MARKER ASSISTED BREEDING TO TRANSFER STRIGA RESISTANCE IN SORGHUM FROM A MAPPED DONOR SOURCE N13 TO A FARMER PREFERRED KENYAN VARIETY OCHUTI

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DECLARATION

I declare that this thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my loved ones for their support and encouragement.

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ABBREVIATIONS

ASARECA: The Association for Strengthening Agricultural Research in Eastern and Central Africa
AUSPC: Area under Striga progressive curve

ASNPC: Area Under the above ground Striga Number Progress Curve

AUDPC: Area under the disease progress curve

BC: Backcross

BMZ: Federal Ministry for Economic Cooperation and Development (BMZ)

cM: Centimorgan

CTAB: Cetyl trimethyl-ammonium bromide

DNA: Deoxyribonucleic acid

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

MAS: Marker Assisted Selection

MABC: Marker Assisted Backcrossing

PCR: Polymerase Chain Reaction

QTL: Quantitative Trait Loci

RNA: Ribonucleic acid

RP: Recurrent parent

SSR: Simple Sequence Repeat

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ABSTRACT

The genus striga (Orobanchaceae) causes substantial losses in sorghum [(*Sorghum bicolor* (L.) Moench] production in sub- Saharan Africa. Yield losses in Western Kenya can range from 20 % in low infested areas to 100 % in highly infested areas. Conventional breeding methods have been used in the past, but have not been successful, however, molecular breeding have proved to be promising . Marker Assisted Selection (MAS) was used to introgress striga resistance Quantitative Trait Loci (QTLs) from the donor source N13, an Indian durra sorghum with mechanical and antibiosis resistance mechanism to striga, into a Kenyan adapted farmer preferred sorghum variety, Ochuti. Five genomic regions and molecular markers associated with stable striga resistance have been identified, these QTL are on chromosomes, A (LG1), B (LG2), I (LG6) and 2 QTLs on J (LG5) and each of these QTL accounts for 12 to 30% of the total variation observed for striga resistance. BC_3F_1 seeds obtained from the previous BMZ project were advanced to BC_5F_1 and BC_3F_3. At the same time, two field trials consisting of eight BC_3F_1 lines were laid out in a randomized complete block design during May 2010 to October 2010 and October 2010 to March 2011 at Alupe and Kibos field stations and they were artificially infested with striga. Eleven polymorphic markers flanking the five QTL were used to select for striga resistance in the BC_3F_2 generation. In the results two plants in the BC_3F_1 generation and three plants in the BC_3F_2 generation with four and one striga resistance QTL were identified respectively. The backcrossed lines 33 and 87 had lower striga scores than the susceptible parental check Ochuti. The study showed the success of MAS to transfer Striga resistance QTL

CHAPTER 1
GENERAL INTRODUCTION

1.0 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the second most important cereal crop after maize in sub-Saharan Africa (Haussmann et al., 2000a, Mutisya, 2004) and it is the fifth most important cereal crop worldwide (Dogget, 1988, FAO stat, 2000). In Kenya sorghum is an important crop with the grain used as a staple food in some parts of Western, Eastern, Central, Coast and parts of Rift valley provinces (Kute et al., 2000., Dogget, 1988., Guiragossian, 1986). Some of the most important uses of sorghum, include beer brewing as malt, making thin porridge (uji), thick porridge (ugali), sorghum syrup and molasses and used as animal feed (Calder, 1955). Sorghum stalks are used for fuel and thatching huts and also as stover for animal feed (Mburu, 1986). The stalks have also been used as an industrial raw material in production of bio fuels in developed countries (ICRISAT, 2007a, 2007b, Laopaiboon et al., 2007). In China the stalks are used to make a decorative millwork material called kirei board. Sorghum is an essential food crop in the semi-arid tropics due to its high nutritional value as illustrated in the Table 1.

Table 1. Comparison of sorghum nutritional value to other food crops

COMPONENTS (per 100g portion, raw grain)	Sorghum	Cassava	Rice	Potato	Cassava	Maize
Water (g)	**9.2**	60	12	82	60	-
Energy (kj)	**1418**	667	1527	288	667	358
Protein (g)	**11.3**	1.4	7	1.7	1.4	9.2
Fat (g)	**3.3**	0.3	1	0.1	0.3	4.6
Carbohydrates (g)	**75**	38	79	16	38	73
Fibre (g)	**6.3**	1.8	1	2.4	1.8	2.8
Sugars (g)	**1.9**	1.7	>0.1	1.2	1.7	-
Iron (mg)	**4.4**	0.27	0.8	0.5	0.27	2.7
Manganese (mg)	**<0.1**	0.4	1.1	0.1	0.4	-
Calcium (mg)	**28**	16	28	9	16	26
Magnesium (mg)	**<120**	21	25	21	21	-
Phosphorus (mg)	**287**	27	115	62	27	-
Potassium (mg)	**350**	271	115	407	271	-
Zinc (mg)	**<1**	0.3	1.1	0.3	0.3	-

Source. Léder, (2004).

1.1 Sorghum production

Sorghum is the most important cereal crops in the semi-arid tropics and globally cultivated due to its unique tolerance to drought, water logging, saline/alkali, infertile soil and high temperatures. Sorghum is grown on an area of 47 million hectares in about 86 countries, with annual grain production of 69 million tonnes (FAO stat, 2004). Major sorghum producers in the world are USA, India, Mexico, Argentina, China and Australia, while (Figure 1) shows the leading sorghum producing countries in Africa.

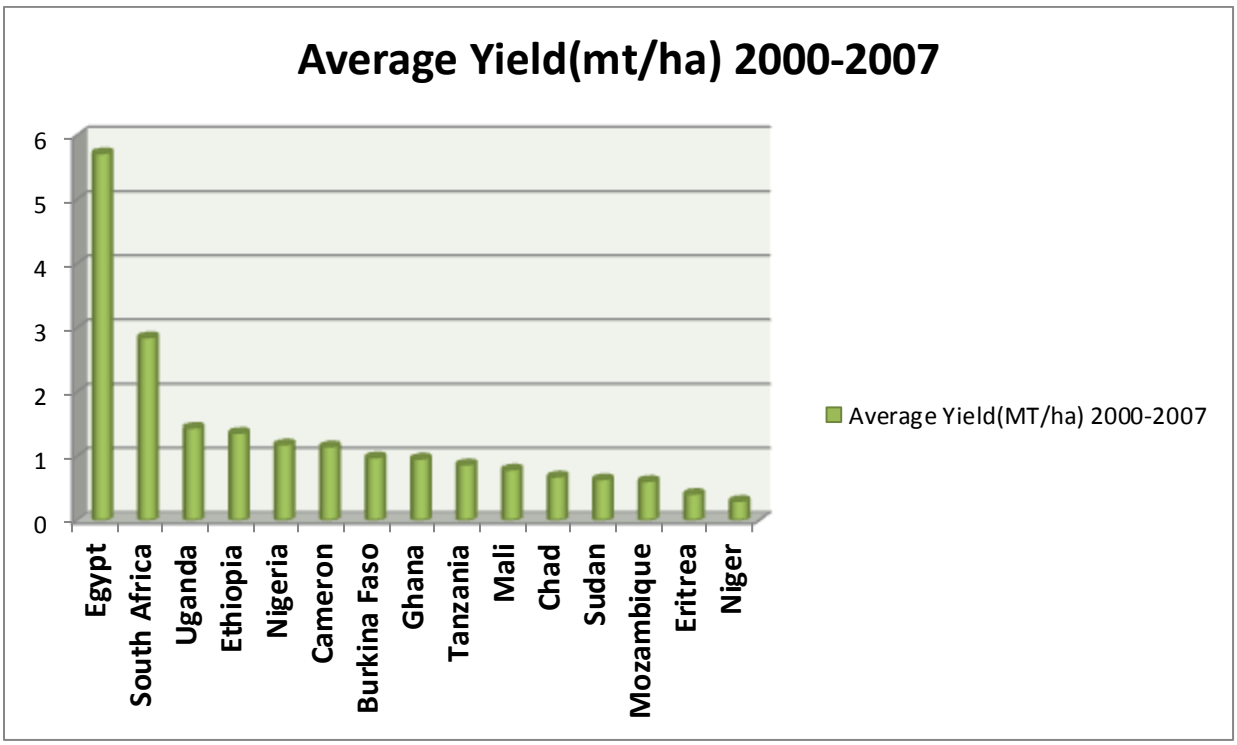


Figure 7. Sorghum yields in Africa countries (Source. FAOSTAT Statistics division 2009)

However, production in Asia and Africa is generally low (500 to 800 kg per ha) compared with yields of up to 7 t / ha recorded (FAO STAT, 2004). Most sorghum production in Africa is in semi-arid areas, productivity in these areas is comparatively low due to various biotic and abiotic stresses.

In Kenya, sorghum is grown in drought-prone marginal agricultural areas of Eastern, Nyanza, Western and Coast Provinces and consumption of sorghum is localized to these growing areas. The

crop performs well in areas between 500 m and 1700 m above sea level, with seasonal rainfall of 300mm and above (Esilaba, 2006). Sorghum production is usually affected by various a biotic and biotic stresses. Some of the most important abiotic stresses include drought, soil fertility and high soil acidity, while the biotic stresses include diseases, insects and most importantly Striga.

1.2 Striga

The Striga genus is among the economically important witch weeds. It was recently placed in Orobanchaceae family (Olmstead et al., 2001) from Scrophulariaceae family. The most important species of Striga are *Striga asiatica* (L.) Kuntze and *Striga hermonthica* (Del.) Benth both which parasitize cereals and *Striga gesnerioides* (Willd.) Vatke which parasitizes cowpeas and other legumes (IITA, 1997).

Striga hermonthica (Del.) Benth (Plate 1) is a hemi-parasitic obligate weed that constrains cereal production in semi-arid, sub-saharan Africa causing huge losses in grain yield (Parker and Riches, 1993) *S. hermonthica* is also known as giant witch weed which is an out crossing species with purple flowers. One Striga plant can produce up to 500,000 seeds, which can remain viable up to 20 years in the soils (Bebawi et al., 1984, Dogget, 1988). The origin of *S. hermonthica* is thought to be in the Nuba Mountains of Sudan and Ethiopia and now it is widespread in many parts of Africa, as well as Yemen and Saudi Arabia (Musselman, 1987).

Striga asiatica (Plate 2) has bright red flowers, but in some regions white, yellow and pink flowers have been observed. Flowers pollinate themselves before opening (Gethi et al., 2005). In Kenya it is localized along the Indian Ocean coast (Frost, 1994).

Striga gesnerioides (Plate 3) is an autogamous species with purple flowers it is an important pest of cowpea and other dicotyledons (Musselman, 1987).

1.2.1 Flowers for different Striga species

Plate 1.*Striga hermonthica* flowers

Plate 2. *Striga asiatica* flowers

Plate 3. *Striga gesnerioides* flowers

Striga is an important constraint to sorghum production in Kenya and sub Saharan Africa (Haussmann et al., 2004). Over 40% of potential sorghum production area in sub Saharan Africa is infested by Striga. In Kenya, Striga infestation is most severe in Nyanza and Western Provinces and it occurs in about 180,000 acres (Odhiambo, 1998). In western Kenya an estimate of 76% of land under sorghum and maize is infested with *S. hermonthica* causing annual losses of about $40.8 million (Hassan et al., 1995, Kanampiu et al., 2002). The Table 2 illustrates the estimated area infested by Striga in the East and Central Africa and Figure 2 shows Striga infestation intensity in Africa.

