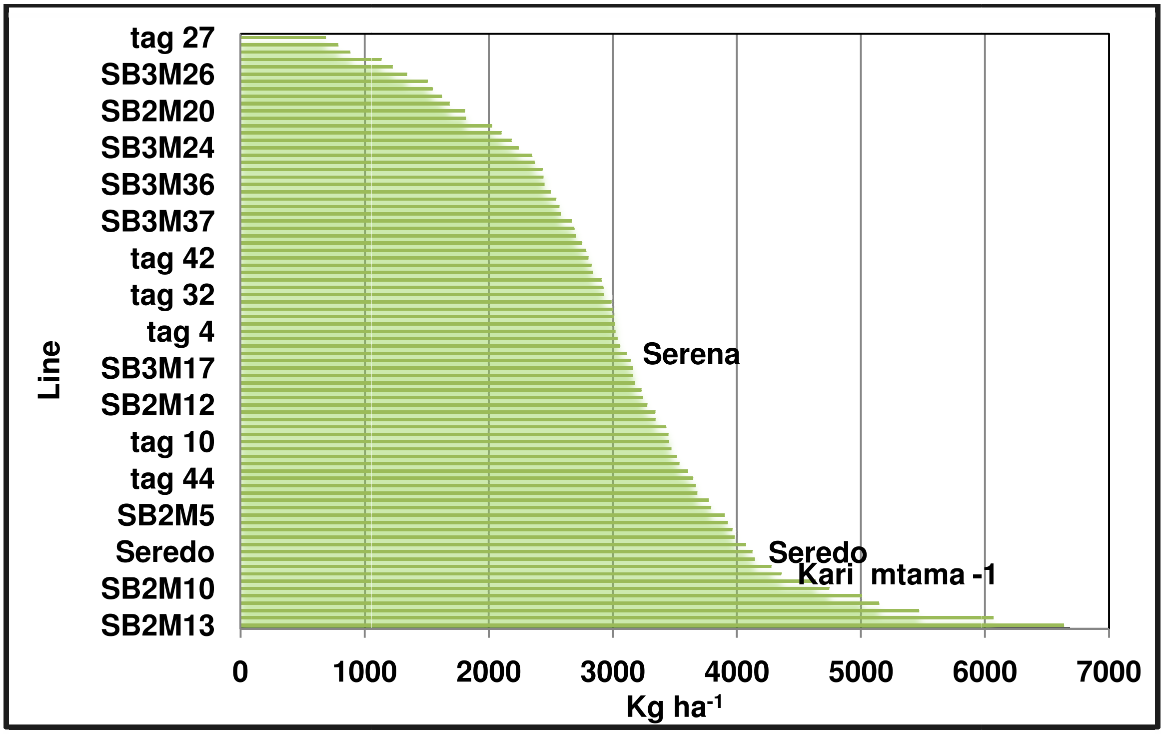

Figure 4.1 Distribution of yield for the mutant lines and checks planted in Kiboko Kenya

Table 4.2: Yields for the top nine and bottom ten mutant lines and the standard check varieties at Kiboko in Kenya during 2008

Variety/Mutant	Yield (kg ha^{-1})	Rank	Percentage relative yield of mutant to			
			Seredo (wild type)	Overall Mean	Best check *Kari/mtama-1*	Mean of checks
SB2M13	6618.8	1	160.8	219.7	152.3	171.7
tag29	6054.0	2	147.1	201.0	139.3	157.1
SB3M1	5454.0	3	132.5	181.1	125.5	141.5
SB2M1	5133.8	4	124.7	170.4	118.1	133.2
SB2M2	4994.3	5	121.4	165.8	114.9	129.6
SB2M10	4731.8	6	115.0	157.1	108.9	122.7
tag 22	4622.3	7	112.3	153.5	106.4	119.9
Kari Mtama-1*	**4345.5**	**8**	**105.6**	**144.3**	**100.0**	**112.7**
SB2M7	4267.5	9	103.7	141.7	98.2	110.7
SB2M3	4132.5	10	100.4	137.2	95.1	107.2
Seredo*	**4114.5**	**11**	**100.0**	**136.6**	**94.7**	**106.7**
Serena*	**3104.3**	**38**	**75.4**	**103.1**	**71.4**	**80.5**
SB2M14	1679.3	72	40.8	55.8	38.6	43.6
tag 12	1617.0	73	39.3	53.7	37.2	41.9
tag 28	1542.0	74	37.5	51.2	35.5	40.0
tag 51	1500.8	75	36.5	49.8	34.5	38.9
SB3M26	1338.0	76	32.5	44.4	30.8	34.7
SB3M6	1221.0	77	29.7	40.5	28.1	31.7
tag 24	1130.3	78	27.5	37.5	26.0	29.3
SB3M2	882.0	79	21.4	29.3	20.3	22.9
tag 41	783.8	80	19.0	26.0	18.0	20.3
tag 27	684.8	81	16.6	22.7	15.8	17.8
$LSD_{(0.05)}$	2073					
Grand Mean	3012					
Mean of checks	3854.8					
C.V. (%)	31					

* Checks

Table 4.3: Performance of some of the best and worst mutant lines and checks for 1000 -seed weight in Kiboko

Mutant line or sorghum variety	1000-seed weight (g)	Overall rank	Percentage relative 1000-seed weight of mutant to	
			Seredo	mean weight
tag27	32.5	1	133.2	132.8
Kari Mtama-1*	**32.3**	**2**	**132.2**	**131.8**
tag 4	28.9	3	118.6	118.2
tag 29	27.9	4	114.3	114.0
tag 51	27.4	5	112.2	111.9
tag 28	27.2	6	111.6	111.3
SB2M7	26.7	7	109.5	109.2
tag2	26.7	8	109.3	109.0
SB2M5	26.6	9	109.1	108.8
SB2M1	26.2	10	107.5	107.2
SB2M9	26.2	11	107.4	107.1
***Serena* ***	**24.5**	**40**	**100.4**	**100.1**
Seredo* (wild type)	**24.4**	**41**	**100.0**	**99.7**
tag10	22.2	72	90.8	90.6
tag12	21.7	73	89.0	88.7
SB2M16	21.6	74	88.5	88.3
tag 24	21.6	75	88.5	88.3
SB3M13	21.6	76	88.4	88.2
SB2M14	20.5	77	84.1	83.8
SB3M38	20.5	78	84.1	83.8
tag 32	20.5	79	83.9	83.6
SB2M20	20.2	80	82.9	82.6
SB3M2	19.9	81	81.6	81.3
$LSD_{(0.05)}$	1.68			
C.V.%	7.3			
Grand Mean	24.47			
Mean of checks	27.07			

*Checks

4.5.2 Scores for different sorghum characteristics

Mean scores for head morphology, head exertion, height uniformity and overall desirability were significant ($P \leq 0.05$) for the different entries (Table 4.4). Figure 4 shows some of the mutant lines for which morphological ratings for the different characteristics were done. Table 4.5 shows the scores for the top ten highest yielding mutants and the checks for various characteristics. The mutant lines tag29 and tag1 had better height uniformity than the wild type *Seredo*, while for head exertion, the mutant lines SB2M7, SB2M10 and tag22 were all rated better than the wild type *Seredo*. The mutant line tag29 and the check *Kari/mtama-1* had a higher score for head architecture than the wild type *Seredo*. For overall desirability, line SB3M1 was rated higher than *Seredo*. In terms of overall ranking, which was the average of all the four characteristics scored, *Seredo* was rated seventh among the top ten highest yielding mutant lines.

Figure 4.2: Some of the mutant lines planted that were rated for different morphological characteristics in Kiboko, Kenya. NB: the difference in height between the mutants (SB3M38 and SB3M39), and between the wild type *Seredo*, and mutants.

Table 4.4: Mean squares for scores of different characteristics of sorghum at Kiboko

Source of variation	df	Head morphology	Exertion	Height uniformity	Desirability
Replication	1	7.62	4.50	2.23	0.03
Rep*Blocks	16	1.92	0.61	0.52	0.46
Line	80	8.05**	0.65*	0.95*	0.62**
Residual	64	3.17	0.40	0.57	0.28

*, ** Significant at 0.05 and 0.001 respectively

Table 4.5: Score of the top 10 highest yielding mutants and checks for various characteristics in Kiboko

Mutant or variety	Height uniformity	Head Exertion	Head architecture	Desirability	Rank[#]
tag29	3.6	3.2	4.5	1.8	1
Serena **(Check)**	**3.1**	**3.8**	**3.3**	**2.7**	**2**
SB3M1	2.4	3.3	2.8	4.2	3
tag 22	2.4	3.6	2.9	3.3	4
Kari/mtama-1 **(Check)**	**2.0**	**3.4**	**4.9**	**1.8**	**5**
SB2M7	2.4	3.7	2.8	2.8	6
Seredo **(wild-type)**	**3.0**	**3.1**	**2.8**	**2.8**	**7**
SB2M13	3.0	2.6	2.9	3.1	8
SB2M1	3.0	2.8	3.2	2.5	9
tag1	4.0	2.6	2.4	2.2	10
SB2M2	3.0	2.6	3.1	2.3	11
SB2M10	2.1	3.5	2.6	2.8	12
SB2M3	2.8	1.7	2.6	2.4	13
$LSD_{(0.05)}$	1.7	1.4	1.5	1.6	
Mean	3.6	3.1	3.8	4	
SED	0.8	0.7	0.7	0.7	
C.V. (%)	21	17	14	27	

Notes: Height uniformity: 1=poor, 5=good; Exertion: 1=good, 5=good, Head architecture: 1=compact head, 5=open head; Desirability: 1=not desirable; 5=highly desirable.
[#] Overall ranking is the mean of the four characteristics scored

4.5.3 Days to 50% flowering at Kibos, Kisumu

Differences of days to 50% flowering for the mutant lines and the checks were significant (P=0.05) (Table 4.6). Table 4.7 shows the means of days to 50% flowering for mutant lines and the checks. The mutant line tag44 had the least number of days to 50% flowering (67.1) but was not significantly lower than the wild type *Seredo* (70.5). The check, *Kari/mtama-1* with 67.5 days to 50% flowering was ranked fourth overall though it was not significantly lower than the *Seredo*. Among the mutant lines significant (P = 0.05) differences in number of days was recorded. There was a difference of ten days between the mutant line with the least number of days and the mutant line with the highest number of days. There was also a significant difference between the mutant line with the highest number of days to 50% flowering and the wild type *Seredo*. In terms of ranking *Seredo* was ranked number 43 overall for this trait.

Table 4.6: Mean squares for days to 50% flowering in Kisumu

Source of variation	df	Mean squares
Replication	1	22.3
Replication* blocks	16	15.9
Lines	80	13.3**
Residual	64	4.2

Table 4.7: Days to 50% flowering for the top 10 and bottom five mutants and checks

Mutant line/variety	Days to 50% flowering	Overall rank
tag44	67.1	1
tag12	67.4	2
tag4	67.5	3
Kari/mtama-1*	**67.5**	**4**
tag1	67.5	5
tag29	68.0	6
SB2M12	68.0	7
SB2M19	68.0	8
tag35	68.0	9
tag2	68.5	10
SB2M20	68.5	11
Seredo*	**70.5**	**43**
Serena*	**73.5**	**73**
tag28	76.0	77
SB3M18	76.0	78
SB3M4	77.5	79
tag24	77.6	80
SB2M9	79.0	81
$LSD_{(0.05)}$	4.9	
Mean	70.97	
SED	2.4	
C.V. (%)	38.6	

* Checks

4.5.4 Above-ground *Striga* counts in Kisumu

Figure 4.2 shows the above ground *Striga* emergence in the field at Kibos for the mutant lines with the least and the highest number of emerged *Striga* plants. Only three mutant lines recorded lower number of *Striga* plants than *Seredo*. The mutant line SB3M34, SB3M10 and SB3M15 all recorded only 3 plants while *Seredo* had 5 *Striga* plants that emerged. However, most of the mutants recorded higher number of *Striga* plants than the controls with the mutant line SB2M5 having the highest number (42) of emerged *Striga* plants. Both the checks, *Kari/mtama-1* and *Serena,* had higher numbers of *Striga* plants than the wild type *Seredo*

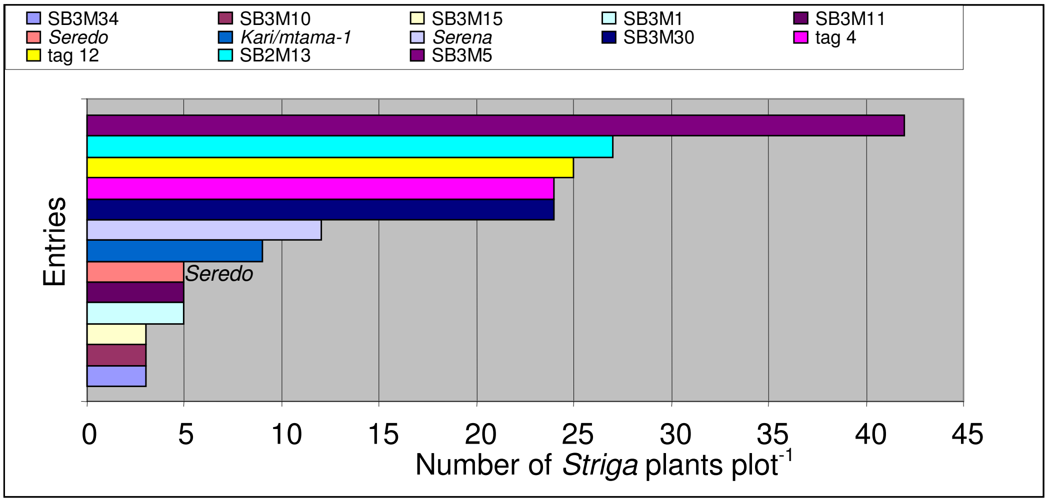

Figure 4.3 Mean number of above ground *Striga* plants for mutant lines with the least and highest number of *Striga* plants. Plants were counted 25cm on each side of the row that were 2m long. Counts are for the 14th week after planting which was the peak for *Striga* emergence.