Table 2. Estimated area under Striga infestation in East and Central Africa

Country	Area Cultivated ('000'ha)	Present crop yields (t/ha)	Striga infested area		Estimated yield loss	yield loss ('000' tonnes)
			000 ha'	% total		
Sudan	6250	0.66	1600	25.6	30	1060
Tanzania	690	0.5	650	90	up to 90	550
Ethiopia	1760	1.27	528	30	25	500
Kenya	150	1.05	80	53.3	35-40	50-60
Eritrea	160	0.62	64	37.5	20-60	30-90
Somalia	500	0.46	150	30	15	30
Rwanda	80	1.05	1.6	2	5	5
Uganda	270	1.5	27	10	10	<1

Data compiled by Obilana, AB. (AATF, 2011)

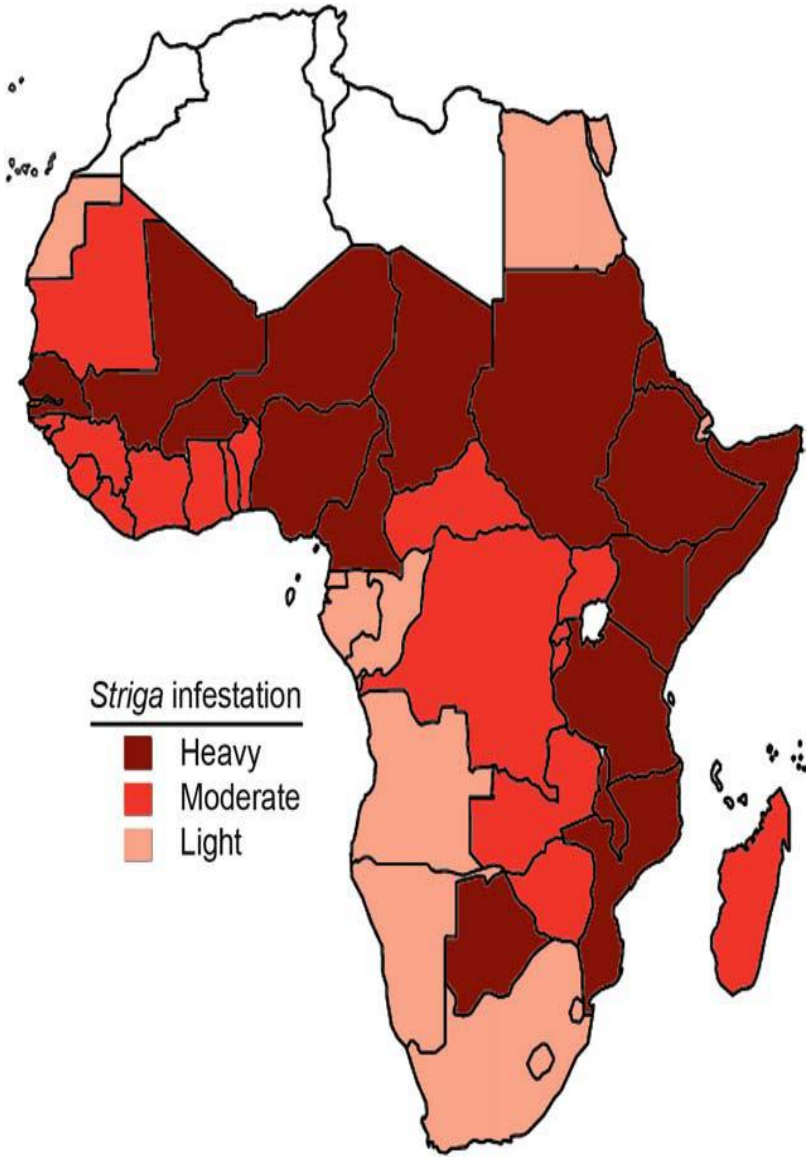


Figure 8. Striga infestation Intensity in Africa (Ejeta, 2007)

1.2.2 Striga in Kenya

There are two Striga species in Kenya *Striga asiatica* (L.) Kuntze and *Striga hermonthica* (Del.) Benth. *Striga hermonthica* is widely distributed in western Kenya, while *Striga asiatica* is found in the coastal region of the country (Odhiambo, 1998). Production of sorghum, maize, rice and sugarcane is affected by Striga infestation (Hassan et al., 1995, Kanampiu et al., 2002).

1.2.3 Striga Biology

Striga weeds lack their own root system thus compensates by penetrating the roots of host plants and depriving essential nutrients for plant growth resulting in host plants having stunted growth and low

yields (Watson et al., 1998). Initial host symptoms are chlorosis, severe stunting and drought like symptoms. Grain yield loss can be 100% in susceptible cultivars under high infestation and under drought conditions (Haussmann et al., 2000a). Striga is an obligate parasite, depending heavily on the host for its survival. Interactions between Striga and its host plant play a crucial role in the survival of the parasite.

After dispersal, Striga seeds remain dormant for several months to avoid germination during the last rains of the season when no host is present (Berner et al., 1997b) a period called after ripening. Striga seeds only germinate when exposed to favourable temperature and moisture (pre-conditioning) and in the presence of germination stimulants from the host and non-host plant roots. Further development include haustorial formation, attachment, penetration and development of the parasite also require signals or resources from the host plant (Ejeta et al., 1992, 2000). Physiological processes during *Striga* infestation in sorghum are complex (Gurney et al., 2000).

Striga control methodologies include reduction of soil seed bank, limitation of Striga seed production and reduction/ prevention of Striga seed dissemination to un- infested field. An effective control strategy should integrate at least one control method from each category (Obilana, 1990). If resistance can be incorporated into adapted, productive cultivar, Striga resistant sorghums can be a major component of integrated Striga control, as resistant cultivar can reduce both new Striga seed production and Striga seed bank in the soil. However breeding progress has been limited due to inadequate information on the genetics of Striga resistance and the difficulty of evaluating resistance in the field. Precise and reliable screening is a must for breeding for resistance to any biotic and a biotic stress (Vasudeva Rao, 1985). Several resistance mechanisms have been proposed by Berner et al., 1995, Ejeta et al., 1992, 1993, Wegmann, 1996; such as low production of germination stimulant, mechanical barriers, inhibition of germ tube exoenzymes by root exudates, pytoalexine synthesis, post-

attachment hypersensitive reactions or incompatibility, antibiosis, insensitivity to Striga toxin and avoidance through root growth habit.

1.3 Marker assisted breeding

Molecular Marker Assisted Selection (MAS) involves use of molecular markers to select for plants with genomic regions of interest (Choudhary and Shekhawat 2008). MAS has become an important tool in plant breeding, it has increased transfer efficiency of genomic regions and the recovery of recurrent parent genome (Ibitoye and Akin-Idowu., 2010).

The advantages of conducting MAS over phenotypic selection include single plants can be selected, selection can be carried out at seedling stage and is simple to screen for difficult and laborious traits than phenotypic screening. There are several MAS schemes marker assisted evaluation of breeding materials, marker assisted backcrossing, marker assisted pyramiding, early generation marker assisted selection and combined marker assisted selection (Collard et al., 2005).

Marker assisted evaluation of breeding materials involves cultivar identity, assessment of purity and genetic diversity, parental selection, study of heterosis and identification of genomic regions under selection. Marker assisted backcross involves use of DNA markers to introgress one or a few genes into an elite/adapted variety. Marker assisted pyramiding is the process of combining several genes together into one genotype and using DNA markers for selection (Ejeta., 2007a).

Factors to consider when choosing DNA markers for use in MAS are level of polymorphism, DNA quantity and quality, Technical procedure, reliability and cost (Mackill and Ni, 2000, Mohler and Singrun, 2004). Simple sequence repeats (SSRs) or microsatellites are the commonly used markers in cereal breeding (Gupta et al., 1999, Gupta and Varshney, 2000). They are reproducible, co-dominant, relatively simple and cheap to use.

Back crossing (BC) is a breeding method used to incorporate one or a few genes into an adapted variety (Allard, 1960). Marker assisted back crossing (MABC) selects for individuals to be used as parents in next generation using DNA results (Semagn et al., 2006b). There are three levels of marker assisted backcrossing (MABC), foreground selection, recombinant selection and background selection (Holland, 2004). Foreground selection is the first level that involves use of markers to screen for target gene (Hospital, 1997). Recombinant selection is level two which involves selection of back cross progeny with target gene and recombination events between linked flanking markers and target loci and it minimizes linkage drag (Hospital, 2005). Background selection is the third level of MABC. Back cross progeny with the highest portion of recurrent parent is selected using unlinked markers to the target locus (Hospital, 1997, Frisch, 1999b). Breeding for Striga resistance in sorghum began in 1920s in South Africa (Saunders, 1933), however breeding progress has been slow due to limited knowledge of the genetics of Striga resistance and the difficulty of evaluating resistance in the field.

1.4 Problem statement and justification

Sorghum is the second most important cereal crop in East Africa as a source of food. However, its productivity is seriously constrained by Striga, leading to a substantial loss of yield. Conventional breeding methods have been used in the past, but have not been successful. Various agronomic practices have also been tried but failed to substantially combat the weed. However, molecular methods hold potential in developing Striga resistant sorghum varieties. This study focused on using marker assisted selection to introgress resistance from N13 variety in to the farmer preferred variety, Ochuti.

1.5 OBJECTIVES

1.5.1 General objective

To enhance sorghum productivity in Striga prone areas by reducing the Striga seed bank in the soil.

1.5.2 Specific objectives

1. To introgress Striga resistance QTL into BC_3F_1 and BC_4F_1 generation.
2. To evaluate the performance of progenies arising from advanced backcrosses

1.6 Hypothesis

Marker assisted selection can be successfully used to introgress Striga resistance QTL into a locally adapted sorghum.

CHAPTER 2
LITERATURE REVIEW

2.1 Sorghum

Sorghum *(Sorghum bicolor* (L.) Moench) is an annual vigorous grass that grows between 0.5 to 5.0 metres in height and it belongs to Poales order of Poaceae family. There are five main races of sorghum, caudatum, bicolor, durra, guinea, kafir and ten intermediates based on the spikelet and grain morphology (Harlan and De wet, 1972). *Sorghum bicolor* is an important race and it is characterized by open inflorescence and long clasping glumes that usually enclose the grain at maturity. Sorghum has a wide diversity and distribution in Africa (Plate 4).

Plate 4. Sorghum diversity in the world (courtesy of ICRISAT India)

Sorghum plant possess some desirable characteristics which makes it a very important crop in areas of its existence, it is a C_4 plant with greater efficiency of dry matter production relative to water use than wheat, indicating it has some tolerance to drought stress (Downess, 1970). It can also endure short periods of waterlogging better than maize (Doggett and Jowett, 1966).

2.1.1 Sorghum Reproductive Biology

Sorghum is a diploid plant (2n=20) which is predominantly self-pollinating, but a cross pollination of 5 to 25% has been reported (Doggett, 1988). The seed is generally round and bluntly pointed and vary in diameter, size, shape and colour depending on the cultivar. Floral initiation takes place 30 to 40 days after germination, and this marks the end of the vegetative phase (House, 1980). Floral initial develops into an inflorescence during the period of rapid cell elongation. Genotype and environment influence largely the time required from vegetative to reproductive phase.