4.6 Discussion

There were significant differences for yield and 1000-seed weight for entries. The mutant line SB2M13 had the highest yield of 6618kg ha^{-1} compared to the wild type *Seredo* with 4114kg ha^{-1}. Eight other mutant lines had higher but not significantly different, yields than *Seredo*. However, these mutants had yields of between 100 and 137% relative to *Seredo* and the lack of significance difference was probably due to the high C.V. value (31%). Most of the mutants with high yield had an above average height uniformity rating (Table 4.5) and this could contribute to their high yield. Plant height variability has been shown to be associated to grain yield decline (Boomsma et al., 2006). For head exertion, the high yielding mutant lines also scored above average ratings (Table 4.5). The mutant line tag29 was ranked highest for the combined overall rating on height uniformity, exertion, head architecture and visual desirability and was ranked second for yield. It was also rated fourth for 1000-seed weight. However, SB2M13, which was ranked first for yield, was ranked seventh for combined overall rating of height uniformity, head exertion, head architecture and desirability. It could, therefore, be assumed that there are also other inherent characteristics not measured in this study that contributed to the high yield of SB2M13. Most probably, a combination of good height uniformity, exertion and head architecture may have contributed to the high yield. Most of the mutant lines displayed lower yields than *Seredo*, probably as a result of the deleterious nature of most mutations (Chopra, 2005). Mutant varieties with increased yields have been generated in many crop species including chickpea, rice (Gaur and Gour, 2002), and barley (Ahloowalia et al., 2004). Many mutants released in India for example, have shown supremacy over other varieties in coordinated trials with eventual release as commercial varieties (Chopra, 2005). The mutants SB2M13, tag29 and SB3M1 all had relative yield of over 130% compared to *Seredo* and would be good candidates for further testing and possible release as direct mutants. On the other hand the same mutants could be used to make crosses with other established varieties for general improvement of yield in sorghum.

Significant differences were observed between entries for 1000-seed weight. The mutant line tag27 had the highest 1000-seed weight but also the lowest mean yield. However, studies have shown a positive correlation between yield and 1000-seed weight for crops including sorghum (Ezeaku and Mohammed, 2006) and wheat (Saleem et al., 2006). The high1000-seed weight for tag27 was most probably because it had very poor seed set and thus more resources were allocated to the fewer grains. However, the extremely low grain fill overrode the benefits of increased 1000-seed weight. The mutant lines tag4, tag29 and tag51 with high 1000-seed weight may be important in breeding for increased yield in sorghum. A significant positive relationship has been shown to exist between 1000-seed weight and yield in oil flax (Copur et al., 2006) and broomgrass (Açıkgöz and Tekeli, 1980) where it was also related to seed size. Here in Kenya, the characteristics farmers want in their varieties include high yield and large grain size. Some of the mutant lines like tag27 and tag4, isolated in this study, will be useful in developing large seeded varieties with high yield for farmers.

The ratings of head morphology, head exertion, height uniformity and overall desirability for mutants and checks were significant (P=0.05). For height uniformity mutant lines tag1 and tag29 had a higher rating than the wild type *Seredo* and can be useful when developing hybrids that have good height uniformity. However, it may still be important to verify the uniformity of the mutant lines with more generations of selfing to make sure the genes are fixed. Head exertion is considered important as it prevents disease and insect damage and some of the mutant lines like tag29 and SB3M1 could be useful in breeding for good head exertion in sorghum. One of the characteristics farmers indicated as important was good head morphology like open head types (Chapter 2). Farmers prefer sorghum varieties with open head types due to the resultant susceptibility to disease and insects of compact head types. Mutant line tag29 was rated the highest for good head architecture and would be useful in breeding for this trait. In rice, mutants with open and semi-open panicles were isolated from parents which had a semi-compact head type (Saddiq and Swaminathan, 1968). In terms of overall desirability, some of the mutants like SB3M1 and tag22 were rated higher than *Seredo*. The outlook of varieties plays an important role as a criterion for farmers, who are generally attracted to varieties with better visual outlook without consideration of yield. As a way of delivering varieties that have the desirable characteristics for farmers, these mutant lines with good overall desirability will have an important role to play in ensuring adoption of varieties by the farmers. Mutant lines like tag29, SB3M1, tag2 and SB2M7, with a higher ranking for all the characteristics, could either be released as varieties after further testing in multi-location trials to confirm their performance or be maintained for further development and for breeding purposes.

Differences in days to 50% flowering were detected among mutant lines and checks. The mutant line with the least number of days was tag44 which took 67 days to flower compared to *Seredo* which required 70.5 days. The difference between the mutant line with least and highest number of days to 50% flowering was 10 days. This was an indication that variability was also generated in the trait. Many varieties exhibiting earliness have been derived from mutations including in rice in Pakistan, where mutation induction in the variety ‘Basmati 370’ produced the early maturing variety ‘Kashmir basmati’ with same aroma and cooking qualities as the parent (Ahloowalia et al., 2004). In environments where the rainy season is short, early maturing varieties are important because they are able to avoid the long spells of drought. Other notable early mutants have also been released in cotton, barley and wheat (van Harten, 1998). In sorghum Bretaudeau and Traore (1990) have been able to generate mutants for earliness in some West African sorghum varieties.

Several mutant lines for example SB3M34, SB3M10 and SB3M15 had fewer numbers of *Striga* plants compared to the parent. Though the difference was not significant the mutants can be useful in further *Striga* research. The mutant line with the highest number of *Striga* plants was SB3M35. This mutant line could be useful as a *Striga* "catchcrop". Plants with high *Striga* stimulating capacity have been put forward as an option, "catchcropping" for *Striga* control. They are planted in very high densities so as to germinate as many *Striga* seeds in the soil as possible. However, the catchcrop is destroyed before the *Striga* flowers thus precluding any *Striga* seed replenishment. Mutant lines like SB3M5 would be ideal for such studies. *Striga* emergence was however low in the season despite artificial infestation having been done and further testing of these mutant lines is recommended. This, however, was not entirely unexpected. A major impediment to *Striga* control is the difficulty in field screening for *Striga* resistance which is generally hindered by large differences in the natural field infestation, sensitivity of *Striga* emergence to prevailing environmental conditions and complexity of host parasite relationship (Haussmann et al., 2000a).

4.7 Conclusions

It was not possible in the time of this study to test the mutant lines in different environments and further testing is recommended in other diverse environments. However, this study identified promising mutant lines including SB3M13, tag29, SB3M1 and SB2M1 for yield improvement. Mutants like tag27, tag4 and tag29 had high 1000-seed weight and may be important in breeding for increased seed size which is an important farmer preferred characteristic. Mutant lines like SB3M1, tag22, SB2M7, among others may be important for improvement in head exertion and overall visual desirability. Though the study did not produce mutants with higher resistance to *Striga* than *Seredo*, the mutants SB3M34 and SB3M10 which had lower numbers of *Striga* can be useful in elucidating the mechanisms of *Striga* resistance. However, most of the mutants in this study performed poorly compared to the wild type for most of the characteristics measured which means that many more individuals should be screened in order to get some that are superior to the wild type. This was a preliminary study and the promising mutant lines require further testing in advanced trials across many locations that represent their target environments. They will also be valuable in increasing the available germplasm for breeding purposes.

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Chapter 5

Development, evaluation and genetic analysis of sulfosulfuron herbicide resistance in sorghum

ABSTRACT

Herbicide tolerant varieties in combination with respective herbicide seed treatments can be used to manage *Striga*. However, there are no herbicide resistant sorghum varieties in Kenya. The objectives of this study, therefore, were to develop herbicide resistance in sorghum; to determine the level of resistance in resultant herbicide tolerant mutants; and to determine the genetic inheritance of herbicide tolerance in sorghum. Five ethyl methane sulphonate (EMS) derived sulfosulfuron tolerant mutants (designated hb6, hb8, hb12, hb56 and hb462,) were identified and selfed to M_4 generation. Varying rates of sulfosulfuron, either as a spray or seed coat, were applied to determine the level of tolerance of the mutant lines. Mutant lines were also crossed with the wild type *Seredo* and among themselves to determine mode of inheritance of the herbicide tolerant trait. Results showed that the susceptible wild type *Seredo* was killed at the lowest herbicide rates of 0.5g ha^{-1} and 1g ha^{-1} sulfosulfuron. Dry matter from the spraying and seed coating experiments showed mutants to be up to 170 times more resistant than the wild type. The LD_{50} values indicated a general trend of hb46 > hb12 > hb462 ~ hb56 > hb8 for level of tolerance under both spraying and seed coating experiments. The F_2 progeny of mutant X wild type crosses segregated in a 1:2:1 fashion for resistant, intermediate and susceptible, which was an indication of semi-dominant inheritance. Intercrosses between mutant lines did not segregate for resistance in the F_2 generation indicating the same mutation could be responsible for the tolerance in all five mutants. It was however not clear from this study why tolerance levels among the mutants differed. The information from this study would be useful for sorghum improvement programme aimed at managing *Striga*.

Keywords: sorghum, mutants, LD_{50}, herbicide tolerance, wild type.

5.1 Introduction

A potential strategy to manage *Striga* is the use of herbicides together with herbicide tolerant varieties. Many studies (Adu-Tutu and Drennan, 1991; Abayo et al., 1998; Kanampiu et al., 2003) have indicated the potential of herbicides to control *Striga* in cereal crops. However, the appropriate mode of application of these herbicides to control *Striga* in sorghum has not been tested under subsistence farming systems. The use of herbicide seed coating to control *Striga* has been demonstrated in maize (Berner et al., 1997; Abayo et al., 1998; Kanampiu et al., 2001). With this technology, the herbicide that is coated onto herbicide tolerant seeds is able to kill any *Striga* plants that attach onto the host roots while sparing the host crop. However, for utilization of herbicide seed coating, the host plant must have tolerance to the herbicide. In maize, where herbicide seed coating has been tested, imidazolinone resistant (IR) maize, which is tolerant to acetolactate synthase (ALS) herbicides, was used (Abayo et al., 1998; Kanampiu et al., 2001; Kanampiu et al., 2002). The tolerance in the host plant ensures that herbicide damage is minimized. In sorghum, herbicide seed coating has not been tested mainly because there are no herbicide tolerant sorghum varieties.

An option available for developing herbicide resistant sorghum varieties is mutagenesis. This has been successful in cereals and other crops where variability for this trait has been limiting. In wheat for example, tolerance to imidazolinone herbicides was achieved via seed mutagenesis (Newhouse et al., 1992; Pozniak and Hucl, 2004). In maize, development of herbicide resistance via mutagenesis has also been reported (Anderson and Georgeson, 1989; Marshall et al., 1992; Newhouse et al., 1992). In soybean, chlorsulfuron resistant soybean mutants were developed via seed mutagenesis (Sebastian and Chaleff, 1987). In sunflower, an imidazolinone resistant EMS derived line has also been generated via EMS mutagenesis (Sala et al., 2008). Apart from mutagenesis, herbicide resistant crops have also been developed by means of genetic engineering as in the case of barley and tobacco (Le et al., 2005; Shimizu et al., 2008), but this method might not be appropriate to develop herbicide resistance in crops, especially in sub-Saharan Africa, where GMOs have generally not been accepted. In weed species, development of herbicide resistance is usually a result of selection resulting from intensive use of herbicides (Bernasconi et al., 1995) and many different weed species are reported to have developed resistance to various herbicides including wild sunflower to imazapyr (Al-Khatib et al., 1998), rye grass to diclofop-methyl (Christopher et al., 1992) and shattercane, the weedy relative of sorghum, to primusulfuron (Anderson et al., 1998). A survey of the literature (Powles and Holtum, 1994; Duke, 1996; Zhang et al., 2003; Ahloowalia et al., 2004; Mulwa and Mwanza, 2006) indicates that the use of herbicide resistant crops is economical and extremely useful especially in weed management suggesting that there is potential to apply the technology in managing *Striga* in Africa.

Currently, many different types of herbicides are in use and this is well illustrated by studies reporting herbicide resistance in different crops. Acetolactate synthase (ALS) inhibiting herbicides are some of the most popular herbicides (Saari et al., 1994). Their popularity stems from the fact that they are highly effective at low dose rates, are not harmful to the environment and have minimal effect on mammals (Christopher et al., 1992; Newhouse et al., 1992; Peterson et al., 2001; Yu et al., 2004). The mode of action of these herbicides is through inhibition of the enzyme acetolactate synthase (ALS), which is involved in biosynthesis of branched chain amino acids (Saari et al., 1994). There are different groups of ALS herbicides that include the sulfonylureas, imidazolinones, triazolopyrimidines (TP) and pyrimidinyl oxybenzoates (POB) (Boutsalis et al., 1999; Peterson et al., 2001).

In many cases where ALS herbicide resistance has been achieved, the mode of inheritance of the trait has normally been found to be semi-dominant (Saari et al., 1994) including in maize, (Newhouse et al., 1991), wheat (Newhouse et al., 1992; Pozniak and Hucl, 2004), Brassica (Swanson et al., 1988) and even very recently in sunflower where it was found to be inherited as a single partially dominant nuclear gene (Sala et al., 2008). However, in very few instances, it has been found to be recessive as in the case of soybeans where tolerance in all four mutant lines developed via EMS mutagenesis was found to be inherited as a single recessive gene (Sebastian and Chaleff, 1987). A genetic and inheritance study is important as it provides information on breeding of the trait, for example, when there is need for introgression of the trait into other varieties.