The spikelets occur in pairs one is sessile, with a perfect flower and the second one is a short pedicel, which possesses only anthers but occasionally have a rudimentary ovary and empty glumes. Sessile spikelets vary in shape from lanceolate to almost round and ovate and sometimes depressed in the middle. The colour changes from green during flowering to different colours like straw, cream, yellow, red, brown, purple or almost black at grain maturity. The seed may be enclosed by the glumes or may protrude from it, while, pedicelled spikelets are much narrower than the sessile spikelets (Aruna and Audilakshmi, 2008).

Anthesis starts with the exertion of the complete panicle. The flower begins to open two days after complete emergence of the panicle. Anthesis or floret opening is followed by the exertion of the anthers and stigmas between the lemma and palea. The sorghum head starts to flower at the tip and anthesis takes place downward over a period of 4 or 5 days and the process takes place first in the sessile spikelets from top to bottom of the inflorescence. When flowering of the sessile spikelets is half way down the panicle, pedicellate (spikelet with anthers only) starts opening at the top of the panicle and proceed downwards. Flowering phase of pedicellate spikelets overtakes that of sessile spikelets before they reach the base of the inflorescence (Maiti, 1996).

At the time of flowering, the glumes open and all three anthers fall free while the two stigmas protrude each on a stiff style. The anthers dehisce when they are dry and pollen is blown into the air. After pollen shedding, pollen in the anthers remains alive for several hours. Pollen diffusion takes place through the apical pore. The pollen drifts to the stigma where it germinates. The pollen tube with two nuclei, grows down the style to fertilize the egg and form a diploid (2n) nucleus. Glumes close shortly after pollination, Anthesis takes place during the morning hours, therefore it is advised to do hand pollination at around 0930 to 1000 hours which can be extended up to 1230 hours on foggy morning (House, 1980).

Seeds are borne on raceme branches on the panicles. After fertilization the ovule begins to develop as light green and ten days later they become darker green. Development of grains takes a sequence of stages comprising of a milky, soft dough, hard dough to the final physiological maturity when a black layer is formed at the hilar region due to the formation of callus tissues (Aruna and Audilakshmi, 2008). It takes about 30 days for the seeds to reach maximum dry weight or physiological maturity. The seed contains about 30% moisture at physiological maturity (Audilakshmi et al., 2005) which reduces to about 10 to 15 % at 20 to 25 days after attaining physiological maturity (House, 1980). Seeds are ready for harvest when they attain physiological maturity to seed dryness. Seeds with more than 12 % moisture must be dried before they are stored (Audilakshmi et al., 2005).

2.1.2 Characteristics of sorghum varieties in Kenya

Farmers in counties from Western part of Kenya prefer growing sorghum varieties which are tolerant to Striga, drought, pests and early maturing. Common landraces in these areas are Ochuti, Nyakabala, Andiwo and Wagita, while common improved varieties are Serena and Seredo (Ndung'u, 2009).

Ochuti is a Kenyan sorghum landrace that is preferred by farmers and has some level of tolerance to Striga (Frost, 1994). Farmers like it due to its colour, taste, drooping heads and Striga tolerance. N13 is an Indian durra sorghum, which is neither high yielding nor drought tolerant but it has Striga

resistance genes for mechanical and antibiosis resistance mechanism. N13 variety stimulates abundant Striga seed germination but forms a mechanical barrier to parasite penetration through lignification of cell walls. The other resistance mechanism, antibiosis, Striga growth is reduced through unfavourable phytohormone supply by the host (Haussmann et al., 2000a, Ejeta, 2007).

A resistant genotype support significantly fewer Striga plant when grown under Striga infestation and produces a higher yield than a susceptible genotype (Doggett, 1988, Ejeta et al., 1992), while tolerant genotypes support a higher number of Striga and show smaller yield reductions than susceptible genotypes under the same pressure. Tolerant genotypes increase Striga seed bank over time (Doggett, 1988), while resistant genotypes reduce new Striga seed production and Striga seed bank in infected soils (Haussmann et al., 2000a).

2.2 Striga

Striga is an obligate hemi-parasite that infects C_3 and C_4 crops especially sorghum, maize, rice and millet. It attaches itself to the host roots by use of the haustorium, weakening the host by wounding the outer root tissues and absorbing the supply of moisture, photosynthates and minerals (Tenebe and Kamara, 2002). Striga is adapted to its environment (Bebawi and Metwali, 1991) and integrated with the host that it only germinate in response to specific chemical cues present in root exudates of the hosts or non-host plants (Parker and Riches, 1993, Yoder, 1999). Striga also causes phytotoxic effects within days of attachment to the hosts (Frost et al., 1997, Gurney et al., 1999).

There are 28 species and 6 sub species of Striga in Africa (Mohamed et al., 2001), *Striga hermonthica* and *Striga asiatica* are the most common in Sub Saharan Africa (Haussmann et al., 2000c). *Striga hermonthica* is thought to have originated in the Nuba Mountains of Sudan and Ethiopia (Musselman, 1987). *Striga hermonthica* is an out crossing species with purple flowers and it is the predominant type as it has affected over 21 million hectares of cereal production areas in Sub Saharan Africa (Sauerborn, 1991).

Various control strategies have been tried, but all have limitations and none has provided complete solution (Oswald, 2005). Striga *c*ontrol has been complicated by the abundant seed production, longevity of the seed bank in the soil (Bebawi et al., 1984) and a complicated mode of parasitism. The change in farming system from shifting cultivation to more permanent cropping, concomitant with loss in soil fertility and frequent cultivation of susceptible host plants are the main factors for increased Striga infestation (Kroschel, 1999). Some control strategies include manipulation of Striga seed numbers either through reduction of Striga seed in the soil seed bank or through limitations of Striga seed production (Haussmann et al., 2000b). Use of Striga resistant crop varieties has vital values especially to the small scale farmers who are adversely affected by Striga infestation. The advantages are reduction in labour for weeding, reduction in the cost of herbicides and a clean environment**.**

2.2.1 Striga germination stimulants

Striga germination stimulant is controlled by a group of sesquiterpene derivatives including strigol (Cook et al., 1972). Germination stimulants found in the root exudates of sorghum, maize and cowpea are from carotenoid biosynthetic pathway and have chemical structures as shown in Figure 3 (Matusova et al., 2005). Haustorial active initiators have been identified to be kinetin, simple phenolic compounds and quinines like 2, 6 dimethoxy1, 4 benzoquinine (DMBQ).

Sorgolactone is the major Striga germination stimulant exuded by sorghum roots (Hauck et al., 1992), maize (*Zea mays*) and proso millets (*Panicum miliaceum* L.). Strigolactone is the Striga germination stimulants (Figure 3) found in root exudates of both host and non-host plants (Matusova et al., 2005).

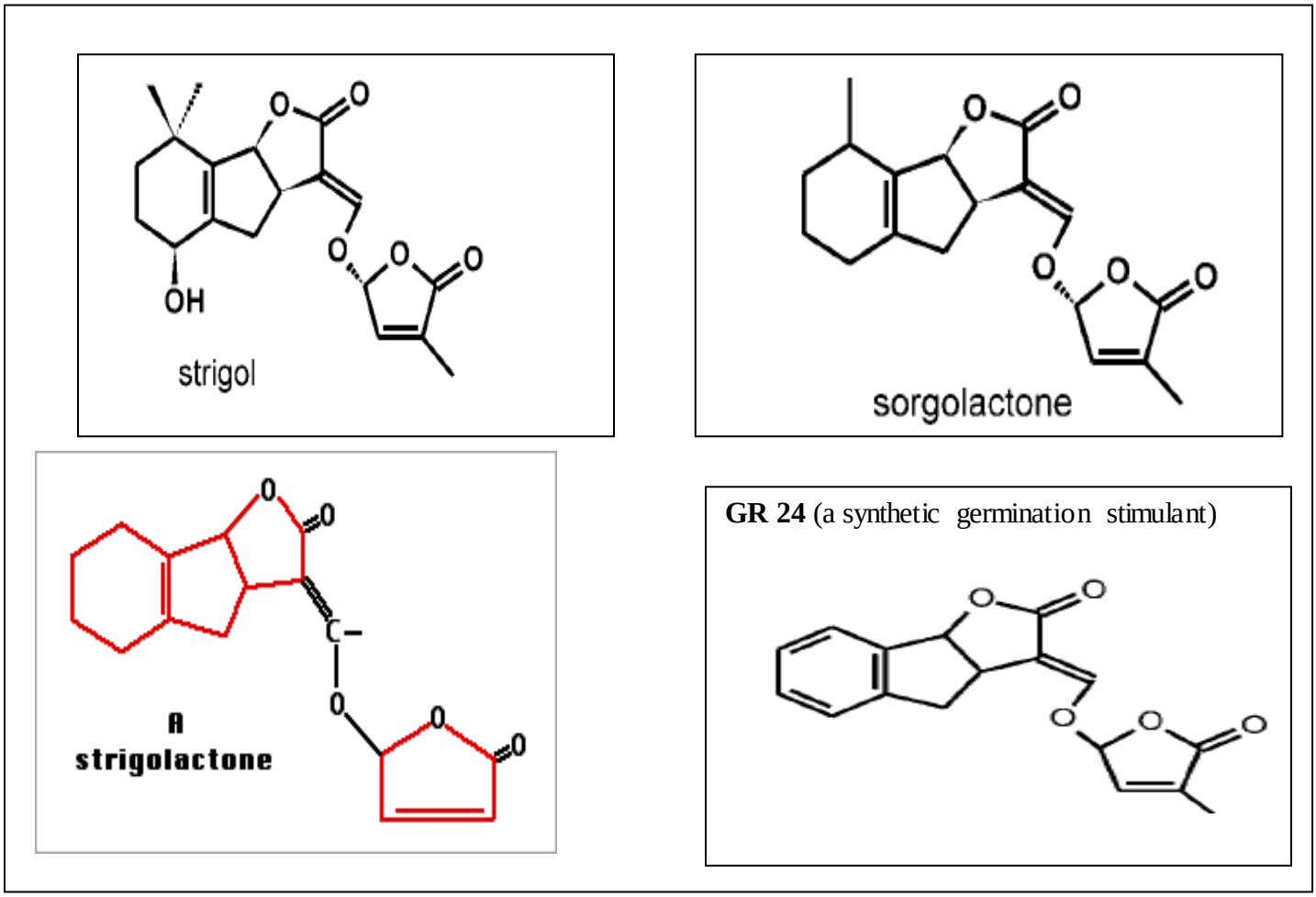


Figure 3. Structures of Striga germination stimulants (Matusova et al., 2005)

2.2.2 Striga Biology

Biology of Striga on how it grows and develops to form an intricate network of coordination with its host is well understood (Ejeta, 2007a). Striga seeds need a conditioning process to be able to respond to external stimuli in order to germinate. The conditioning process requires exposure of Striga seeds to warm and moist environment, so that the imbibed seed may respond to chemical stimulants of germination. Essential pathways take place during conditioning process leading to respiration and synthesis of protein and hormones (Joel et al., 2007).

Conditioned and after ripened Striga seed will germinate in response to minute levels of exudates released by host roots. Conditioned Striga seeds have the ability to revert back to dormancy if they lack host stimuli in close proximity through wet dormancy (Mohamed et al., 1998). Close proximity of host plants roots is necessary to provide trigger signals for development of Striga haustorium, which attaches to the host roots. Formation of haustorium is the start of the parasitic process, as the parasite begins to tap water and nutrients from the host plant, which is a crucial step to the eventual development of the parasite. Parasitic attachment to host root surface takes place immediately and it is facilitated by secretion of a hemicelluloses based adhesive substance that fixes the parasite to the host root (Baird and Riopel, 1985). This binding is strong but not very host specific as the haustoria can attach to non-host roots (Hood et al., 1998).

Attachment of haustoria to host root is a requirement for penetration phase of haustorial development and this may involve additional chemical or tactile signals from the host root. Penetration of Striga roots to the host to tap nutrients and water takes place immediately as the Striga seed has small reserves which are expended during germination and haustorial differentiation. Connection to the vascular core of the host root is aided by enzymatic activity that breaks down the wall components of host cortical cells. After penetration of the xylem, haustorial cells lose their protoplast transforming them into water-conducting elements that are continuous with host xylem. The parasitic seedling develops a tubercle to assist with accumulation of nutrients soon after its attachment to host tissue. The cotyledenous Striga leaves emerge from the seed coat within a day after vascular connections have been established with the host (Hood et al., 1998). After six weeks of above ground emergence the parasitic seedling forms flowers which later mature to seeds after two weeks of pollination. Survival of Striga as a parasite and its successful development as a plant depends on its interactions with the host plant. Figure 4 summarizes the biology and the lifecycle of Striga.

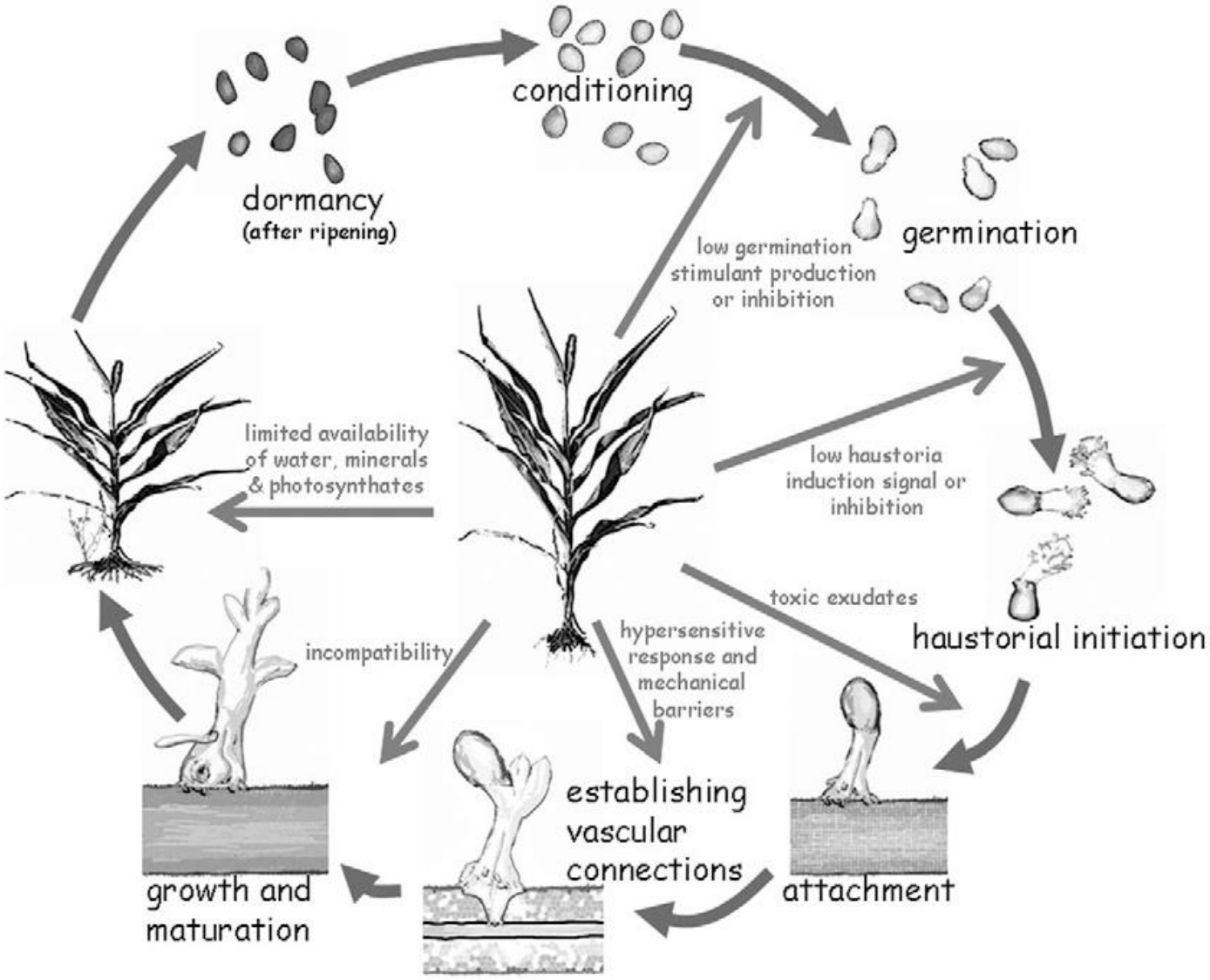


Figure 4. Summary of Striga lifecycle (Ejeta, 2007a)

2.2.3 Mechanisms of Striga Resistance

Several resistance mechanisms to Striga have been proposed, mechanical barriers, inhibition of germ-tube exoenzymes by root exudates, phytoalexin synthesis, post–attachment hypersensitive reactions or incompatibility, antibiosis, insensitivity to Striga toxin and avoidance through root growth habit(Ejeta and Butler, 1993).

2.2. 3. 1 Low Germination Stimulant (LGS) production

Sorghum genotypes with low germination stimulant (LGS) produce insufficient amounts of the exudates required for germination of conditioned Striga seeds. These genotypes producing low levels of germination stimulants have been found to be resistant to Striga in field tests (Ramaiah, 1987, Hess et al., 1992). All highly susceptible sorghum genotypes are high producers of the germination stimulant. There are several classes of chemical signals for Striga seed germination, the most common and important one is sorgolactones (Hauck et al., 1992). This low germination stimulant (LGS) trait is inherited as a single, nuclear, recessive gene with largely addictive gene action. Cultivars with this mechanism are Framida, SRN 39, 555, SAR lines, IS 15401 and IS 9830 (Ejeta and Butler, 1993, Heller and Wegmann, 2000, Haussmann et al., 2000b).

2.2.3.2 Low Production of the Haustorial Initiation Factor (LHF)

Germinated Striga near the roots of sorghum genotypes with low production of the haustorial initiation factor mechanism do not form haustoria and therefore die from their inability to attach to their potential host. Unlike the signals for germination of Striga seeds, signals for haustoria induction has not be identified yet, but a large number of phenolic compounds have been shown to function as haustoria initiators in Striga. A simple quinone, 2, 6 dimethoxy1, 4 benzo quinine (DMBQ), though not present in host root exudates has been shown to act as a strong haustorial initiating factor (Lynn and Chang 1990). Extended agar gel assay (EAGA) was used to study this mechanism of resistance. Extended agar gel assay (EAGA) distinguishes host genotypes qualitatively on their ability to induce haustorial formation. Inheritance of this trait is through a dominant allele of a single gene. Example of sorghum with this mechanism is Accession P-78 of *Sorghum drummondii* (Haussmann et al., 2000b, Ejeta, 2007a).

2.2.3.3 Hypersensitive Response (HR)

Hypersensitive response resistance involves localized necrosis of host tissues surrounding the site of attachment, it is coupled with release of phytoalexins that kill the attached Striga, hence does not penetrate host tissues or develop further. Mohamed et al., 2003, has described hypersensitive response and its association with failed Striga parasitism. Hypersensitive response expression has been studied extensively in a number of host-parasite system, it is generally characterized by the appearance of necrotic zones around the site of attempted infection (Agrios, 1988). Death of host cells results in unsuccessful establishment of the parasite hence its ultimate demise. Examples of sorghum genotypes with this mechanism are Framida, Dobbs, SAR 16, SAR 19, SAR 33, *Sorghum versicolor* and wild sorghum accession P47121 (Ejeta, 2007, Haussmann et al., 2000b).

2.2.3.4 Incompatible Response (IR)

This host response is similar to hypersensitive response (HR), in that it discourages further development beyond attachment (Greiner et al., 2001). Striga seedlings that manage to penetrate the host tissues do not develop beyond first emergence of leaves. Some Striga appear to develop normally at first but later show stunted growth. Incompatible response reactions may develop as a result of failure to establish adequate vascular connections caused by lack of vital factors or because of the production of toxic factors that disrupt growth and development of the parasite. An example of a cultivar with this mechanism is SRN 39 (Ejeta, 2007, Haussmann et al., 2000b).

2.2.3.5 Mechanical barriers

Mechanical barrier to penetration has been reported in certain host-parasite associations by increased lignification (Maiti et al., 1984), deposition of cellulose layers (Olivier et al., 1991) and encapsulation (Labrousse et al., 2001). Example of varieties with this mechanism is N13 and Framida (Haussmann et al., 2000b, Ejeta, 2007).

2.2.3.6 Antibiosis

Antibiosis mechanism reduces Striga development through unfavourable phytohormones supply by the host. This mechanism is present in SRN 39 and N13, while insensitivity to Striga toxin through maintenance of stomatal aperture and photosynthetic efficiency and avoidance through root growth habit is achieved by having fewer roots in the upper 15 to 20 cm (Haussmann et al 2000).

2.3 Genetic markers

Molecular markers are identifiable DNA sequences found at specific locations of the genome and transmitted from one generation to the next, following standard laws of inheritance (Collard et al., 2005, Semagn et al., 2006 a). DNA based markers can be utilized effectively in marker assisted selection (MAS) for tracing favourable alleles (dominant or recessive) across generations and identifying the most suitable individuals among the segregating progeny based on allelic composition across a part or the entire genome.

There are several genetic marker types. Morphological markers are based on visible/phenotypic characteristics for instance plant height, whereas biochemical markers are based on gene products for instance isozymes. Molecular markers are based on DNA sequence variation an example being microsatellite repeats (Jones et al., 1997). Morphological and biochemical markers are limited in number and are influenced by environmental conditions, developmental stage/ tissue type, while molecular markers are virtually unlimited in number and are not affected by environment or tissue type.

DNA markers are valuable tools in various analyses in plant breeding. They are used in early generation selection, in choosing donor parents in backcrossing, recovering of recurrent parent genotype in backcrossing, germplasm characterization/fingerprinting, among other uses (Varshney et al., 2009).

DNA-based molecular markers can be classified into three categories depending on how the polymorphism is revealed. Hybridization based polymorphisms, PCR-based polymorphisms and sequence- based polymorphisms (Gupta et al., 2002).

Polymorphic markers reveal differences between individuals of the same or different species. Co-dominant markers indicate differences in size whereas dominant markers are either present or absent. In addition co- dominant markers have many different alleles whereas a dominant marker only has two alleles (Collard et al., 2005).

2.3.1 Hybridization-based molecular markers

2.3.1.1 Restriction fragment length polymorphism (RFLP)

RFLP is the most widely used hybridization-based marker. The technique is based on restriction enzymes that reveal pattern differences between DNA fragment sizes in individual organisms. Organisms of the same species have almost identical genomes but there are differences on nucleotides due to point mutation, insertion/deletion, translocation, inversion and duplication. These differences in DNA sequences can result in gain, loss or relocation of restriction sites. Therefore digestion of DNA with restriction enzymes results in fragments whose number and size vary among individuals.