In sub-Saharan Africa there is very limited use of herbicides. In Kenya for example, herbicides are mainly used for high return crops like tea, coffee or in the horticulture and floriculture industry and rarely in cereals. One of the reasons is the high cost of herbicides which deters most small-scale farmers from using them. Most small-scale farmers also grow a variety of crops within the same small piece of land and use of herbicides is not feasible because apart from killing the unwanted weeds, it may also kill other crop plants being grown close to the crop of interest. Also, many herbicides like roundup are mainly non-crop selective and can only be used on crops which have tolerance or resistance genes. Generally, development of crops with herbicide resistance may offer farmers a wider choice of crops to grow as herbicides can be applied to control weeds without damage to the crop (Duke, 1996). There are very few herbicide tolerant varieties being grown in Kenya because there is very limited use of herbicides in the country. However, the development of herbicide seed coating technology for *Striga* management provides a niche for herbicide resistant crops and resultant increase in herbicide use. This low cost technology also makes herbicides affordable to the small-scale farmers in Africa.

5.2 Objectives of the study

The main objective of this study was to develop, select and characterize the tolerance to the herbicide sulfosulfuron in sorghum.

The specific objectives were:

- to compare the tolerance levels of mutants to the wild type when the herbicide was applied as a spray or as a seed coating; and
- to determine the mode of inheritance of sulfosulfuron tolerance in herbicide resistant sorghum mutants.

5.3 Hypotheses tested:

- Different concentrations of herbicide have the same effects on the different herbicide tolerant mutant lines.
- Herbicide application either as a seed coating or as a spray can be used to discriminate the different levels of resistance to herbicide for herbicide tolerant sorghum mutant lines.
- Sulfosulfuron herbicide tolerance is simply inherited.

- Different herbicide resistant mutants have different mutations for herbicide tolerance.

5.4 Materials and methods

5.4.1 Seed mutagenesis

Fifty thousand seeds of the variety *Seredo* were mutagenized using the procedure by Koornneef (2002). The sorghum seeds were soaked in water for 14h at 27^oC. Seeds were then soaked in 0.3% v/v EMS for 12h at 27^oC after which they were washed with 1M sodium hydroxide (NaOH) to neutralize the mutagen. The seeds were then rinsed thoroughly with tap water for 3h to ensure the mutagen was completely washed off and the seeds were safe for handling.

5.4.2 Herbicide used

The herbicide used to select for herbicide tolerance is a product of Monsanto, under the brand name Monitor used as a wheat selective herbicide. It is in the form of water dispersible granules with a formulation of 75% sulfosulfuron active ingredient. Sulfosulfuron is a sulfonylurea herbicide which is among the group of herbicides collectively called acetolactate synthase or ALS inhibiting herbicides as their mode of action is inhibition of the ALS biosynthesis pathway (Le et al., 2005) in the plant. The herbicide was purchased locally in Kenya from a chemical company. Monitor is applied as a selective post-emergence foliar herbicide at the recommended rate of 40g ha^{-1}.

5.4.3 Selection and generation of herbicide resistance

Mutagenised seeds were planted in the field with no fertilizer. Fertilization has been shown to increase tillering which minimizes the chances of getting seed yield by the main tiller (Koornneef, 2002). Mutagenized M_1 seed gave rise to M_1 plants and seeds harvested from the M_1 plants were M_2 seeds. Over four million M_2 seeds were drilled into plant rows. Plants arising from these seeds were the M_2 plants. After three weeks, the M_2 plants were sprayed with sulfosulfuron at 20g ha^{-1} using a knapsack sprayer. This rate was chosen as it was the half dose of the recommended rate and it was envisaged it could select for mutants with good resistance to the herbicide. Very high dose rates are capable of overwhelming even those mutants with some level of tolerance. A surfactant was added at the rate of 0.25% of the herbicide solution. Herbicide tolerant mutants that survived the herbicide treatment were advanced to M_3 generation through self pollination.

5.4.4 Experimental design and management

5.4.4.1 Greenhouse herbicide spraying assessment for herbicide tolerance

Seeds from the herbicide resistant mutants and the wild type *Seredo* were planted in flats containing a sandy loam soil from the field station at Katumani in Machakos, Kenya. The germinating plants were watered after every two days. Two weeks after planting (WAP), when the plants were in the second leaf stage, emerged seedlings were sprayed with a calibrated hand sprayer with varying concentrations of the herbicide. The concentrations used were 1, 5, 10, 20 and 40g ha^{-1} sulfosulfuron. The controls were treated with tap water mixed with a surfactant only. Two weeks after spraying (WAS), plants were harvested by cutting them at soil level and dried at 70^{o}C for 48h in the oven before weighing to determine the shoot dry weights.

5.4.4.2 Field Assessment

Mutant seed was also planted at the Kenya Agricultural Research Institute (KARI), Kiboko station in Makueni district in Kenya. Standard planting procedures and protection of seed with chemical were applied but no fertilizer was used. Three WAP, when plants were in the third leaf stage, they were sprayed with sulfosulfuron at concentrations of 1, 5, 10, 20 and 40g ha^{-1}. Spraying was done using a knapsack sprayer. Two WAS, five plants for each of the concentrations used were cut at the soil level and dried in an oven at 70^{o}C for 48h to determine shoot dry weight.

5.4.4.3 Seed coating in the field

Seed from the five herbicide resistant mutant lines and the wild type *Seredo* were coated with varying concentrations of the herbicide. The different concentrations of herbicide used were 0.5, 1, 10, 20 and 40g ha^{-1} sulfosulfuron. The lower rate of sulfosulfuron was included as herbicide seed coating, as indicated by Kanampui et al., (2003) ensures a very high concentration of herbicide around the germinating seed which may kill the germinating seed. It was, therefore, important to include a lower herbicide concentration than in the spraying experiment. The control was treated with the herbicide binder only. To bind the herbicide onto the seed, murtano, which is a seed treatment compound with insecticidal properties, was used. Murtano is a dry compound but gets sticky when water is added. A little amount of murtano was put into a small beaker and the required amount of herbicide added. The herbicide and murtano were mixed thoroughly forming sticky slurry. The required number of seeds was added and mixed into the herbicide/murtano slurry to ensure even coating for all the seeds. Seeds were dried and packed in small seed packets ready for planting. Plot sizes were 1.4m X 2.0m. The distance between rows was 0.7m while the distance between adjacent hills was 0.25m. Three WAP, the number of germinating plants was recorded for each of the different treatments.

5.4.5 Experimental design

In all the above experiments, the experiments were laid out in split-plot in randomized complete blocks design. The herbicide concentrations were the main plots and mutant lines and wild type the sub-plots. This was important especially in the field where herbicide spray drift would affect nearby plants. Spraying in the field was done early in the morning when it was not windy. In all the spraying experiments, a surfactant, agral 90, a non-ionic wetting and spreading agent was applied at the rate of 0.25% v/v of the spray mixture.

5.4.6 Tests for the mode of inheritance of herbicide tolerance and allelism

The five herbicide tolerant mutant, hb46, hb12, hb462, hb56, and hb8 were crossed with the herbicide susceptible wild-type *Seredo* by hand emasculation and pollination. Crosses were also made among the different mutant lines to investigate whether the mutant lines had different alleles or genes for resistance. The F_1 progeny were selfed to advance them to F_2 generation seed for screening of resistance. Inter-crosses between the different mutants lines were also made to investigate allelism.

All the F_2 populations from the crosses of *Seredo* X mutant, the inter-crosses between the mutants, the susceptible *Seredo* and the tolerant mutants were planted in the field at Kiboko in Makueni district. For each of the crosses, approximately 100 seeds were planted in small plots of 2m X 2m with the distance between the rows and between holes being 0.2m. Seeds were planted one in a hole. The plants were irrigated after every two to three days to ensure good plant growth. Two WAP, the plants were sprayed at the rate of 10g ha^{-1} sulfosulfuron. Using this rate, it was possible to discriminate three different mutant reactions of resistant (R), intermediate (I) and susceptible (S) (Figure 5.4). Three WAS, the plants were scored for level of damage. All plants were scored into these three phenotypes according to the level of damage. Plants that were regarded as resistant showed very minimal damage. Plants considered to have an intermediate response were stunted with some necrosis, while plants that were regarded as susceptible were killed by the herbicide.

5.4.7 Data analysis

Data were subjected to ANOVA using general linear model procedures in Genstat 2000 version 11 (Payne et al., 2007). Means were separated using the least significant difference (LSD). Shoot dry weights and number of emerged plants were expressed as a percentage of their respective controls. Mortality assessments were done using generalized non-linear probit analysis (exponential decay) where LD_{50} values were calculated as the herbicide rates required reducing dry matter or germination by 50%. For the genetic study, data of the segregation of plants into phenotypic classes was tested for goodness of fit to Mendelian segregation patterns of 1:2:1 (R:I:S) for the single gene model for the mutant X *Seredo* (wild type) crosses and for 15:1 (R:S) pattern for two gene model for the mutant X mutant crosses using the Chi-square test procedure at the 0.05 probability level using GenStat.

5.5 Results

Out of approximately four million M_2 plants sprayed with herbicide, five plants survived the herbicide treatment. These plants were designated hb46, hb12, hb462, hb56, and hb8. Each of these mutants was selfed and seed kept separately. In the next season, the seed from each of the mutants was planted in single rows. The wild type *Seredo* was also planted and the plants sprayed with sulfosulfuron at 20g ha^{-1}. All the mutants survived while all the wild type plants were killed by the herbicide (Figure 5.1). These M_3 mutant lines were also selfed, and seed kept separately. The seed harvested from these plants was M_4 seed, which was used for evaluation of herbicide tolerance by spraying and seed coating.

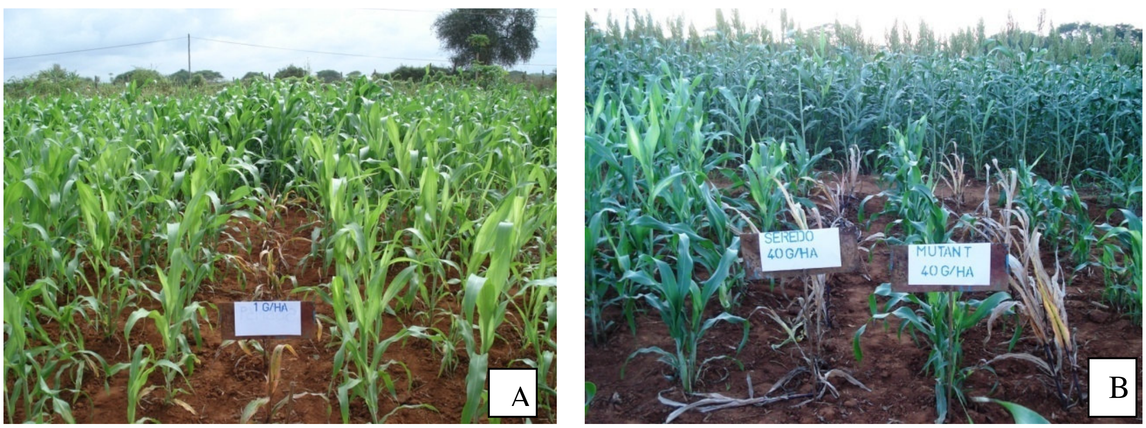

Figure 5.1: Developing sulfosulfuron resistance in sorghum. Reaction of herbicide resistant mutants and susceptible progenitor *Seredo* to herbicide sprays of (A) 1g ha^{-1} and (B) 40g ha^{-1} sulfosulfuron, three WAS. Nb. The rows with dead plants are for the wild type *Seredo*.

5.5.1 Greenhouse assessment

The herbicide concentration and variety main effects, and their interaction effects were all highly significant ($P < 0.01$) (Table 5.1) for shoot dry weight. Generally, there was reduction in percentage dry matter with an increase in the herbicide concentration for all the mutant lines and for the wild type (Table 5.2). For *Seredo*, the reduction in dry biomass was more pronounced than for all the mutant lines. For example at 1g ha^{-1}, the reduction for the mutants was between 3% and 48%, while for the wild type *Seredo*, it was 70%. Reduction in dry biomass also varied within the mutant lines with some mutant lines exhibiting more tolerance than others. At 5g ha^{-1}, biomass reduction for hb46 was

27%, while it was over 50% for hb8. At 40g ha^{-1}, biomass for the mutant line with the highest level of tolerance (hb46) was reduced by 48% and by 80% for hb8. Differential tolerance was also shown by the LD_{50} values (Table 5.3). The herbicide concentration required to decrease the dry matter of the mutant line hb46 by 50% was 16.8g ha^{-1} of sulfosulfuron, while that required to decrease the wild type *Seredo* was 1.1g ha^{-1}. The LD_{50} values for the mutant lines were also different with mutant line hb46 having the highest tolerance and hb8 the lowest. Mutant lines hb46, hb12 and hb56 were over 15 times more tolerant to the herbicide than the wild type.

Table 5.1: Analysis of variance for shoot dry weight of sorghum lines at different herbicide concentration in the greenhouse experiment

Source of variation	df	Mean squares
Replication	2	226.8
Concentration	4	16340.8**
Error (a)	8	152.5
Variety	5	2507.8**
Concentration X Variety	20	665.6**
Error (b)	50	196.2
Total	89	

** Significant at P=0.001

Table 5.2: Effect of herbicide concentration on dry matter[#] reduction in the greenhouse spraying experiment

Herbicide Rate (g ha^{-1})	Mutant/variety					
	Hb46	Hb462	Hb56	Hb8	hb12	*Seredo*
0	100	100	100	107.6	100	100
1	90	52.2	86.6	67.3	97	30
5	73.5	80.1	59.4	43.5	54.8	16.9
20	37.6	42.7	18.9	30.2	23.7	11.7
40	51.9	32.6	27.1	20.7	41.7	6.1
$LSD_{(0.05)}$	22.5					

[#]- dry matter calculated as a percentage of the control

Table 5.3: LD_{50}[#] (g ha^{-1}) values for greenhouse experiment

Mutant line	LD_{50} (g ha^{-1})	Standard error	Confidence interval lower 95%	Confidence interval upper 95%
hb46	16.8	0.5	15.7	17.8
hb12	15.6	0.5	14.7	16.6
hb462	6.5	0.5	5.6	7.4
hb56	15.3	0.7	14.0	16.5
hb8	3.9	0.5	3.0	4.8
Seredo (wild type)	1.1	0.4	0.2	1.9

[#] Herbicide concentration required to reduce dry matter by 50%

5.5.2 Herbicide spraying experiment in the field

The analysis of variance for dry matter in the field shows that the effects of concentration, variety and the interaction of variety by concentration were highly significant ($P < 0.01$) (Table 5.4). The reduction in dry matter calculated as percentage of the control for mutants and the wild type are shown in Figure 5.2. Biomass for the wild type *Seredo* was highly reduced at all the herbicide rates compared to the tolerant mutant lines. Mutant lines also differed markedly in their response to the different concentrations of herbicide. For example, the mutant line hb56 was greatly reduced in biomass up to 5g ha^{-1} but a further increase in the concentration of the herbicide resulted in more gradual decline. On the other hand hb46 showed more tolerance to the herbicide than the other mutant lines at almost all the different herbicide rates. The differential resistance of mutants was evident since the reduction rates were not constant for the mutant lines at the different herbicide rates. For example, at 20g ha^{-1}, mean reduction of biomass for hb46 was 50% while the mean reduction for the mutant line hb462 was approximately 70%. At 5g ha^{-1} the mutant line with the least reduction in biomass was hb12, and the mutant line with highest reduction was hb8. However, at 20 g ha^{-1}, the mutant lines hb462 and hb46 had the highest and lowest reduction, respectively. The differential resistance of the mutant lines was also shown by the calculations of LD_{50} (Table 5.5). The mutant line with the highest LD_{50} of 22g ha^{-1} was hb46, while the mutant line with the lowest LD_{50} was hb8 (11.8g ha^{-1}). *Seredo*, which was not resistant to the herbicide, had an LD_{50} of 0.53g ha^{-1}.

Table 5.4: Mean squares from analysis of Variance for biomass in the field

Source of variation	d.f.	Mean squares.
Replication	2	61.85
Concentration	4	14956.01**
Error (a)	8	31.51
Variety	5	2044.71**
Concentration X variety	20	426.9**
Error (b)	50	64.66
Total	89	

** Significant at P=0.001

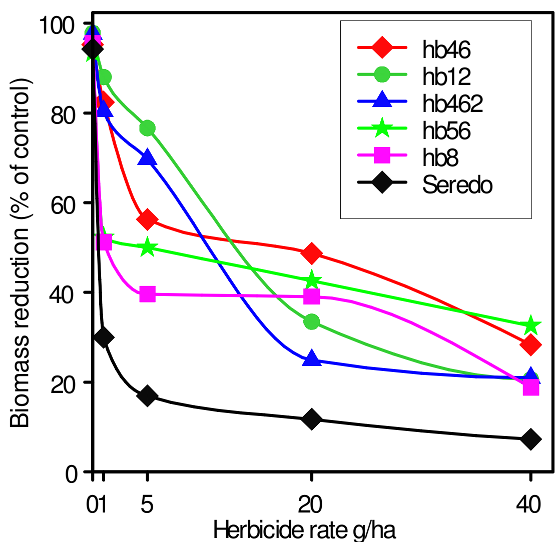

Figure 5.2: Response of mutant lines and the wild type *Seredo* to different concentrations of herbicide in the field

Table 5.5: LD_{50} values from biomass measurements in the field for spraying experiment

Mutant line/variety	LD_{50}	Standard error
hb8	11.8	0.42
hb46	22.01	0.53
hb12	20.33	0.24
hb56	17.45	0.49
hb462	17.79	0.41
Seredo (wild type)	0.53	0.17

5.5.3 Seed coating experiment in the field

No emergence was recorded in treatments where the wild type *Seredo* was coated with the herbicide (Figure 5.3). However, there was a general decline in germination percentage of mutant lines with increasing herbicide rates. At 0.5g ha^{-1} reduction in germination varied from approximately 5 to 25% while at 40g ha^{-1} it varied from approximately 85 to 95%. From the LD_{50} values for emergence (Table 5.6), the mutant line hb46 had the highest tolerance while hb8 was the most sensitive. The wild type *Seredo* had an LD_{50} value of 0.13. The general trend from the LD_{50} values for reduction in germination from herbicide seed coating was hb8 > hb462 > hb56 > hb12 > hb46.

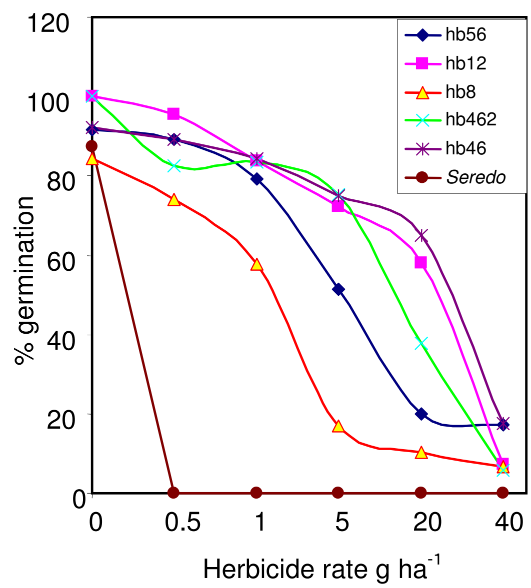

Figure 5.3: Effect of herbicide seed coating on emergence of herbicide resistant mutants and *Seredo* in the field

Table 5.6: LD_{50} values for different mutants and the wild type for plant emergence of seed coated with herbicide

Mutant line/Variety	LD_{50}	Standard error	Confidence limits	
			Lower 95%	Upper 95%
hb462	9.0	1.692	6.018	12.42
hb12	20.0	1.903	16.777	24.13
hb8	2.9	1.705	-4.028	2.35
hb56	13.2	1.847	10.09	17.13
hb46	23.1	3.054	18.41	30.1
Seredo (wild type)	0.13	0.083	0.07	0.38

5.5.4 Inheritance study

In the inheritance study the use of the herbicide rate of 10 g ha^{-1}, enabled three distinct phenotypes to be distinguished (Figure 5.4). Plants were recorded to be resistant if they showed negligible or very little damage, intermediate if they looked stunted and showed signs of necrosis, and susceptible if they were killed by the herbicide treatment. In all the cases when the spraying was done, the wild type *Seredo* and the homozygous resistant lines were also used as controls for better discrimination of the three phenotypes. Three WAS plants were scored for level of damage. The F_1 for the crosses of Mutant X *Seredo* were all intermediate in phenotype (Table 5.7). All the plants for the different mutant lines had some level of stunting and necrosis. Using the controls it was easy to discriminate for the different reactions. Even for the more tolerant hb46 it was possible to make a distinction for the three phenotypes. For the F_2 generation, the chi-square goodness of fit from observed and expected ratios of resistant, Intermediate and susceptible individuals, indicated a good fit for the 1:2:1 segregation ratio (Table 5.8). Screening of the F_2 generation of the intercrosses between the different mutant lines did not produce the expected ratio of 15:1 for resistant: susceptible (Table 5.9) for any of the crosses.

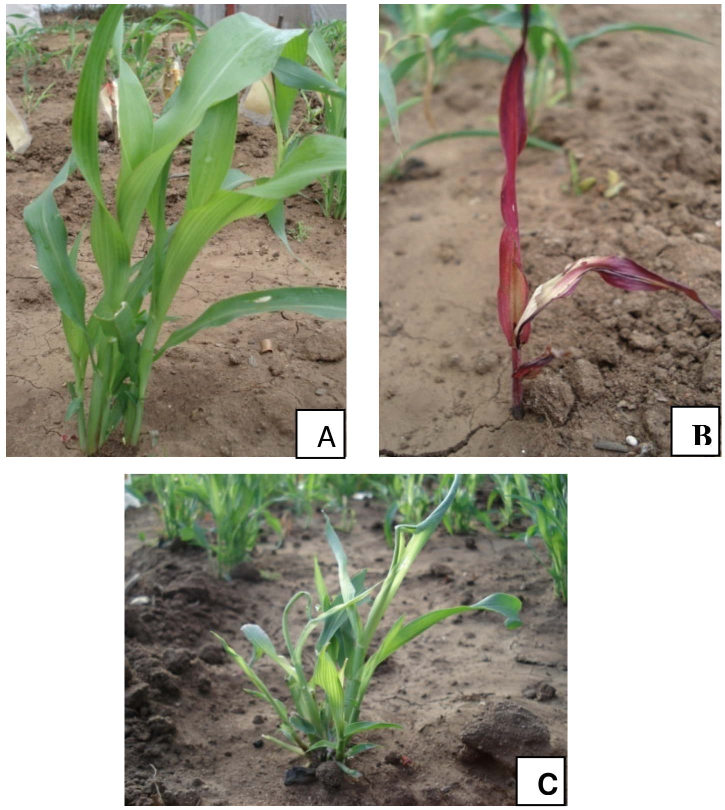

Figure 5.4: Three different reactions to herbicide sprays used to distinguish between the Resistant (A- no visible damage), susceptible (B- dead plant), and Intermediate (C- plant is stunted and shows chlorosis on leaves).

Table 5.7: Reaction of the F1 plants from crosses between the mutant lines and the susceptible *Seredo*

Cross	Total number of plants	Resistant	Intermediate	Susceptible
hb46 X *Seredo*	20	0	19	1
hb12 X *Seredo*	14	0	12	2
hb462 X *Seredo*	15	0	15	0
hb56 X *Seredo*	9	0	9	0
hb8 X *Seredo*	7	0	7	0

Table 5.8: X^2 goodness of fit for 1:2:1 segregation of the F2 generation of mutant X *Seredo*

Cross	Total plants	Resistant		Intermediate		Susceptible		X^2
		O	E	O	E	O	E	
hb46 X *Seredo*	88	20.0	22.0	44.0	44.0	24.0	22.0	0.36ns[#]
hb12 X *Seredo*	96	24.0	24.0	46.0	48.0	26.0	24.0	0.25ns
hb462 X *Seredo*	76	17.0	24.0	40.0	48.0	19.0	24.0	4.42ns
hb56 X *Seredo*	82	24.0	21.5	44.0	43.0	14.0	21.5	2.93ns
hb8 X *Seredo*	96	20.0	24.0	40.0	48.0	30.0	24.0	3.50ns

O, E= Observed and expected values for segregation, respectively
[#] ns- Indicates that the observed segregation is not significantly different from the expected segregation at the 0.05 probability level when tested by the X^2 distribution under 2 degrees of freedom. An X^2 value of 5.99 or larger would be needed to reject the 1:2:1 segregation ratio of the null hypothesis of no difference between the observed and expected ratio.

Table 5.9: The observed segregation of resistant: susceptible for intercrosses between the mutant lines.

	Second parent			
First parent	hb12	hb462	hb56	hb8
hb46	98:0	92:0	86:1	81:0
hb12		86:0	89:2	82:0
hb462			69:0	87:0
hb56				89:0

5.6 Discussion

5.6.1 Development of herbicide resistant mutant lines

The mutant lines developed in this study were shown to be tolerant to the herbicide sulfosulfuron at the rates used. In the spraying experiments in the greenhouse and in the field, the rates of herbicides required to reduce dry matter in the mutant lines was generally higher than in the wild type *Seredo*. The increased tolerance to herbicide was also evident in the seed coating experiment where the wild type *Seredo* did not emerge even for treatments where seed was coated with the lowest concentration of 0.5g ha^{-1}. There was also a decrease in plant biomass with increase in the herbicide rate.

However, the reduction in the susceptible *Seredo* was much more pronounced. The wild type *Seredo* was killed by herbicide sprays at the lowest concentration while mutant lines survived the highest concentration of 40g ha^{-1} (Figure 5.1). This led to the conclusion that improved tolerance to herbicide had been conferred on to the mutant lines through mutagenesis. This is the first known report of EMS derived sulfosulfuron tolerant sorghum mutants generated via chemical mutagenesis.

In both the greenhouse and the field spraying experiments, significant mutant by concentration interaction effects were evident (Table 5.1, 5 4). For example, at 5g ha^{-1}, the mutant line hb8 had the highest reduction of biomass among the five mutants while the mutant line hb12 had the least reduction (Figure 5.2). However, at 20g ha^{-1}, the line with the highest reduction was mutant line hb462 while the mutant line with the least reduction was hb46. The reasons why specific mutants showed different tolerance levels at different herbicide rates is not clear from this study, though it has been reported to occur in the weed species *Bidens pilosa* that is resistant to ALS-inhibiting herbicides (López-Ovejero et al., 2006). The interaction effects were probably due to inherent differential resistance of the mutant lines to the herbicide.

5.6.2 Dry matter reduction

In both the greenhouse and the field spraying experiments *Seredo* was killed at the lowest rates applied. Mutants survived the highest rate of application of 40g ha^{-1} both in the field and the greenhouse. The mutant lines and the wild type responded differently to application of sulfosulfuron as indicated by the different LD_{50} values (Table 5.3, 5.5), though for all of them there was a general decline in shoot dry matter with increasing herbicide rate. A similar trend for glyphosate resistance has been observed for Sorghum *halepense* (the weed relative of grain sorghum) (Kintzios et al., 1999). Mutant lines showed differential sensitivity to herbicide treatment in both the greenhouse and the field experiment as shown by the LD_{50} values. From the LD_{50} values in the greenhouse experiment, mutant line hb46 had the highest tolerance to sulfosulfuron. Approximately 16.8g ha^{-1} of sulfosulfuron was required to reduce dry matter by 50% for hb46, which was 15 times the resistance of the wild type that had an LD_{50} value of 1.1g ha^{-1}. In the field, the same mutant line hb46 had the highest tolerance with an LD_{50} value of 22.1g ha^{-1}. The LD_{50} for *Seredo* was 0.5g ha^{-1}. All the mutant lines recorded higher LD_{50}

values in the field than in the greenhouse probably because plants were more robust in the field than in the greenhouse where plant space is restricted. Also, the experiments in the greenhouse were more controlled and the efficacy of the herbicide was maximal whereas application in the field is prone to wind drifts thus requiring more herbicide for the same effect. From LD_{50} values in the greenhouse, it was deduced that the general trend in the level of resistance was hb46 > hb12 > hb56> hb462> hb8, while in the field, the general trend was hb46> hb12> hb462> hb56> hb8. In the greenhouse and the field experiments, the general trend was the same with mutant lines hb46 and hb8 recording the highest and the lowest tolerance levels, respectively. Since the results of the greenhouse and the field concur, it can be deduced that rating of tolerance can be done in the field or greenhouse with more or less similar results.