There are several advantages of using RFLPs. These markers are co-dominant and can be detected by many detection systems, they can also be converted to SCARs and no sequence information is required for synthesis. RFLP requires presence of high quality and quantity DNA. The technique is not amenable to automation and the level of polymorphism is low. Also the technique is time consuming, laborious, expensive and requires radioactively labelled probes (Tanksley et al., 1989, Beckmann and Soller 1986, Kochert, 1994).

2.3.2 PCR-based markers

Polymerase Chain Reaction (PCR) is a molecular biology technique that uses DNA polymerase enzyme to amplify small quantities of DNA. The amplification of target DNA can be doubled from the

previous cycle provided there is sufficient amount of DNA polymerase, primers and deoxynucleotide triphosphate (dNTPs) in the reaction solution. There are two types of PCR-based markers depending on the primer used for amplification. One type consists of the arbitrary or semi-arbitrary primed PCR techniques, which are developed without prior sequence information such as arbitrarily primed polymerase chain reaction (AP-PCR), DNA amplification fingerprinting (DAF), random amplified microsatellite polymorphism (RAPD), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeat (ISSR). The second type consists site-targeted PCR techniques developed from known DNA sequences for instance Expressed Sequence Tags (EST), Cleaved Amplified Polymorphic Sequence (CAPS) Simple Sequence Repeats (SSR), Sequence Characterized Amplified Region (SCAR) and Sequence Tagged Site (STS) (Semagn et al., 2006 a).

2.3.2.1 Random amplified polymorphic DNA (RAPD)

RAPD markers require small amounts of DNA and no sequence information. RAPD have good polymorphirsm and high genomic abundance. The technique can be automated. RAPD markers are highly sensitive to laboratory changes, therefore low reproducibility within or between laboratories. RAPD is a dominant marker hence, it cannot be used for either across populations or across species (Williams et al., 1990, Welsh and McClelland, 1990).

2.3.2.2 Amplified fragment length polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) technique combines the power of Restriction Fragment Length Polymorphism (RFLP) with the flexibility of PCR technology by ligating primer recognition sequences to the restricted DNA. The AFLP technique can be automated, no sequence information is required and small DNA quantities are required (Vos et al., 1995).

2.3.2.3 Cleaved amplified polymorphic sequence (CAPS)

Cleaved Amplified Polymorphic Sequence (CAPS) is a combination of the PCR and Restriction Fragment Length Polymorphism (RFLP) (Maeda et al., 1990). This technique uses the PCR to

amplify the target DNA and restriction enzymes for digestion. CAPS markers are single locus, species specific and requires small amount of DNA.

2.3.2.4 Sequence characterized amplified region (SCAR)

Sequence Characterized Amplified Region (SCAR) marker is a genomic DNA fragment that is identified by PCR amplification using a pair of specific oligonucleotide primers. SCARs are derived by cloning and sequencing the two ends of RAPD marker that appeared to be diagnostic for specific purposes. The advantages of using SCAR are that the marker can be converted into a co-dominant marker, it is less sensitive to reaction conditions and can detect a single locus (Paran and Michelmore, 1993).

2.3.2.5 Sequence Tagged Site (STS)

Sequence Tagged Site (STS) was first developed by (Olsen et al., 1989), STS are short, unique sequence whose exact sequence is not present anyway else in the genome. STS markers are co-dominant, highly reproducible, suitable for high throughput and automation and simple for use. The disadvantages of STS are that they require sequence information and decreased levels of polymorphism (Reamon-Buttner and Jung, 2000).

2.3.2.6 Single Nucleotide Polymorphism (SNP)

Single Nucleotide Polymorphism (SNP**)** are robust, suitable for high throughput, they can be automated and the polymorphism is identifiable by different methods. The marker can be technically challenging as it requires high development costs and sequence information (Gupta et al., 2001).

2.3.2.7 Simple Sequence Repeats (SSR)

Simple Sequence Repeats (SSRs) are short sequences of nucleotides (2 to 6 units in length) that are repeated in tandem. SSRs are also known as sequence tagged microsatellite site (STMS) marker (Davierwala et al., 2000, Huettel et al., 1999, Mohapatra et al., 2003). The SSR are highly

polymorphic, robust, can be automated, need small quantity of DNA, multi-allelic, require radioactive labelling and co-dominant. The disadvantages of using SSR is that markers are species specific, usually single loci even in polyploids, has high start-up costs and the results can sometimes be difficult to interpret due to stuttering. Having said that, SSR markers are excellent for use in MAS, in fingerprinting and in MAB (Semagn et al., 2006 a).

2.3.2.8 Diversity Array Technology (DArT)

Diversity Array Technology (DArT) is a micro-array hybridization based technique that enables the simultaneous typing of several hundred polymorphic loci spread over the genome. With this technique no sequence information is required, is highly automated, the results are obtained quickly and highly reproducible. However, the method requires extensive investment both in laboratory facilities and in skilled man power (Wenzl et al., 2004, Jaccoud et al., 2001).

2.4 Marker assisted selection (MAS)

MAS involves selection of plants with genomic regions involved in expression of trait of interest (Choudhary et al., 2008). MAS is possible for traits controlled by major genes as well as quantitative trait loci (QTL) due to the availability of an array of molecular markers and dense molecular genetic maps (Semagn et al., 2006a). MAS is gaining importance as it would improve the efficiency of plant breeding through precise transfer of genomic regions of interest (foreground selection) and accelerating the recovery of the recurrent parent genome (background selection) (Semagn et al., 2006 a, Choudhary et al., 2008, Ibitoye and Akin-Idowu, 2010). MAS has also been widely used for the selection of simply inherited traits.

Most genomic regions selected using MAS are often chromosome segments carrying quantitative trait loci (QTL). In case of polygenic traits, it is preferable to have two polymorphic DNA markers flanking the target gene or QTL or a marker within a QTL.

2.4.1 Factors affecting MAS

Several factors affect the success of MAS, the critical being the number of target genes to be transferred, the distance between the flanking markers and the target genes and the number of genotypes selected in each breeding generation (Francia et al., 2005). MAS is normally applied where there is a genetic map with adequate number of polymorphic markers to accurately locate desired QTL or major genes where there is close linkage between the QTL or a major gene of interest and adjacent markers.

The success of MAS also depends on the location of the marker with respect to genes of interest. Dekkers, (2004) classified three kinds of relationships between the markers and respective genes, the first is when the molecular marker is located within the gene of interest, which is favourable for MAS and it is ideally referred to as gene-assisted selection. This kind of relationship is the most preferred, but it is difficult to find these types of markers. The second relationship is when the marker is in linkage disequilibrium (LD) with the gene of interest throughout the population. Linkage disequilibrium (LD) is the tendency of certain combination of alleles to be inherited together. Population-wide linkage disequilibrium (LD) is found when markers and genes of interest are physically close to each other. Selection using these markers is called LD-MAS. The third option is when the marker is in linkage equilibrium (LE) with genes of interest throughout the population. This is the most difficult and challenging situation for applying MAS (Choudhary et al., 2008).

2.4.2 Foreground and background selection

Marker assisted foreground selection was first proposed by Tanksley, 1983 and first used in introgression of resistance genes by Melchinger, 1990. Foreground selection uses molecular markers to trace the presence of target genes, thus, ensuring precise transfer of genomic regions of interest from a donor parent into a recipient parent (Semagn et al., 2006c).

Background selection accelerates the recovery of recipient parent genome and this reduces linkage drag. The recovery of recurrent parent genome is achieved by selecting for individuals that are heterozygous at the target locus and homozygous for the recurrent parent alleles at two markers flanking the target locus(Hospital and Decoux, 2002). Flanking markers should be closely linked to the target loci to reduce linkage drag (Hospital, 2001).

Marker assisted Breeding (MAB) is very useful when phenotypic screening is expensive or when the trait has low heritability and is highly influenced by the environment, or for traits expressed late in plant development (Han et al., 1997, Huang et al., 1997). Gene pyramiding is a useful approach for simultaneously introgressing genes for different agronomic traits or multiple QTL (Hash and Senthilvel, 2008).

MAS have been widely used for simply inherited traits than for polygenic traits, although there are a few success stories in improving quantitative traits. There are several examples of use of MAS in introgression of trait and in gene pyramiding. These include introgression of *y*d2 gene conferring resistance to barley yellow dwarf virus through two cycles of marker assisted backcrossing (Jefferies et al., 2003), pyramiding of different resistance genes for barley yellow mosaic virus (*rym*4, *rym*5. *rym*9 and *rym*11) in barley (Friedt and Ordon 2007) use of yield-related QTL for MAS in maize in private sector (Koebner 2004) and pyramiding of blast and blight resistance in rice simultaneously (He et al., 2004, Narayanan et al., 2002, Sanchez et al., 2000,Singh et al., 2001). Examples when MAS has been used to accelerate varietal development include release of US barley variety Tango that contains two QTL for adult resistance to stripe rust (Toojinda et al., 1998), development of quality protein maize (QPM) through marker aided transfer of *opaque*2 gene (Dreher et al., 2000) and release of Indonesian rice cultivars ‘Angke’ and ‘Conde’, in which marker assisted selection (MAS) was used to introduce *xa*5 into a background of *xa*4 (Toenniessen et al., 2003).

2.5 Sorghum mapping

Sorghum genome mapping based on DNA markers began in the early 1990s (Mace et al., 2008). Maps of sorghum published in the last decade were initially based on RFLP markers, then AFLPs and SSRs and very recently they have been based on DArT markers (Mace et al., 2009).

QTL mapping requires contrasting parents, large population size, markers for genotyping and availability of statistical packages for linkage analysis (Semagn et al., 2006c). Mapping populations are usually obtained from controlled crosses, commonly used mapping populations such as F2, Backcrosses, double haploid (DH), recombinant inbred lines (RILs) and near isogenic lines (NILs) (He et al., 2001, Doerge, 2002).

Genetic mapping is based on the principle that genes/markers/loci segregate via chromosome recombination during meiosis thus allowing their analysis in the progeny (Paterson, 1996). Map construction is computerized and it involves linkage analyses and mapping. Several computer packages are presently available for genetic linkage mapping, commonly used programs include, JoinMap, GMENDEL, MAPMAKER/EXP, LLINKAGE, Map manager QTX, QTL cartographer, QTL cartwin, PLABQTL, Epistat, MQTL and MAP L 98. These statistical programs do the linkage analyses which include calculating pair wise recombination frequencies between markers, establishing the linkage groups, estimating map distance and determining map order (Manly et al., 2001).

After linkage analyses the test for segregation distortion is done. Segregation distortion is a deviation of the observed genotypic frequencies from the expected in a given genotypic class within a segregating population. For each segregating marker a chi-square analysis is performed to test for deviation from the expected segregation ratio for the mapping population (Lyttle, 1991). An important step is establishing the linkage groups markers are assigned using the odds ratio (LOD score). LOD score (logarithm of odds) zero (0) is the probability that two loci are linked with a given recombination value over a probability that the two are not linked (Stam, 1993. Critical LOD score values are used to

establish linkage groups and calculate map distances are called Linklod and Maplod respectively (Stam, 1993, Ortiz et al., 2001). Linklod value of three as minimum threshold value between two markers, indicate that linkage is 1000 times more likely than no linkage (Stam, 1993). The final step of linkage mapping is determining map distance and locus order (Semagn et al., 2006c).