Mutant lines displayed different tolerance levels to herbicide as shown by the significant ($P = 0.05$) differences in biomass (Figure 5.2 and 5.3) and the LD_{50} values (Tables 5.3 and 5.5). This different sensitivity to herbicide would seem to suggest that the resistances in the different mutants are conferred by different genes or alleles. In a study on development of herbicide resistance in spring wheat using mutagenesis, resistance in four of six lines that had tolerance, was found to be allelic to an already characterized resistance gene in wheat while the resistance in the other two lines was found to be due to new genes which were designated *Imi1* and *Imi2* (Pozniak and Hucl, 2004). Also, in maize, three different reactions of maize lines generated from cell culture from the same variety were regarded to be three mutations conferring different sensitivities to the maize lines (Newhouse et al., 1991). It is possible that the five mutant lines have different mutations that are conferring different levels of resistance. Different point mutations in the ALS gene, which is the active binding site for the ALS herbicides, is thought to result in different sensitivities (Preston and Mallory Smith, 2000). However, crosses between the mutant lines did not segregate for resistance in the F_2, an indication that probably the same gene was responsible for the resistance. Therefore the different sensitivities of the mutant lines could be due to other mechanisms of tolerance in the mutants which this study was not able to establish. Different factors such as the rate of absorption of herbicide, herbicide metabolism, and translocation within the plant are all thought to have major effects on the level of tolerance to any herbicide (Newhouse et al., 1991). Though this study did not determine the mode of action of the resistance, most cases of ALS herbicide resistance have been shown to be due to an insensitive ALS where the mutation modifies the binding site of the herbicide on the ALS locus, thus rendering it ineffective. Herbicide resistance due to an insensitive ALS has been shown to occur in maize (Newhouse et al., 1991; Neuffer et al., 1997), wheat (Newhouse et al., 1992), sugarbeet (Wright et al., 1998), common sunflower (Al-Khatib et al., 1998) and even in weed species like shattercane, which is a weed relative of cultivated sorghum (Anderson et al., 1998).

5.6.3 Herbicide seed coating

No emergence of herbicide coated seed was recorded for *Seredo* in the field. Mutant lines survived the highest concentration of 40g ha^{-1} although there was a gradual decrease of emergence with increasing herbicide rate (Figure 5.3). From the LD_{50} values, the general trend in tolerance can be deduced to be hb46> hb12> hb56> hb462> hb8. As in the greenhouse and the field, the general trend is similar with hb46 recording the highest tolerance level and hb8 the lowest tolerance level. The LD_{50} for *Seredo* was 0.1g ha^{-1} while that of hb46 which had the highest tolerance level was 23.1g ha^{-1}. The mutant line was, therefore, approximately 176 times more tolerant to the herbicide than the wild type. Herbicide resistant sugarbeet lines have been shown to be between 40 to 1000 times more resistant to different ALS herbicides than the parent wild type (Wright et al., 1998). In a study to determine the level of tolerance of different biotypes of Johnson grass (*Sorghum halepense*) to different herbicides, Burke et al. (2006) recorded resistance levels of between 6 and 20 fold for the different herbicides.

5.6.4 Genetic inheritance study

All the F_1 populations resulting from crosses between *Seredo* and the mutant lines were intermediate in reaction (Table 5.7). All of them showed some level of damage and necrosis on treatment with herbicide. The indication here is that all the F_1 populations are heterozygous for tolerance to sulfosulfuron. A cross between a homozygous resistant (RR) and a susceptible genotype (rr) for a single semi-dominant gene is expected to have all the F_1 as intermediate in reaction (Rr). In this case, all the F_1 displayed an intermediate reaction and is indicative of semi-dominant inheritance. Since the resistant parent (RR) displayed increased level of resistance than the heterozygous, it can also be concluded that effect of the genes is additive. This has been reported by

Newhouse et al. (1991) in maize and Chaleff and Ray (1984) in tobacco. The expected segregation for the F_2 segregating for a single resistance gene would be 1(RR): 2(Rr): 1(rr). The heterozygous individuals are intermediate in reaction to the homozygous resistant and the susceptible. Chi-square analysis of the F_2 populations from the mutant X *Seredo* crosses showed a good fit for 1:2:1 for resistant: intermediate: susceptible (Table 5.8), which was an indication of a semi-dominant singe gene segregation. The conclusion, therefore, was that the resistance in the five different mutant lines is partially dominant. These results are similar to those reported by Pozniak and Hucl (2004) in wheat, who also concluded that the resistance mechanisms are additive owing to the fact that the heterozygous individuals display high level of resistance. Semi-dominance type of inheritance for ALS resistance has also been reported for other crops. Imidazolinone resistant mutant lines of wheat developed via mutagenesis were shown to display a semi-dominant type of reaction (Newhouse et al., 1992; Pozniak and Hucl, 2004). In Maize, resistance in three Imidazolinone resistant maize lines was also found to be inherited as a single semi-dominant allele (Newhouse et al., 1991). However, resistance to ALS herbicides has also been found to be conferred by a recessive gene as in the case of soybean where resistance of chlorsulfuron resistant mutant lines developed via mutagenesis were found to be conferred by a single recessive gene (Sebastian and Chaleff, 1987).

The selfed F_2 progenies from intercrosses between the mutant lines that were screened did not show segregation for tolerance to the herbicide. The three plants that were susceptible were most likely as a result of contamination of the seed or other mechanisms that this study was not able to explain and so they were not considered part of the ratio of for segregation. For two independent genes segregating for resistance (R_1 and R_2) the segregation ratios expected are $9(R_1\_R_2\_)$: $3(R_1\_r_2r_2)$: $3(r_1r_1R_2\_)$: $1(r_1r_1r_2r_2)$. That is, nine out of 16 individuals would have both the two dominant genes (R_1 and R_2), 3 would have at least one of the dominant genes (R_1), 3 would have at least one of the other dominant genes (R_2) and one individual would have none of the dominant genes, but instead possess all the recessive genes. Therefore, if the presence of at least one resistance gene (R) confers resistance, then the reasoning would be that one individual would be susceptible to herbicide in the F_2 treatment since it would be lacking in any of the two resistance genes giving a ratio of 15:1 for resistant: susceptible. No segregation was observed in the F2 progenies of crosses among the mutants. This was an indication that the resistance in the five lines was probably due to the same resistance gene. Different herbicide resistant mutant lines of wheat, that were developed via mutagenesis, were also found not to segregate and it was concluded that the mutations conferring the resistance were alleles at the same locus or that they were very closely linked (Newhouse et al., 1992). In the present study, it was however surprising that the mutant lines did not show segregation as they differed in their sensitivity to the herbicide which would have been explained by the fact that they were allelic or that the different tolerance levels were due to different genes. One of the explanations would be that other inherent mechanisms apart from an insensitive ALS, which is assumed in this case, may determine the level of tolerance of the different mutant lines. Whereas, resistance to the ALS herbicides has generally been shown to be conferred by an insensitive ALS (Newhouse et al.,1991; Newhouse et al., 1992; Bernasconi et al., 1995), in some species, it has been shown to be conferred by other mechanisms including increased metabolism of the herbicide (Christopher et al., 1992; Kuk et al., 2002). An analysis of the mechanism of tolerance for the different mutants was not possible in the time of this study though it is envisaged to be done in the near future.

5.7 Conclusion

- Five herbicide tolerant mutants, hb46, hb12, hb56, hb462, and hb8 were identified and isolated all carrying the same gene which behaves in a semi-dominant fashion.
- Differential sensitivity to the herbicide was detected among the mutants, but the causes of these differences could not be discerned.
- Other inherent mechanisms including herbicide translocation and metabolism may be responsible for the differential sensitivity.
- The seed coating experiment showed mutants to be up to 176 times more tolerant than the wild type parent *Seredo* and so can be utilized for herbicide seed coating of sorghum seed to test the whether the technology is effective for sorghum.
- Herbicide spraying and herbicide seed coating were shown to be effective in discriminating between the levels of tolerance for the different mutant lines with more or less similar results.

- The inheritance study provides insight into the possible breeding of the trait into other varieties.

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Chapter 6

Effect of sulfosulfuron seed coating of herbicide tolerant sorghum on *Striga* infestation in Kenya

Abstract

Herbicide seed coating of herbicide resistant maize has been found to be effective in the control of *Striga*. However, this has not been tested in sorghum. Seeds of five EMS derived sulfonylurea tolerant sorghum mutant lines (designated hb46, hb12, hb462, hb56 and hb8) and the wild type *Seredo* were coated with varying concentrations (0.5, 1, 5, 20 and 40g ha^{-1}) of the herbicide sulfosulfuron and planted in a *Striga* infested field in order to establish if herbicide seed coating was effective in reducing *Striga* parasitism. *Seredo*, the susceptible control, did not survive herbicide treatment. Mutant lines survived herbicide treatments but plant stand count and biomass were reduced with increasing herbicide rate. Seed coating significantly ($P = 0.05$) reduced *Striga* emergence with the highest herbicide rate reducing *Striga* emergence the most. Use of 0.5g ha^{-1} or 1g ha^{-1} sulfosulfuron did not reduce sorghum biomass but decreased *Striga* emergence by up to 54% at 10 WAP, and up to 47% at 12, 14 and 16 WAP. Therefore, a herbicide dose rate of between 0.5g ha^{-1} and 1g ha^{-1} would be the recommended dose for the tested sorghum mutants. Number of *Striga* plants with flowers and seed capsules was significantly lower for all treatments where seed was coated with herbicide indicating seed coating affected reproductive capacity of *Striga* that successfully parasitized the host. 1g ha^{-1} sulfosulfuron reduced *Striga* flowering and seed set by 52% and 77% at 14 WAP, respectively. Mutant lines also displayed differential herbicide tolerance and *Striga* resistance and those with better *Striga* resistance and higher herbicide tolerance would be best suited for herbicide seed coating

Keywords: sulfosulfuron, mutants, *Striga,* herbicide tolerance

6.1 Introduction

One of the most constraining factors in sorghum production in Kenya presently is the *Striga* weed. In the first chapter of this thesis, a participatory rural appraisal in a *Striga* endemic area in Western Kenya highlighted the magnitude of the *Striga* weed problem for the predominantly low income subsistence farmers. In the four villages that the *Striga* survey was carried out, the *Striga* weed menace was listed as one of the major constraints to sorghum and in general cereal farming. Control options farmers indicated they utilized varied from intercropping, handweeding, use of tolerant varieties or landraces like *Ochuti*, and in some cases even use of wood ash. However, the farmers considered most of these control options ineffective. There is need for a more comprehensive solution to *Striga*.

Striga, a root hemi-parasitic weed in the family scrophulariacea is considered the most serious biotic constraint to cereal farming and has great economic impact on the subsistence farming systems of sub-Saharan Africa (Haussmann et al., 2000b; Gurney et al., 2002; Kanampiu et al., 2003; Scholes and Press, 2008). It is parasitic on cereals including maize, sorghum and millets. These are the among the most important staple food crops in Kenya. Through root exudates stimulation, the parasite seed germinates and using a haustorial root attaches on to the host roots, where it creates a nutritional sink and draws essential plant photosynthates from the host plant (Ejeta and Butler, 1993; Berner et al., 1995; Press et al., 2002). Apart from this, the plant is also reported to increase the level of damage through phytotoxins (Gurney et al., 1995; Sinebo and Drennan, 2001). Grain yield loss is varied but in situations where infestations are high, yield losses of 100% have been recorded (Ejeta and Butler, 1993; Haussmann et al., 2000a). In Africa, estimated grain yield loss resulting from *Striga* infestations is to the tune of 4.1 million tons while the estimated area infested by *Striga* is 21 million ha (Sinebo and Drennan, 2001).

Most strategies directed towards *Striga* control have not provided tangible results for the subsistence farmer to conclusively deal with the *Striga* menace. Control options advocated include cultural methods like trap cropping, fallowing, intercropping (Berner et al., 1995; Haussmann et al., 2000a), reduction of soil seed banks, (Haussmann et al., 2000a; Ransom, 2000), chemicals, (Gworgwor et al., 2002) and tolerant or resistant varieties (Haussmann et al., 2001; Gurney et al., 2002; Gurney et al., 2003; Rich et al., 2004). Most of these methods have not fitted well with subsistence farming systems practiced in much of the African continent. Cultural control methods for example, have been shown to take too long for actual benefits to be realized, which discourages the financially constrained African farmer who is looking for a quick solution. Others like fertility enhancement and fumigation are too expensive for the small-scale farmers while resistance and tolerance is complicated by the complex and prolific nature of the *Striga* weed which has ensured that up to now, no completely tolerant varieties are found in the cereals (Gurney et al., 2002). What farmers need is a control strategy that has immediate and tangible results (Kanampiu et al., 2003) and is easy to apply within their complex system of subsistence farming (Berner et al., 1995).

Use of herbicides for *Striga* control has been known for quite a while and many studies have shown the effectiveness of herbicide sprays to kill *Striga.* However, the initial mode of application was use of post-directed sprays on growing *Striga* plants. For example, Adu-Tutu and Drennan, (1991) investigated the effect of metsulfuron on *Striga* emergence applied pre and post-emergence and found that it gave good control of *Striga hermonthica* in sorghum. However, the herbicide was found to be highly toxic to the susceptible sorghum variety resulting in little benefit of reduced *Striga* infestation. Carsky et al. (1994) have also compared *Striga* hand weeding and post directed application of herbicides and found that herbicide sprays gave good control of *Striga* as significant reduction in the number of *Striga* plants was observed. Post-emergence directed sprays of imazapyr have also been shown to delay *Striga asiatica* emergence and to give good control (Abayo et al., 1998).

Despite the good prospects of post-directed herbicide sprays in *Striga* control, the use of post-emergence herbicides has been found unsuitable owing to the fact that by the time the herbicide is applied, host damage has already occurred as most of *Striga* damage occurs while *Striga* is still under the ground (Berner et al., 1995; Kanampiu et al., 2003). It follows then, that the real benefit would be in reducing *Striga* seed bank as *Striga* plants are killed by the herbicide. While, this would be suitable for the long term, it is usually not favourable with farmers, who out of financial considerations are looking for an option with instantaneous benefits. Also, the technicalities involved in mixing the herbicide into knapsack sprayers have health, technical as well as financial implications for the farmer. Many small-scale farmers lack the necessary know-how to apply the herbicides correctly. As a remedy to these challenges, seed coating of herbicide tolerant seed was introduced. The technology which uses herbicide tolerant maize seed has been found to give season-long *Striga* control, and is affordable to small-scale farmers as very little amounts of the herbicide are required for *Striga* control. Another advantage of the technology, unlike other herbicide forms of control, is that there is no need of herbicide sprays, since the seed coating can be applied at the source where seed is packaged. Several studies have been carried out to determine suitable herbicides or to optimize herbicide rates (Berner et al., 1997; Abayo et al., 1998; Kanampiu et al., 2001; Kanampiu et al., 2003). Relatively good prospects have been reaped from herbicide seed coating technology as the herbicide coated maize seed variety, Ua Kayongo, is already available to farmers in Kenya.

Herbicide seed coating relies on the fact that the cereal crop to be coated has resistance to the herbicide. Sorghum mutants in this study were developed for sulfonylurea resistance. The sulfonylurea herbicides are some of the most widely used herbicides, with their popularity stemming from the fact that they have low application rates, broad spectrum activity against many weeds and low mammalian toxicity (Saari et al., 1994). These herbicides are in the group of herbicides that are collectively called the acetolactate synthase (ALS) inhibiting herbicides whose mode of action is inhibition of the ALS, the first enzyme in the biosynthesis of amino acids, valine, leucine and isoleucine (Saari et al., 1994). Binding of the herbicide onto the ALS enzyme stops essential biosynthesis of proteins. An insensitive ALS can be conferred on to plants through point mutations in the ALS gene which renders the plant resistant to the herbicide (Preston and Mallory-Smith, 2000). The selector herbicide for this study was the sulfonylurea herbicide sulfosulfuron. Apart from the reasons advanced above on popularity of SU herbicides, the other reason for choosing sulfosulfuron was that it was easily available in Kenya where it is mainly used for weed control in wheat.

Use of herbicide seed coating can thus only be utilized if the crop of interest has resistance to the herbicide. In maize, this has been made possible through the development of Imidazolinone resistant (IR) maize which has acetolactate synthase (ALS) target-site resistance (Berner et al., 1997; Abayo et al., 1998; Kanampiu et al., 2003). In Africa, there are no sorghum herbicide resistant varieties that have been developed, which has excluded the testing of this technology in the crop. However, this has been made possible with the development of herbicide tolerant sorghum mutants in this study. Development of ALS resistance in sorghum in this study was accomplished through ethyl methane sulfonate (EMS) mutagenesis which has been known to cause point mutations in the ALS gene (Pozniak and Hucl, 2004; Sala et al., 2008). This study, therefore explored the possibilities of utilizing herbicide seed coating of herbicide tolerant sorghum to protect sorghum against the parasitic weed *Striga*. There are no other documented studies on using herbicide seed coating for herbicide tolerant sorghum for *Striga* control.

6.2 Objectives

The objectives of this study were to determine the effects of sulfosulfuron coated seed of herbicide tolerant sorghum on *Striga* infestation and to determine the effects of different herbicidal concentrations on *Striga* emergence, *Striga* reproductive capacity, and sorghum biomass and plant stand.

6.3 Hypotheses of the study

- Sulfosulfuron seed coating of herbicide tolerant sorghum mutants is effective in the control of *Striga hermonthica* infestation.
- Different concentrations of the herbicide have similar effects on the *Striga* weed and on sorghum host.

6.4 Materials and methods

6.4.1 Herbicide tolerant Mutant lines

Five different herbicide tolerant sorghum mutant lines were used in this study. The mutant lines designated hb46, hb12, hb462, hb56 and hb8 were at M_4 generation obtained via ethyl methane sulfonate (EMS) mutagenesis of the sorghum variety, *Seredo*. This is an improved variety popular in the sorghum growing areas of Kenya, where it is recommended for its earliness and high yield. The variety is highly susceptible to *Striga* infestation. Mutant lines were generally found to display the same phenotype as the wild type apart from herbicide resistance. A study on herbicide tolerance for the mutants (Previous chapter), indicated that mutant lines had varying tolerance levels to sulfosulfuron. At the M_4 generation the resistance in all the mutant lines was found to be relatively stable.

6.4.2 Herbicide

The herbicide used in this study was sulfosulfuron, which is a sulfonylurea (SU) herbicide. The mode of action of SU herbicides is the inhibition of acetolactate synthase (ALS), the first enzyme that catalyses the biosynthesis of branched-chain amino acids, valine, leucine and isoleucine (Brown, 1990). In Kenya, the herbicide is registered under the brand name Monitor, and is used as a selective herbicide in wheat.

6.4.3 Experimental design, seed coating and growing conditions

Seed from the different herbicide resistant mutant lines and the parent *Seredo* were coated with different concentrations of the herbicide viz, 0.5, 1, 5, 20, and 40g ha^{-1} of sulfosulfuron. As indicated in the preceding chapter, murtano, a commercial seed dressing compound was used to bind the herbicide on to the seed. The control was seed coated with murtano only with no herbicide. To determine if the seed binding chemical had any effect, a treatment where the seeds were not coated with murtano or herbicide was also included. The experiment was set up as a split plot in randomized complete blocks design with herbicide concentrations as the main plot and the mutant lines and the variety as the sub-plots. The seed for the different treatments were planted in plots of size 2.1m X 2.0m. The distance between adjacent rows was 0.7m and the distance between the planting holes was 0.2m. There were three rows in a plot but the third row in each plot was not planted so as to preclude interference from adjacent plots. This design ensures that no land is lost to border rows and also minimizes plot interference which is important to preclude shading which has been shown to have an effect on *Striga* emergence (Haussmann et al., 2000a). All treatments were replicated three times.

Artificial infestation of *Striga* seed was done to improve precision of *Striga* counts as *Striga* density has been found to be highly erratic in most fields (Haussmann et al., 2000a). A known number of *Striga hermonthica* seeds were mixed with finely sieved sand and using measured scoops, this *Striga* seed/sand mixture was mixed within the top 15cm in the planting hole to give an infection density of 2000 seeds per planting hole. This inoculation is recommended to ensure uniform infestation levels (Haussmann et al., 2000a).

Planting was done using a hoe. Fertilizer was applied at the recommended rate of 50Kg ha^{-1} of di-ammonium phosphate (DAP) at planting. To protect the seed from insect damage, an insecticide, carbofuran, was applied at planting at the rate of 1g per planting hole. Three weeks after planting (WAP), plots received nitrogen in the form of calcium ammonium nitrate (CAN) (26% N) at the rate of 18kg N ha^{-1}.

6.4.4 Experiment management

Normal weeding using a hoe was done after every two weeks for the first six WAP. After this, there were three more weeding sessions at the 8^{th}, 10^{th} and 12^{th} week, which were done by hand so as not to interfere with any germinating *Striga* plants. All weeds except *Striga* were removed. Insecticide sprays, when necessary, were carried out in order to protect the growing plants from sorghum shoot fly. Supplementary irrigation was applied when necessary throughout the duration of the trial.

6.4.5 *Striga* counts and reproductive capacity of *Striga* measurements

Striga counts were initiated as from the 8^{th} WAP and continued at two weeks intervals until the 16^{th} WAP. Counts were done on the two rows of the plot. At 14 WAP, which was the peak of *Striga* emergence in the field, the number of *Striga* plants with flowers and with seed capsules was also recorded to determine the effect of herbicide seed coating on the reproductive capacity of *Striga*.

6.4.6 Other measurements

The number of sorghum plants per plot was also recorded at three WAP so as to determine the effect of herbicide seed coating on host plant emergence. There was no yield data as the experiment was conducted off season and sorghum midge damage was extensive despite use of insecticides. This resulted in very poor seed set. Instead, plant biomass data was taken at harvest time. In each of the plots the plants were cut at the soil level and weighed. Plants were then dried at 70°C for approximately 48 h or until they were completely dry and the above-ground biomass data recorded.

6.4.7 Data analysis

Treatment effects (the response to varying rates of herbicides) were analysed using a general analysis of variance procedure in Gensat version 11. Regression analysis using the general linear models procedure was also carried out to detect the relationship between herbicide concentration and *Striga* density. Means were separated using the least significant difference (LSD) test procedure at the 5% level of significance.

6.5 Results

Seredo, which does not have resistance to sulfosulfuron, did not emerge in any of the treatments where it was coated with the herbicide and it was, therefore, not included in the analysis. The effect of murtano, the binding agent was found to be non-significant (data not shown) from the control where seed was not coated and, therefore, the data for murtano-only and no-murtano treatments were combined.

6.5.1 Effect of herbicide seed coating on sorghum

The effects of the herbicide concentration and the mutant line were highly significant ($P < 0.001$) for sorghum plant stand and biomass (Table 6.1). There was decreased sorghum plant emergence and biomass yield with increasing herbicide concentration (Table 6.2). Plant density and biomass yield were not significantly different for the control and the rates of 0.5g ha^{-1} and 1g ha^{-1}. However, both biomass and plant density were significantly lower at 5g ha^{-1} than at 0.5g ha^{-1} and 1g ha^{-1} herbicide rate. Biomass yield and plant density were severely reduced at 40g ha^{-1} herbicide concentration. Plant emergence and biomass yield was also significantly ($P=0.05$) different for different mutant lines (Table 6.3). The mutant line hb46 had the highest plant density and plant biomass. Plant densities for hb46, and hb12 were not significantly different. However, hb56, hb462 and hb8 all had significantly lower plant densities than hb46. Plant biomass yield for all the mutant lines were not different except for hb8 which had significantly lower biomass yield than the rest.

Table 6.1: Mean squares for the number of sorghum plants per plot and above ground dried biomass and *Striga* emergence

		Sorghum		*Striga* emergence			
				Weeks			
Source of Variation	d.f.	Plants plot^{-1}	Biomass (kg plot^{-1})	10	12	14	16
Replication	2	7.66	0.19	45.22	40.9	16.31	120
Concentration	5	313.35**	2.26**	90.74*	389.17*	1085.62**	933.1**
Error (a)	10	6.46	0.09	11.09	42.08	89.24	96.7
Mutant	4	125.44**	0.7201**	10.04	53.44*	198.71*	215*
Concentration X Mutant	20	17.23	0.16	5.41	23.3	67.23	58
Error (b)	48	8.78	0.15	4.69	14.39	61.1	42.3
Total	89						

*,**: Significant at P=0.05 and 0.001, respectively

Table 6.2: Effect of herbicide concentration on sorghum plant density and biomass yield

	#Sorghum		*Striga* density plot^{-1}			
			Weeks			
Herbicide rate (g ha^{-1})	Plant Stand	Biomass (kg plot^{-1})	10	12	14	16
0.0	12.3	1.15	5.50	12.50	22.20	22.20
0.5	10.6	0.99	2.53	9.47	18.87	13.50
1.0	12.6	1.13	3.07	6.60	13.80	13.70
5.0	6.13	0.71	1.31	3.83	9.36	11.50
20.0	7.07	0.69	0.14	3.02	5.64	7.80
40.0	1.33	0.19	0.26	0.91	1.15	2.40
LSD	1.79	0.21	2.34	4.57	6.65	6.93
C.V.	25.6	29.1	31.6	36.1	28.2	32.7

per plot. (Plot size=2.1m X 2.0m)

Table 6.3: Plant emergence and biomass yields for the different mutant lines

Mutant line	Number of plants $plot^{-1}$	Biomass yield (Kg $plot^{-1}$)
hb56	7.9	0.83
hb46	11.29	1.07
hb8	5.19	0.58
hb462	9.0	0.83
hb12	10.76	0.98
$LSD_{(0.05)}$	1.8	0.24
C.V. (%)	21	18

6.5.2 ***Striga*** **plant density**

The effect of herbicide concentration on *Striga* plant density at 12, 14, and 16 WAP was highly significant ($P < 0.001$) while at 10 WAP it was significant ($P = 0.05$) (Table 6.1). There were also significant ($P = 0.05$) differences between mutants effect on *Striga* emergence at 12, 14, and 16 WAP. Generally, there was a decline in the number of *Striga* plants that emerged with increasing herbicide concentration for all the record dates (Table 6.2, Figure 6.1)). At 10 WAP, *Striga* counts in all the treatments where sorghum seed was coated with herbicide were significantly lower than the controls. *Striga* density at the herbicide concentration of 1g ha^{-1} and above was significantly lower than the control at 12 and 14 WAP, but not different for herbicide concentration of 0.5g ha^{-1}. *Striga* counts at 16 WAP were significantly lower for all treatments where seed was coated with the herbicide compared to the control. Regression of herbicide rate versus *Striga* density at 14WAP, which was the *Striga* emergence peak, indicated a gradual decline in the number of *Striga* plants with increasing herbicide rate (Figure 6.1).