2.5.1 STRIGA QTL MAPPING

Molecular markers for resistance to *Striga hermonthica* in sorghum were mapped in two recombinant inbred population (RIP) of F3:5 lines from a cross between IS 9830 x E36-1 and N13 x E36-1. IS 9830 resistance mechanism has low stimulation of Striga seed germination where N13 has mechanical resistance (Maiti et al., 1984). Recombinant inbred population 1 was a cross IS 9830 x E36-1 with a genetic map of 1498cM with 137 markers distributed over 11 linkage groups while recombinant inbred population 2 was from the cross of N13 x E36-1 with a genetic map of 1599cM with 157 markers spread in 11 linkage groups (Haussman et al., 2004). Five QTL mapped from these populations were validated across environment and years (Haussmann et al., 2004).

These five QTL have been identified in linkage group 1, 2, 5 (2 QTL) and 6 using the revised linkage group (Kim et al., 2004) as shown in figure 5 and each of this QTL accounts for 12 to 30% of the total variation observed for Striga resistance. This variation is quantitative therefore the resistance conferred is expected to be broad and durable (Kim et al., 2004). Simple sequence repeats (SSR) markers (table 3) to the QTL are available for use in Marker Assisted Selection. This study utilized the five genomic regions (Quantitative Trait Loci) to select for Striga resistance (Haussmann et al., 2004).

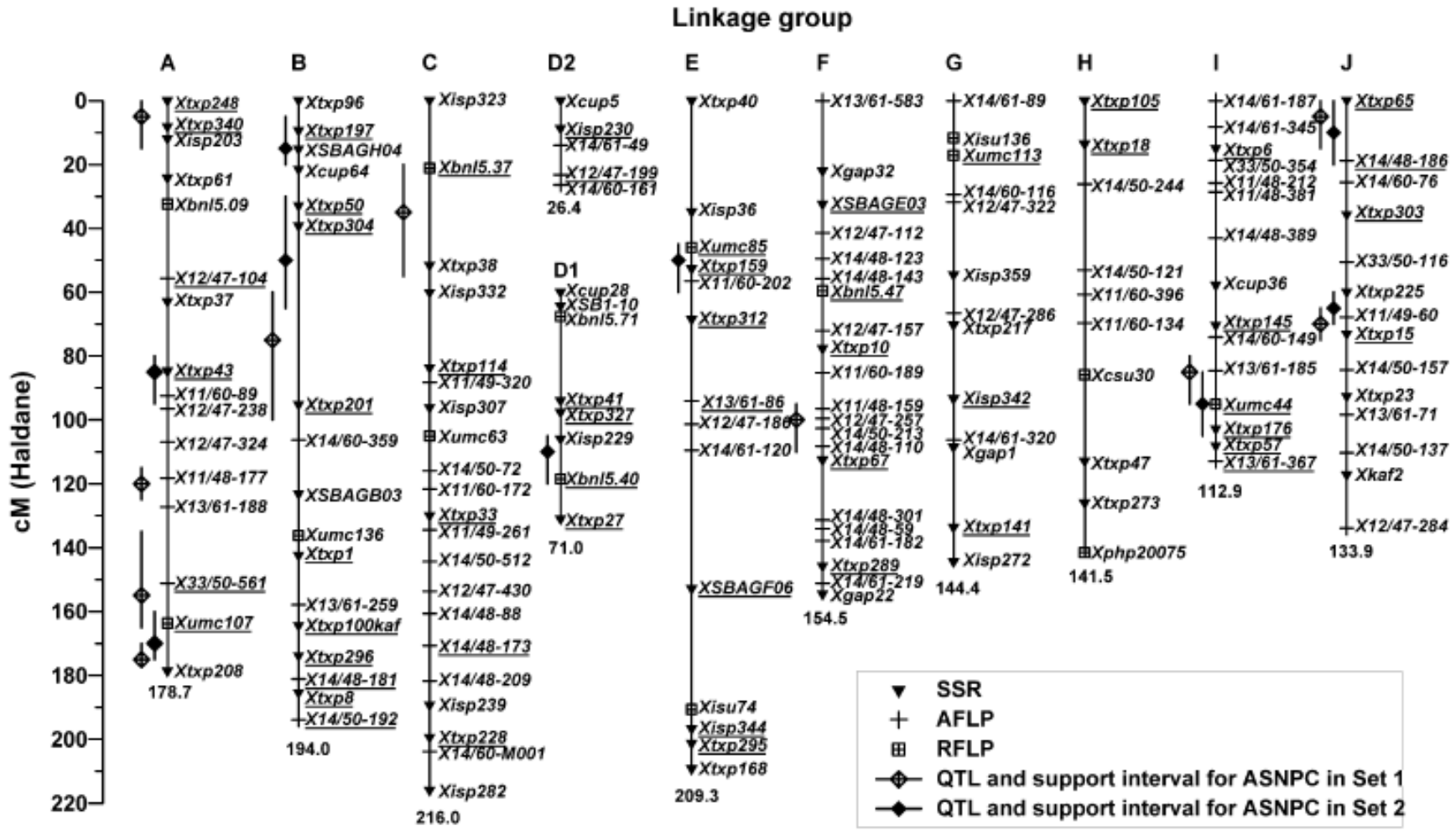


Figure 5. Genetic linkage map of RIP (N13 X E36-1) (Haussmann et al., 2004b). Linkage groups are named according to Bhattramakki et al., 2000.

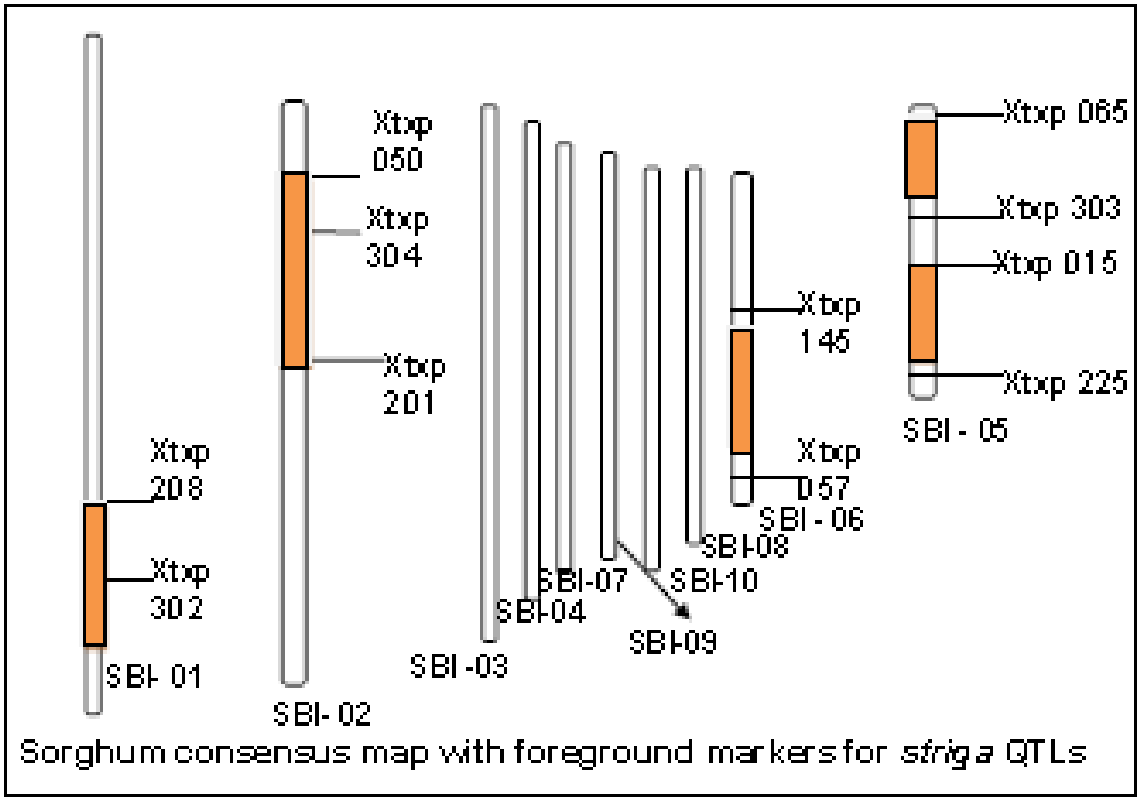


Figure 6. Summary of the foreground SSR markers used

The Figure 6 shows the polymorphic SSR markers used in this study for foreground selection. The SSR markers shown were selected from the genetic map in Figure 5.Table 3 below shows the location and interval length of the polymorphic SSR markers used in this study.

Table 3. Summary of SSR markers used in the study from the above genetic linkage

QTL	Linked SSR marker	Interval Length (cM)
Chromosome A - Linkage Group 1	Xtxp208 and Xtxp302	45
Chromosome J_1 – Linkage Group 5	Xtxp303 and Xtxp065	40
Chromosomes B – Linkage Group 2	Xtxp201, Xtxp50 and 304	50
Chromosome I – Linkage Group 6	Xtxp145 and Xtxp057	40
Chromosome J_2 – Linkage group 5	Xtxp015 and Xtxp225	20

Table 3 shows the chromosome location and the distance in centimorgan units between the polymorphic SSR markers used for foreground selection.

CHAPTER 3

Backcross generation and Striga QTL analysis in the backcrosses

3.0 ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is the second most important cereal crop in Eastern Africa after maize. Among other constraints its production is greatly affected by the parasitic weed Striga. *Striga hermonthica* is the most important species in Kenya. Marker Assisted Selection (MAS) was used to introgress Striga resistance Quantitative Trait Loci (QTL) into a Kenyan adapted farmer preferred sorghum variety, Ochuti. N13 an Indian durra sorghum with mechanical and antibiosis resistance mechanism to Striga, was used as a donor parent. Five genomic regions and molecular markers associated with stable Striga resistance have been identified and these QTL are on Chromosomes A (LG1), B (LG2), I (LG6) and 2 QTLs on J (LG5). The seeds of BC_3F_1 obtained from the previous cross of N13 x Ochuti were advanced to BC_5F_1 and BC_3F_3, this work was done in a greenhouse at the University of Nairobi, Upper Kabete campus. BC_5F_1 were generated by backcrossing to Ochuti and BC_3F_1 seeds were selfed to BC_3F_3.

Molecular work was done at the BecA laboratories using 11 polymorphic SSR markers for foreground selection. Five plants were identified with Striga resistance QTL introgressed, two plants in BC_3F_1 and three plants in BC_3F_2 having four to one QTL. Hence from the study, it is possible to use MAS to transfer Striga resistance QTL.

3.1 INTRODUCTION

Striga has been a major constrain in sorghum production. Many breeding advances have been geared towards resistance of the crop to this weed. Sorghum being among crops with perfect flower (both sexes in the same floret), its effective crossing involves removal of anthers from the florets/emasculation to be crossed before dehiscence (House, 1980). Crossing between normal male fertile lines can be accomplished by hand emasculation or treatment with hot water or use of plastic bag technique. However, effective use of these techniques on one hand require skills as floret can easily be damaged at the same time during hand emasculation resulting in no seeds forming or self-pollination, on the other hand use of hot water requires the head to be soaked for the correct period of time at correct temperature, so as to kill the male organs and not the female organs (House, 1985).