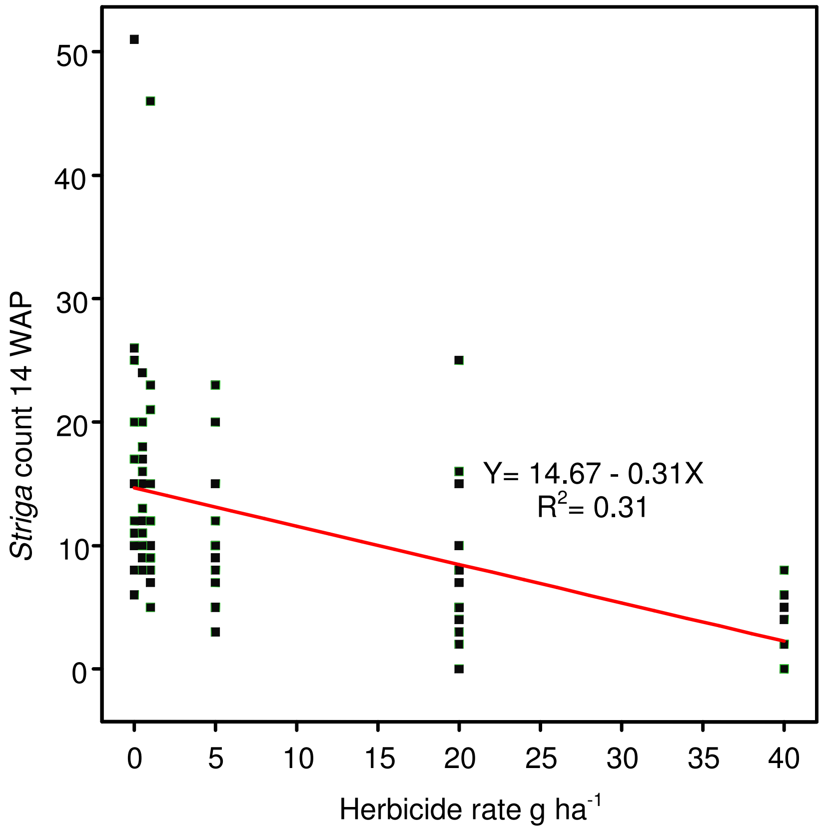

Figure 6.1. Effect of herbicide rate on the *Striga* plant density in the field 14 WAP in Kibos Kisumu

There were significant differences among mutant lines for *Striga* emergence at 12, 14 and 16WAP, but not at 10 WAP (Table 6.1). The mutant line hb12 had the highest number of *Striga* plants at 12, 14 and 16WAP (Table.6.4). However, at 16 WAP, all the other mutant lines had significantly lower *Striga* emergence than hb12. At 12 and 14 WAP, only the mutant line hb8 had significantly lower number of *Striga* plants than hb12.

Table 6.4. The effect of variety on above ground *Striga* density

	Weeks		
Mutant line	12	14	16
hb56	8.43	14.38	13.50
hb46	6.61	12.38	12.80
hb8	4.74	8.18	9.80
Hb462	6.47	12.73	11.90
hb12	8.62	16.52	18.50
LSD	2.36	4.86	4.05
C.V. (%)	14.6	18.3	21.2

6.5.3 *Striga* reproduction

The effect of herbicide concentration was highly significant (P<0.001) for number of *Striga* plants with flowers and seed capsules (Table 6.5). Figure 6.2 shows the reduction in number of *Striga* and with flowers in untreated and using 20g ha^{-1} seed coating. The effect of mutant line on both *Striga* flowering and *Striga* plants with capsules was not significant. All treatments where herbicide was used had lower percentage of *Striga* plants that flowered and with seed capsules at 14WAP (Figure 6.3). Generally, there was a decline in the percentage of flowering *Striga* plants and with capsules with increasing herbicide rate. The herbicide concentration of 40g ha^{-1} lowered the percentage of flowering *Striga* plants by approximately 90%. The herbicide concentration of 0.5g ha^{-1} lowered the percentage of flowering *Striga* plants by approximately 37.5%. The same trend was also evident for the number of plants with *Striga* capsules. Figure 6.4 shows how herbicide seed coating delayed the emergence of *Striga*. At 10 WAP, emerged *Striga* plants in the control were approximately seven plants and almost zero for the herbicide rate of 40g ha^{-1}. Generally, increasing the herbicide rate also increased the delay in emergence of *Striga*.

Table 6.5: Mean squares for *Striga* plants with flowers and capsules in the field

Source of variation	d.f.	% Plants with	
		flowers	capsules
Replication	2	12.5	67.5
Concentration	5	897.2**	783.1**
Residual	10	112.3	148.2
Mutant	4	34.6	58.0
Concentration X Mutant	20	25.1	45.6
Residual	48	23.6	41.4
Total	89		

**: Significant at P=0.001

A B

Figure 6.2: Effect of herbicide seed coating on *Striga* emergence and flowering in the field in Kibos, Kenya. A, control-mutant line with no herbicide seed coating showing flowering *Striga*, and B- the same mutant line that was coated with 20g ha^{-1} herbicide, with no *Striga*.

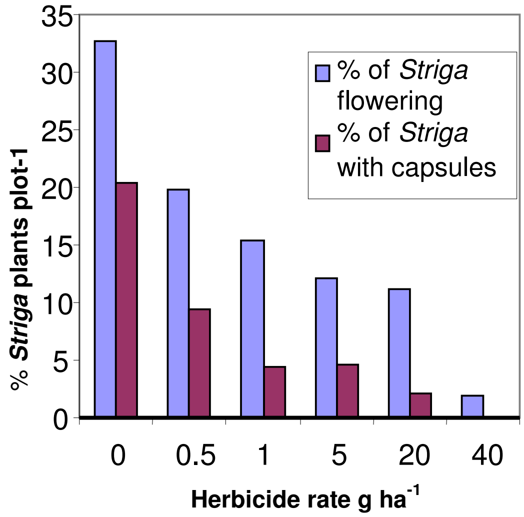

Figure 6.3: Effect of herbicide seed coating on *Striga* reproductive capacity 14 WAP in Kibos Kenya.

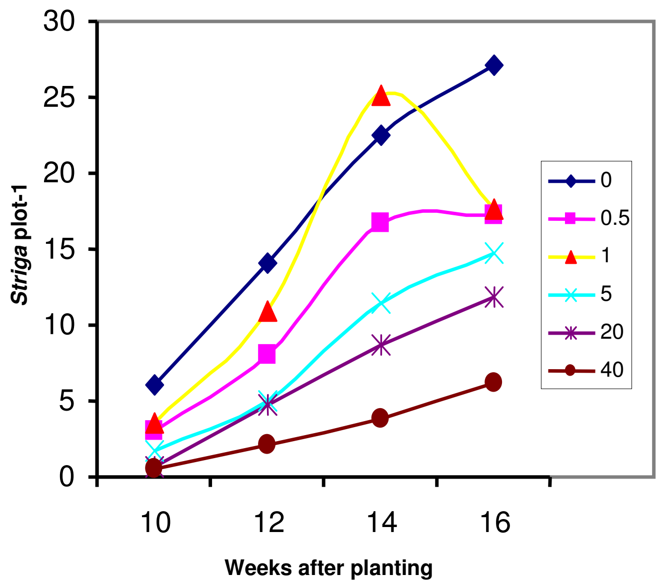

Figure 6.4: Delay of *Striga* emergence by herbicide seed coating in Kibos, Kisumu.

6.6 Discussion

The main objective of this study was to determine the effectiveness of using herbicide seed coating on herbicide tolerant sorghum seed for protection against *Striga*. Apart from the effect of the herbicide on *Striga* infestation it was also important to determine the effect herbicide seed coating had on the biomass yield of the crop plants or mutants which is important to detect phytotoxicity effects on the mutant lines. Generally, there was a corresponding decrease in plant stand and biomass with increasing herbicide rate. At 40g ha^{-1}, plant biomass and plant stand were severely reduced (Table 6.2). Plant biomass for example at the rate of 40g ha^{-1} was reduced by 83% compared to the control, while plant stand was decreased by 89%. However, sorghum plant stand and biomass were not different between the control and herbicide rates of 0.5g ha^{-1} and 1g ha^{-1}. Generally, reduced plant stand and biomass is probably due to phytotoxicity effects of herbicide, because although the mutants display increased tolerance to the herbicide as compared to the wild type, they are not completely resistant and very high herbicide rates would also probably kill them. However, different mutant lines also displayed different tolerance levels to the herbicide (Table.6.3) which had also been confirmed from the previous chapter (see chapter 5). From the biomass yield data, mutant line hb46 had the highest tolerance as it was least affected by the herbicide, while hb8 had the lowest tolerance to the herbicide. Though not much research has been carried out on these recently developed herbicide tolerant mutants, a possible reason for the differential sensitivities of the mutant lines is thought to be differential metabolism of herbicide as the mutation was found to be the same in all the five mutants (Chapter 5).

Generally for all the mutant lines, phytotoxicity was evident from the stand count and plant biomass. There was a general trend of decreasing sorghum stand and biomass with increasing herbicide rate. This effect of herbicide phytotoxicity is a challenge as it means that while the technology of coating with herbicide may be important for decreasing *Striga* infestation, the resultant decrease in plant biomass may outweigh its benefits and a proper optimization of herbicide dose rate, sorghum biomass or yield and *Striga* infestation reduction should be done to ensure the most effective herbicide dose rate is utilized. Abayo et al. (1998) have also found some ALS-inhibiting herbicides like sulfometuron to be phytotoxic to imidazolinone resistant (IR) maize lines resulting in low maize yields. Herbicide phytotoxicity effects have also been reported by Kanampiu et al. (2003) on IR maize in instances when there is very little moisture in the soil leading to high concentration of herbicide around the germinating seed plant. As a remedy to the problem of phytotoxicity, the authors recommended the use of slow release or controlled formulations of herbicides which allow for use of lower doses thus reducing phytotoxicity, while also extending the protective period of the herbicide on *Striga* infestation. The herbicide resistant lines in this study have only recently been developed and are targeted for further improvement mainly to increase the level of tolerance so that more effective dosage rates of the herbicide can be used without damage to the crop. However, since mutant lines also displayed differences in tolerance levels to herbicide, then at the moment the mutant lines with the least reduction of biomass should be utilized for herbicide seed coating as more mutants with increased herbicide tolerance are sought. The mutant lines were found to have the same mutation, but there is need for further investigation into the other mechanisms that enhance tolerance in some of the mutant lines including factors such as absorption, metabolism and translocation of herbicide, all which are thought to influence herbicide tolerance (Newhouse et al., 1991).

Herbicide effect on *Striga* density was highly significant and a general decrease in above ground *Striga* density with increasing herbicide concentration was evident (Table 6.2, Figure 6.1). Effect of herbicide rate on *Striga* emergence was highly significant at 12, 14, and 16 WAP and significant for 10 WAP, mainly because there were less *Striga* plants that had emerged by the 10 WAP, and the effect became more significant as *Striga* plant emergence increased. However, on all the dates that *Striga* emergence data was recorded, there were more *Striga* plants in the control plots than in the plots where seed was coated with the herbicide. Other studies involving maize (Abayo et al., 1998; Kanampiu et al., 2001; Kanampiu et al., 2003) have also shown that herbicide seed coating reduces *Striga* density in herbicide treated plots. Though, there was also a decrease in sorghum plant biomass (Table 6.2), which may have contributed to reduced number of *Striga* counts from fewer roots to parasitize, the effect of herbicide seed treatment was still discernable. For example, at 10 WAP, there were significantly less number of emerged *Striga* plants in treatments where 0.5g ha^{-1} and 1g ha^{-1} herbicide rates were applied compared to the control, but the biomass decrease for the two rates was not significantly different from the control treatment. Likewise, at 12 and

14WAP, *Striga* density was lower by 47% and 37% respectively in the treatment that received 1g ha^{-1} herbicide seed treatment, while the corresponding biomass yield was not different between this rate and the control. Again at the 16 WAP, *Striga* density was significantly lower in all treatments where herbicide was applied but the biomass reduction for herbicide treatments with 0.5g ha^{-1} or 1g ha^{-1} were not different from the control. However, the reduced *Striga* density at 40g ha^{-1} may also have been excercabated by the reduced biomass yield. Though decreased biomass yield with increasing herbicide rates have also been shown to occur (Abayo et al., 1998; Kanampiu et al., 2003) a major challenge in fully developing this technology in sorghum will be to increase the level of tolerance in the herbicide resistant material so that sufficiently high herbicide dosage rates could be utilized without a corresponding damage to the crop. However, from the data presented here use of 1g ha^{-1} sulfosulfuron does not lead to decreased biomass yield, but decreases *Striga* density by approximately 40% which in itself is a good indication of the effectiveness of the technology to control *Striga*. Also, the reduced infestation means there is reduced replenishment of *Striga* seed bank in the soil. One of the priorities for *Striga* control is the reduction in *Striga* seed replenishment and it has been mentioned that a comprehensive control package must include an option that reduces *Striga* seed banks (Haussmann et al., 2000a).The low amounts of herbicide required for *Striga* emergence reduction are an indication of the efficacy of the sulfonylurea herbicides. However, this study was not able to determine the yield benefit accruing from use of the technology as there was no yield data, which would have given a good overall quantification of the benefits of the technology in sorghum.

The effect of different mutant lines was also significant for *Striga* emergence. This effect was probably not related to the dry matter yield of the mutants as mutant line hb12 had the highest number of *Striga* plants for all the dates that *Striga* counts were recorded (Table 6.4) but it was not the mutant line with the highest biomass yield (Table 6.3). At 16 WAP for example, all the mutant lines had significantly lower numbers of emerged *Striga* plants than hb12, but only mutant line hb8 had a lower biomass. From the foregoing, it may be inferred the mutant lines have inherent *Striga* resistance differences. Further studies under no herbicide application would be helpful in determining if some of the mutants have *Striga* resistance. A combination of herbicide seed coating and inherent *Striga* resistance would greatly improve *Striga* management.