The use of Marker assisted selection (MAS) technique requires genotyping of large number of individuals. This technique ensures high throughput genotyping which is essential to obtain sufficient quality DNA necessary for generating robust and easily scored data with minimal repeats, hence cutting down cost for DNA extraction and molecular work (Mace et al., 2003). The efficiency of Marker assisted backcrossing (MABC) depend on, number of markers and their position in relation to the targeted gene, experimental design and selection strategy (Matthias and Albrecht, 2005).

The objective of laboratory work was to identify and confirm Striga resistant QTL using the polymorphic SSR markers (foreground selection) and the backcrosses were being generated to fix the identified Striga resistant QTL and also increase the background of the donor parent (Ochuti) in the backcrosses.

3.2 PLANT MATERIALS FOR GENERATION OF BACKCROSSES

Four genotypes were used in this study. Two back cross lines 87 and 33 both at BC_3F_1 with supposed four QTL each (they are a product of N13 x Ochuti), while N13 was used as the donor parent and Ochuti was used as the recurrent parent. BC_3F_1 seeds were obtained from the previous Federal Ministry for Economic Cooperation and Development (BMZ) project (Both genotypic and phenotypic data was not available for this study). Back crosses were generated in a greenhouse at the University of Nairobi, Upper Kabete Campus. Two back crosses were done to advance the materials to BC_5F_1 and BC_3F_1 seeds were selfed twice to BC_3F_3. The work was done between December 2009 and March 2011.

3.2. 1 GREEN HOUSE ACTIVITIES

The parental line (Ochuti) was planted one week earlier to synchronize flowering because it flowers later than N13 and backcrosses. Planting of back crosses was staggered to avoid synchronous flowering and also reduce the work load of hand emasculation. The seeds were sown directly in the buckets which had a mixture of manure, soil and sand in the ratio of 2:1:1 respectively. After two weeks of seedling emergence, leaves of all plants were cut, put in eppendorff tubes with 96% ethanol and taken to BecA laboratories for DNA extraction and analysis.

Hand emasculation technique used in this study because only a small quantity of seed was needed. Sorghum florets were emasculated a day before anthesis, toothpick was inserted between the outer glumes of the sessile spikelets to tease out the anthers. The remaining fertile spikelets on the panicle were removed and the sorghum head were bagged with a mafuco bag with the date of emasculation to prevent foreign pollen from landing on the stigmas and pollinating the next morning (Reddy and Kumar 2008). Pollen for crossing were collected from Ochuti in the mornings usually between 0700 and 1200hours when anthers dehisce, the bag with the collected

pollen was put over the emasculated sorghum head, tapped to release the pollen to the stigma. The bag was pinned and date of pollination was indicated. Ochuti was used as the pollen donor at this stage of backcrossing in order to recover the maximum amount of its characteristics (House, 1985 ,Reddy et al., 2008).

3.3 MATERIALS AND METHODS

Materials used in this study are described in table 4 below.

Table 4. Number of samples genotyped per generation

Generation	Line 87	Line 33	Parents	Total	Location
BC_3F_1	24 plants	42 plants	6 plants	72 plants	Green house
BC_4F_1	112 plants	176 plants	6 plants	294 plants	Green house
BC_3S_1 (BC_3F_2	57 plants	72 plants	6 plants	135 plants	Green house
BC_3S_2 (BC_3F_3)	45 plants	32 plants	6 plants	83 plants	Field at Alupe & Kibos

The starting material for this study was the BC_3F_1 generation whereby seventy two samples were genotyped. Some of the plants in this population were selfed to produce BC_3S_1 (BC_3F_2), and the remaining BC_3F_1 population plants were backcrossed to Ochuti to generate BC_4F_1. 294 plants of BC_4F_1 were genotyped and these materials further backcrossed to BC_5F_1 which were not genotyped as this fell outside the scope of this study (Table 4). The number of samples genotyped for BC_3S_1 (BC_3F_2) was 135 plants. Both BC_3F_1 and BC_4F_1 were sampled from the plants in the Green house and BC_3S_2 (BC_3F_3) was sampled from the field trials plots at Kibos and Alupe.

3.4 FOREGROUND ANALYSIS

3.4.1 DNA extraction

Genomic DNA was extracted using the high through-put protocol modified from Mace et al., 2003. All the leaf samples collected from University of Nairobi green house were cut into smaller pieces and approximately 50mg was put in the extraction tubes with two steel beads. The samples were dipped in liquid nitrogen to freeze them and make them brittle for efficient grinding.

CTAB extraction buffer was pre-heated at (65^{o}C) and 400 µl was added in each extraction tube with the leaf samples and placed in a Genogrinder at 1500 revolutions per minute until it was finely ground. The extraction buffer contained 3% (w/v) Cetyl trimethyl Ammonium Bromide (CTAB). The macerated substance was transferred to clean tubes and incubated for 15 minutes at 65^{o}C with occasional mixing. Solvent extraction/phase separation was done by adding 350 µl of chloroform isoamyl alcohol (24:1) to each sample and inverting twice to mix. It was then centrifuged at 3000 rpm for 15 minutes at 24^{o}C. About 400 µl of supernatant of each sample was transferred to clean extraction tubes and 400 µl of isopropanol (stored at -20^{o}C) was added and inverted to mix well and stored overnight at -20^{o}C. Isopropanol facilitates precipitation of DNA to give visible DNA strands. The next day the tubes were centrifuged for 20 minutes at 3000rpm at 4^{o}C to pellet the nucleic acids. The supernatant was decanted and the pellet left on the bench to dry. The pellet consisted of crude DNA, low -salt TE buffer (10mM Tris and 0.1mM EDTA) 200 µl and 3 µl RNase A (10mg/ml) was added to each sample and incubated overnight, the RNase digests RNA (modified Mace et al., 2003, Doyle and Doyle ,1987)

Another solvent extraction was done by adding 200µl of chloroform isoamyl alcohol (24:1) to each sample and inverted twice to mix. It was centrifuged at 3000 rpm for 10 minutes. The

aqueous layer approximately 180 µl was transferred to clean eppendorrf tubes and 315 µl of ethanol sodium acetate solution for purification was added to each sample and placed at -20 ^{o}C for 5 minutes. Centrifugation was done and supernatant was decanted and each pellet washed with 200 µl of 70% ethanol. The pellet was air dried for one hour and re-suspended in 100 µl of low-salt TE buffer and stored at -20 ^{o}C for long term storage and 4 ^{o}C for immediate use. Low salt buffer was used to maintain the integrity of the DNA as it protects it from degradation (Mace et al., 2003)

3.4.2 DNA quality and quantity check

DNA quality check was done using 0.8% (w/v) agarose gel electrophoresis stained with gel red (Biotium, USA) (5µl/100ml). 0.8 g of agarose was weighed and mixed with 100ml 1X TBE buffer (Tris borate-EDTA-{EDTA disodium, Tris base and Boric acid}(Mace et al., 2003). The mixture was heated in the microwave until the agarose dissolved in the buffer, and 5µl of gel red was added. The gel tanks were prepared with the appropriate comb and the solution was poured into the casting gel trays and left to solidify, the combs were slowly and carefully removed. 6 µl (4µl of DNA and 2µl of loading dye) were loaded into each well and the first wells of each row in the gel only 1.5µl of 100bp DNA ladder was put. DNA ladder was used to determine the size of the genomic DNA from 100 base pairs to 10,200 base pairs. The gel was run for around 1 hour at 120 volts and the gel was then scanned under UV light transilluminator. DNA with solid high molecular weight bands indicated non-degraded DNA and smeared bands indicated degraded DNA (CIMMYT, 2005).

Nanodrop spectrophotometer (Thermo Fischer Scientific) was used to determine the DNA quantity and quality at absorbance ratio of 260/ 280 nm and 230/280 nm (Mace et al., 2003). Pure DNA reading range between 1.8 and 2.0 (Semagn et al., 2006a, Thermo Fisher Scientific Inc, 2008). These Nanodrop readings were used to determine the amount of T E buffer (Tris –cl

and EDTA) to be used to dilute each sample to an even stock solution of 100ng/µl and a constant working concentration of10ng/µl for PCR reactions.

3.4.3 Foreground selection

The SSR markers used were both directly labelled and M13 tailed. The M13 forward primers were 5- tailed with 19 base pairs with M13 universal sequence (CACGACGTTGTAAAACGAC) and the M13 universal sequence primer was also 5' tagged with VIC, NED, FAM and PET fluorescent dyes (Markus Schuelke, 2000). Eleven SSR markers were used for foreground selection, presented in Table 5. Background selection was not done as all the samples had Ochuti alleles in the foreground selection. The foreground markers were sourced from Bioneer Company.

Table 5. Details of the SSR markers used for foreground selection

Primer Name	Primer type	Dye	Colour	Repeat Motif	ALLELE 1 (N13)	ALLELE 2 (OCHUTI)	Chromosome/ Linkage group
Xtxp 302	Directly labelled	VIC	Green	(TGT)8	237	196	Chromosome A/ Linkage Group 1
Xtxp 303	Directly labelled	NED	Yellow/ Black	(GT)13	150	152	Chromosome J1/ Linkage Group 5
Xtxp 201	Directly labelled	VIC	Green	(GA)36	183	188	Chromosome B/ Linkage Group 2
Xtxp 015	Directly labelled	NED	Yellow/ Black	(TC)16	217	219	Chromosome J2/ Linkage Group 5
Xtxp 208	Directly labelled	FAM	Blue	(GGA)8	260	257	Chromosome A/ Linkage Group 1
Xtxp 304	M13 - Tailed	FAM	Blue	(TCT)42	323*	231*	Chromosome B/ Linkage Group 2
Xtxp 225	M13 - Tailed	NED	Yellow/ Black	(CT)9(CA)8CCC(CA)6	183*	187*	Chromosome J2/ Linkage Group 5
Xtxp 145	M13 - Tailed	PET	Red	(AG)22	262*	232*	Chromosome I/ Linkage Group 6
Xtxp 057	M13 - Tailed	PET	Red	(GT)21	261*	268*	Chromosome I/ Linkage Group 6
Xtxp 065	M13 - Tailed	VIC	Green	(ACC)4+(CCA)3CG(CT)8	149*	151*	Chromosome J1/ Linkage Group 5
Xtxp 050	M13 - Tailed	NED	Yellow/ Black	(CT)13(CA)9	316*	314*	Chromosome B/ Linkage Group 2

*They have 19 extra base pairs from their actual allele size they are M13-tailed. **(**Haussmann et al., 2004b and Bhattramakki et al., 2000)

3.4.4 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) amplification was done using GeneAmp PCR systems 9600 (PE-Applied Biosystems). PCR components for directly labelled SSR markers included, 1X PCR buffer without $MgCl_2$, 2 mM $MgCl_2$, 0.2 µM forward primer, 0.20 µM reverse primer, 0.04 mM of each of the four dNTPs, 0.2 U DNA polymerase (Applied Biosystems) and 30 ng template DNA. Double distilled water was used to top up the reaction to 10 µl. PCR components for M13 tailed SSR markers included, 1X PCR buffer without $MgCl_2$, 2 mM $MgCl_2$, 0.08 µM forward primer, 0.12 µM of fluorescent-labelled M13 primer, 0.20 µM reverse primer, 0.04 mM of each of the four dNTPs, 0.2 U DNA polymerase (Applied Biosystems) and 30 ng template DNA. Double distilled water was used to top up the reaction to 10 µl. After PCR, a few samples from each primer pair were randomly selected and checked for amplification in a 2% (w/v) agarose gel (modified from Dilworth and Frey., 2000 and Paris and Carter., 2000)