Overall *Striga* emergence in this study was low, despite artificially infesting the field. *Striga* is known to be very erratic in emergence with high variability between the seasons. This is one of the major challenges that make field evaluation for *Striga* difficult. This variability in space and time can be substantial and has been reported in various studies (Efron, 1993; Haussmann et al., 2000a). Some of the reasons for observed variability include non-uniformity of soil fertility within the plots and differences in *Striga* base level before infestation (Haussmann et al., 2000a). In this study the experiment was conducted off-season and *Striga* dormancy in the soil may have contributed to the low *Striga* emergence.

The effect of herbicide concentration was significant for both *Striga* plants with flowers and with capsules (Table 6.5). The percentage of *Striga* plants with flowers and with seed capsules was significantly lower in the treatments where the herbicide seed coating was applied than in the control (Figure 6.3). Generally, there was a decline in the percentage of flowering *Striga* plants and those forming seed capsules with increasing herbicide rate. The percentage of flowering *Striga* plants was not significantly different among treatments that had herbicide, except for the rate of 40g ha^{-1}, which was probably caused by the very low numbers of *Striga* plants in this treatment. Lower numbers of *Striga* plants with flowers and seed capsules were recorded in all treatments where sorghum seed was coated with herbicide. Abayo et al. (1998) also found that imazapyr, chlorsulfuron and sulfometuron herbicides greatly reduced the number of *Striga* seed capsules. The implications of reduced *Striga* reproductive capacity are exciting because of concomitant decrease in *Striga* seed replenishment into the soil. It seems then, that even when *Striga* plants are successful in parasitizing the sorghum plant, the translocated herbicide in the plant affects their reproductive capacity. This effect would probably be enhanced if the sorghum seed was able to carry more herbicide and if the inherent tolerance level of the sorghum mutants was increased to allow of higher herbicide dosage rates. Generally, the benefit of seed coating would be two fold - reducing the number of *Striga* plants that emerge and reducing the number of new *Striga* seeds introduced into the soil. *Striga* has been very difficult to control owing to the prolific nature in which it multiplies. One *Striga* plant has the capacity to produce 40,000 and 90,000 seeds (Ejeta and Butler, 1993) though other studies put it at relatively higher numbers. These extremely high *Striga* seed numbers in the soils are thought to contribute to the failure of plant host resistance to make any meaningful impact as even tolerant varieties are overwhelmed by high *Striga* seed densities (Kanampiu et al., 2003). Any comprehensive *Striga* control strategy should always involve those options that reduce the *Striga* seed banks.

Figure 6.4 shows the effect of delayed *Striga* emergence treatments when seeds were coated with herbicide. At 10 WAP, there were very few *Striga* plants that had emerged in the treatment where 40g ha^{-1} herbicide concentration was used while at the same time there were on average more than five *Striga* plants in the control plots. Although the current numbers of *Striga* were low in this study, in seasons where the *Striga* infestation is high, this delay in *Striga* emergence, which is probably an indication of delayed attachment, may also delay parasitic effects on the host crop. The delaying in the time of *Striga* attachment has been shown to increase yield considerably (Abayo et al., 1998). The ALS herbicides imazapyr, chlorsulfuron and sulfometuron have been found to delay *Striga* attachment by three to four weeks (Abayo et al., 1998). A Significant decrease in *Striga* emergence and an increase in yield by delaying *Striga* attachment has also been shown in sorghum seedlings transplanted from *Striga* free nurseries (Berner et al., 1995). This study has shown the possibility of herbicide seed treatments in sorghum to delay *Striga* emergence. However, a more comprehensive study is needed to optimize the best herbicide rate that increases the time between planting and the time of *Striga* emergence.

6.7 Conclusions

This study has effectively shown that coating herbicide tolerant seed with the sulfonylurea herbicide sulfosulfuron can effectively reduce *Striga* infestation in sorghum fields.

- Coating herbicide tolerant sorghum seed with herbicide decreased *Striga* infestation.
- Herbicide seed coating reduced *Striga* reproductive capacity which was indicated by the lower number of flowering *Striga* plants and those with seed capsules in treatments where herbicide was used.
- Phytotoxicity effects of herbicide on crop host were, however, evident as shown by reduced sorghum biomass and plant stand. Mutants displayed differential tolerance to the herbicide with mutant line hb46 being the most tolerant.

- Mutant lines displayed differential *Striga* tolerance with the mutant line hb8 having the least number of *Striga* plants among the five mutant lines.
- The most effective dosage rate was 1g ha^{-1} sulfosulfuron as it decreased *Striga* infestation by 47% without concomitant decrease in sorghum biomass yield. However, a more thorough optimization with the mutants with the highest level of herbicide tolerance and higher *Striga* tolerance is recommended.
- Herbicide seed coating technology can reduce *Striga* damage in sorghum *Striga* seed bank replenishment in the soil.

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Chapter 7
General overview

7.1 Introduction

Sorghum is the second most important cereal crop in Kenya today. Despite its relative importance especially for the semi-arid zones of the country, production is heavily constrained by the *Striga* weed menace among other challenges. No comprehensive control method is available to manage *Striga,* but herbicide seed coating has been shown to offer season long protection against the weed in maize. Therefore, the main objective of this study was to develop, through ethyl methane sulfonate mutagenesis, herbicide resistant and other valuable mutants of sorghum for protection against *Striga* and for general improvement of the crop.

7.2 The specific objectives were:

- to determine the effect of *Striga* and other constraints on sorghum production in two, rural, sorghum growing, *Striga* endemic, districts of western Kenya;
- to develop an effective mutagenesis protocol for effective mutagenesis in sorghum;
- to evaluate EMS derived sorghum mutants for important agronomic characteristics;
- to develop sulfosulfuron herbicide resistance in sorghum and
- to determine the effect of herbicide seed coating in sorghum on *Striga* infestation in the field.

7.3 Summary of research findings

7.3.1 Literature review

The literature review established that:

- Sorghum is an important cereal crop in Kenya, especially, for the arid and semi-arid zones, and the *Striga* endemic districts of western Kenya and coast province.

- The *Striga* weed is one of the major challenges requiring attention. A novel control option for the protection of sorghum against *Striga* would be coating seed with acetolactate synthase inhibiting herbicides, which are able to preclude *Striga* attachment on to roots of the host crop.
- Mutation breeding, specifically, EMS mutagenesis, could be used for development of herbicide resistant mutants and other mutants of agronomic value in sorghum. However, mutagenesis optimization is important before embarking on a mutation breeding programme.
- The genetics and inheritance of herbicide resistance are important for breeding of the trait.

7.3.2 Significance and impact of *Striga* and other constraints on sorghum production

The PRA study established that:

- The local landraces like *Ochuti*, *Nyakabala* and Jowi Jamuomo were grown by more farmers than the improved varieties *Serena* and *Seredo*.
- Reasons for choosing local varieties were drought and *Striga* tolerance, resistance to bird damage and storage pests, and reliability under adverse conditions.
- Important constraints to sorghum production in the two districts included *Striga*, drought and unpredictable environmental conditions, storage pests, long distances to agricultural commodities markets for seeds and fertilizer, and socio economic constraints like poverty and poor infrastructure.
- The most important characteristics farmers wanted in new varieties were *Striga* resistance/tolerance, drought tolerance, resistance to storage pests, early maturity, good taste and resistance to bird damage.
- Farmers did not consider cultural methods of *Striga* control as effective as chemicals or inorganic fertilizers
- Availability and cost of a control option are important if farmers are to adopt a control option and therefore it was concluded that herbicide seed coating in sorghum would be easily adopted as it was cheap and effective.

7.3.3 Development of an EMS mutagenesis protocol and genetic variability enhancement

- The LD_{50} (mutagen dose required to reduce a growth parameter by 50%) was used to determine the concentration of EMS to employ for effective mutagenesis in sorghum. The LD_{50} based on shoot length reduction was 0.35% and 0.4% for *Seredo* and *Kari/mtama-1*, respectively, for 6h exposure time, while highest mutation frequency was induced at 0.3% EMS for 6h.
- Ethyl methane sulfonate mutagenesis was also found to be effective in generating variability for panicle length and width, panicle and seed weight, and for head architecture. However, varietal and exposure time differences were evident for variation in certain characteristics necessitating genotype optimization for some of these traits.
- Generally, the protocol should be adequate for sorghum EMS mutagenesis and is available for sorghum mutation breeding programmes. Mutants generated in this study will be available to breeders for sorghum improvement for important farmer preferred characteristics.

7.3.4 Agronomic performance of EMS derived sorghum lines

- There were significant effects of mutants on yield, 1000-seed weight and rating of different morphological characteristics like head exertion and plant height uniformity.
- Nine mutant lines had higher yield than the wild type *Seredo*. Relative yield of the mutant line “SB2M13” was 160% relative to the wild type, while eight other mutant lines had yields of between 100% and 147% relative to the wild type.
- Mutant line “tag27” had the highest 1000-seed weight which was 133% relative to the wild type *Seredo* but was the lowest yielding.
- The rating for head architecture, plant height uniformity, head exertion and desirability indicated that six of the ten highest yielding mutant lines were also superior to the wild type for scores on these characteristics.
- The high yielding mutant lines may be released as direct mutants after multilocational trails while the other mutant lines will serve as breeding material

for important traits like large seeds, loose panicle, head exertion, height uniformity, and overall desirability.

7.3.5 Development, evaluation and genetic characterization of resistance to Sulfonylurea herbicide in sorghum

- Resistance, was developed by screening, over four million M_2 plants derived from the variety *Seredo*, using the ALS inhibiting herbicide sulfosulfuron., Five mutants (hb46, hb12, hb56, hb462, hb8) were confirmed to be tolerant to the herbicide.
- Seed coating and spraying experiments indicated mutants to be between five and 170 fold more resistant than the wild type, which meant they could be used for testing the herbicide seed coating technology which requires a high level of tolerance to the herbicide.
- Crosses between the mutant lines and the wild type indicated an inheritance pattern consistent with a semi-dominant gene which confirmed the findings of many other studies that have also mainly reported on ALS resistance being conferred by a single semi-dominant gene.
- The mutant lines exhibited differential sensitivity to the herbicide. A genetic study to determine allelism did not come up with the expected segregation of 15:1 for resistant: susceptible, indicating that the resistance in the five lines was probably due to the same gene mutation. The differential sensitivity of the mutants was probably caused by other inherent mechanisms like herbicide metabolism or translocation.

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7.3.6 Herbicide seed coating of herbicide tolerant sorghum for protection against *Striga*

- There were significant herbicide concentration effects with increasing herbicide rate resulting in reduced *Striga* density.
- Though increase in herbicide concentration also resulted in concomitant decrease in sorghum plant stand and biomass, there was evidence that herbicide effect was effective in reducing *Striga* infestation in treatments where seed of herbicide resistant mutant lines was coated with herbicide.

- Use of 1g ha^{-1} sulfosulfuron reduced *Striga* density by up to 47% while *Striga* seed flowering and seed set was reduced by 52% and 77%, respectively. This rate of herbicide would be the recommended rate to use as it did not result in decreased biomass yield.
- However an increase in tolerance to the herbicide in the mutants can minimize host damage and allow for use of higher rates that would be more effective in *Striga* control.

7.3.7 Implications for breeding

- Farmers' landraces can be a good source of genetic variation for improvement of traits like *Striga* tolerance, pest and drought resistance, and adaptability. The PRA study clearly showed that farmers' selection criteria are important and breeders should consider their opinions first before embarking on developing varieties intended for them. While breeding for *Striga* has proved exceptionally difficult over the years, partly because of the complex nature of the parasite, learning to live with the parasite may be the next better option and in this regard then, tolerance genes which allow farmers landraces such as *Ochuti* to survive well under heavy *Striga* infestations may be the right place for breeders to source these genes.

- This study has shown that mutation breeding can be useful in generating valuable genetic variability and new varieties. In chapters three and four, valuable genetic variation for such characteristics as head length, head width, head morphology and overall yield increment in mutant line SB2M13 for example, gave the indication that there exists a niche for mutation breeding in sorghum improvement. This is especially important for traits like nutritional quality where natural variability is known to be lacking in the sorghum gene pool (Axtell et al., 1979).

- Generally most of herbicide resistant crops (HRCs) have been developed via genetic transformation. This study has shown that genes for ALS-herbicide resistance can also be easily developed via chemical mutagenesis. In Africa and other countries where GMOs are not accepted, the development of HRCs through mutagenesis may be the most economical option to develop resistance in many other crops of interest. The inheritance study will be useful for breeding and introgression of the trait into other varieties.

7.3.8 The Way forward

- The participatory Appraisal has highlighted the difficulties of farmers in sorghum production and government intervention is required to help alleviate poverty, improve infrastructure and educate the masses in order to improve sorghum production in western Kenya.
- The mutants generated in this study can be used for further development of sorghum for diverse characteristics. In particular, the mutants need to be screened for such characteristics as drought tolerance and nutritional quality that this study was unable to undertake.
- Other mutants such as "SB2M13" need to be evaluated in multilocational trials to confirm their yield superiority and for possible release as direct mutants.
- Segregation of the gene for resistance indicated that the resistance was a semi-dominant gene. However, a more thorough study should be done to verify the biochemical nature of the inheritance in order to determine if it is caused by an insensitive ALS. Whereas most resistances to ALS herbicides have been known to be caused by alteration of the binding site of the herbicide on the ALS molecule, other studies have also found that increased resistance may also be caused by increased herbicide metabolism. Biochemical elucidation of the mutation trait would help to fully understand the mutation with a view of enhancing the tolerance levels of the herbicide tolerant mutant lines.
- There is need for more work to optimize the dose requirement for effective *Striga* control. In this regard, the possibility of employing slow release formulations of herbicide that ensure adequate protection against *Striga* throughout the season needs to be explored. Also, while herbicide seed coating has been shown to be effective in reducing *Striga* infestation, it should not be used on its own but should be part of a comprehensive *Striga* control strategy employing other forms of control including resistance and sanitation

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