3.4.5 Genotyping

Genotyping was done through fragment analysis using a fluorescent fragment detection system on an ABI 3730 DNA Sequencer. For high throughput and low cost genotyping, PCR products were co-loaded in set of 3 markers. Samples for genotyping were prepared as follow; 0.125 µl of GeneScan™ LIZ 500 internal lane size standard (Applied Biosystems) and 8µl of HI-DI™ Formamide (Applied Biosystems) were added in each co-loaded sample. Liz standard and HIDI mixture was prepared in the ratio 49:1. Liz standard was used to size DNA fragments in the Applied Biosystems fluorescence - based DNA electrophoresis system and HIDI was used to ensure that the DNA fragments stayed single stranded after they were denatured. The PCR products to be sent for genotyping were determined by the type of dye used and the strength of the band as seen on the 2% agarose gel. For PET and NED dyes more PCR product was picked

as they are weaker dyes hence fluoresce less, hence a minimum of 3.0 µl and a maximum of 3.5µl. For VIC and FAM since they are strong dyes and fluoresces more, a minimum of 1.8 µl and a maximum of 2.5 µl was used. The mixture was denatured at 94°C for 5 minutes then cooled on ice immediately for denaturation purposes. Samples were sent to Segoli laboratory at the BecA/ILRI hub for capillary electrophoresis. The denatured DNA fragments were size-fractioned using an ABI 3730 Capillary DNA Sequencer (PE-Applied Biosystems) using the default parameters but with an injection time of 40 seconds. The peaks were sized and the alleles analyzed using GeneMapper V 4.0 software. The co-loading sets used in this study is shown in table 6

Table 6. Co-loading sets used in the study

MARKER	Amount used	Dye used	N13 allele	Ochuti Allele	Primer type
Co-loading Set 1					
Xtxp 065	2.5 µL	VIC	149*	151*	M13-tailed
Xtxp 208	2.0 µL	FAM	260	257	Directly labelled
Xtxp 050	3.0 µL	NED	316*	314*	M13-tailed
Co-loadingSet 2					
Xtxp 057	2.5 µL	FAM	261*	268*	M13-tailed
Xtxp 145	3.5 µL	PET	262*	232*	M13-tailed
Xtxp 015	3.5 µL	NED	217	219	Directly labelled
Co-loading Set 3					
Xtxp 302	2.0 µL	VIC	237	196	Directly labelled
Xtxp 303	3.5 µL	NED	150	152	Directly labelled
Xtxp 304	2.0 µL	FAM	323*	231*	M13-tailed
Co-loading Set 4					
Xtxp 225	3.5 µL	NED	183*	187*	M13-tailed
Xtxp 201	2.5 µL	VIC	183	188	Directly labelled

* allele size for M13 tailed primer, i.e. it has extra 19 base pairs)

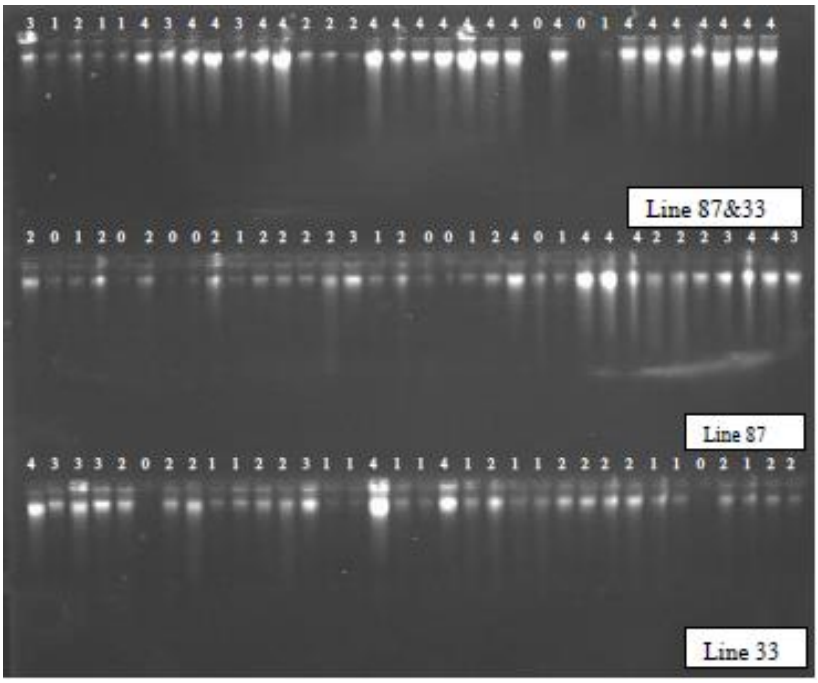
3.4.6 Data analysis

GeneScan^{TM} LIZ 500 internal lane size standard (Applied Biosystems) is capable of sizing DNA fragments/ PCR product in the 35-500 base pairs range, therefore providing 16 single-stranded labelled fragments. Each of the DNA fragments is labelled with the LIZ fluorephore, which results in a single peak when run under denaturing conditions. GeneMapper V4.0 software was used to perform size calling which includes peak detection and fragment size matching. The software uses reference data to construct bins where peaks are detected and the alleles are automatically assigned allele calls based on the bin definitions. Algorithms determine if peaks represent alleles. When a peak from a data sample matches the location of a bin, an allele call was done manually. Once the analyses were complete the results were exported in table form to Microsoft Excel (AppliedBiosystems, 2005).

3.5 Results

3.5.1 DNA quality check.

The extracted DNA quality was checked using 0.8% (w/v) agarose gel stained with Gel Red (Biotium, USA) (5µl/100ml). Figure 8 shows the quality of the extracted DNA. Sharp DNA that are separated as solid bands indicate good quality DNA that was not degraded and faint bands or smears indicate presence of sheared DNA or presence of RNA (Figure 7).



0.8%agarose gel run for 30 min at 110 V

Figure 7. Agarose gel showing the quality of genomic DNA from BC_3F_1

The key for interpreting the gel, 4 indicated very good DNA, 3 for good DNA, 2 for fair DNA and 1 for sheared/degraded DNA. The results show that isolated genomic DNA was of high molecular weight as indicated by the presence of intense bands. Faint bands indicate that the amount of DNA is low while some bands show presence of sheared DNA/ degraded DNA or presence of RNA.

The DNA quantity results from Nanodrop readings for BC_3F_1 on Figure 8 showed that 49 samples had a range of 1.8 to 2.0 at A260/280nm, which is pure DNA, 23 samples had below 1.8 at A260/280nm, 15 samples had 1.7, 5 samples had 1.6 and the other 3 had 1.5, 1.4 and 0.8. Figure 9 shows BC_4F_1 samples with absorbance 260/280nm ranging from 1.8 to 2.0 was 276 samples with pure DNA, 6 samples had absorbance ratio of above 2.0 and 6 samples had below 1.8. Samples having good standard quality were diluted to a standard concentration for use as template in PCR reactions with the 11 foreground primer pairs.

DNA samples with low quality were discarded and DNA extraction repeated from fresh tissues to replace them. Nanodrop readings are attached in the appendix I and II.

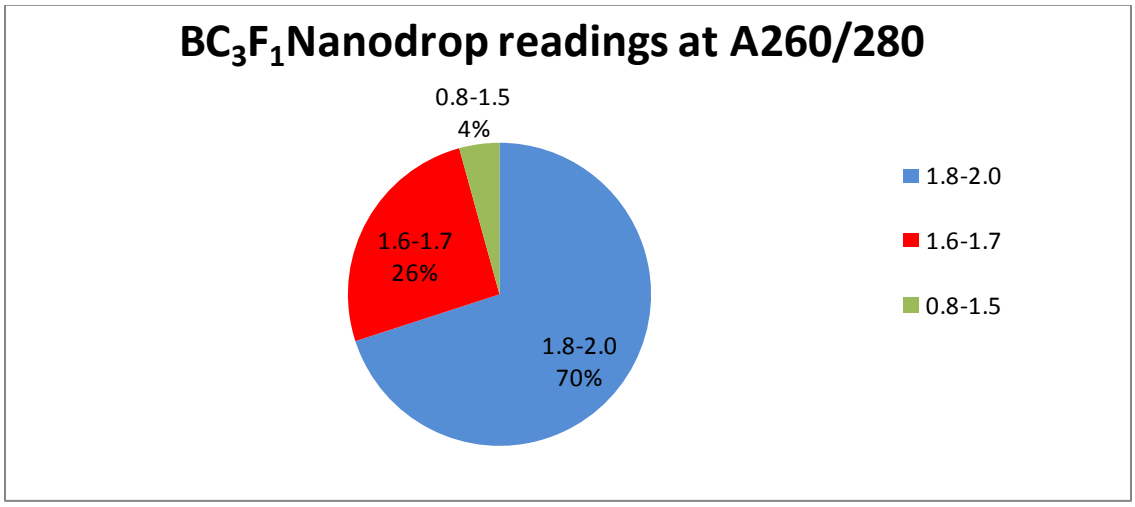


Figure 8. A pie chart showing Nanodrop readings of 72 samples of BC_3F_1

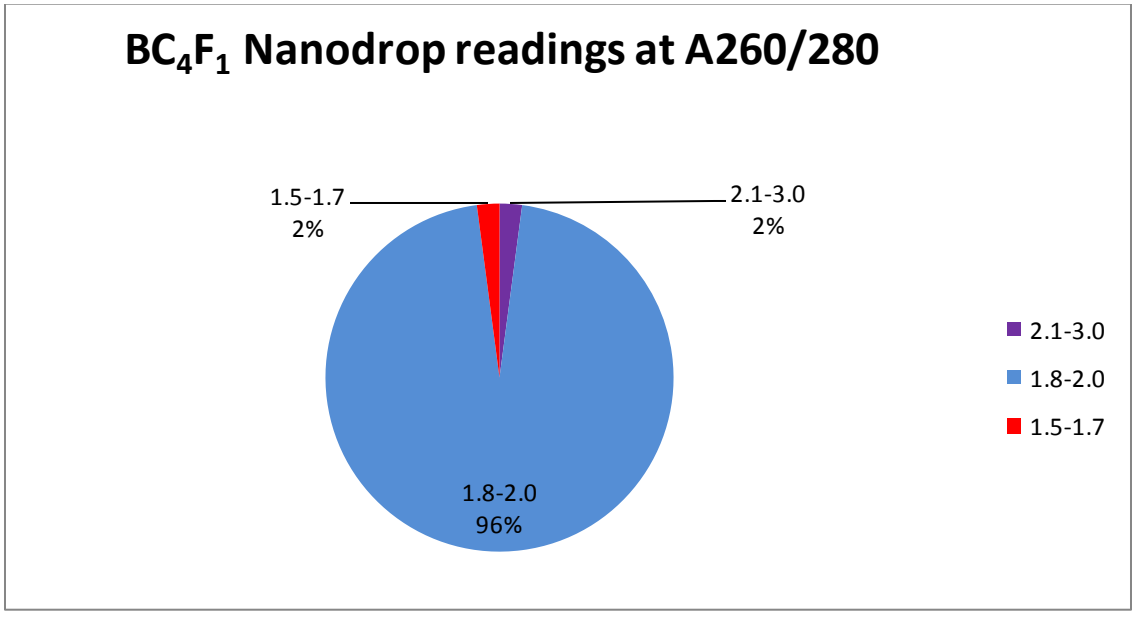
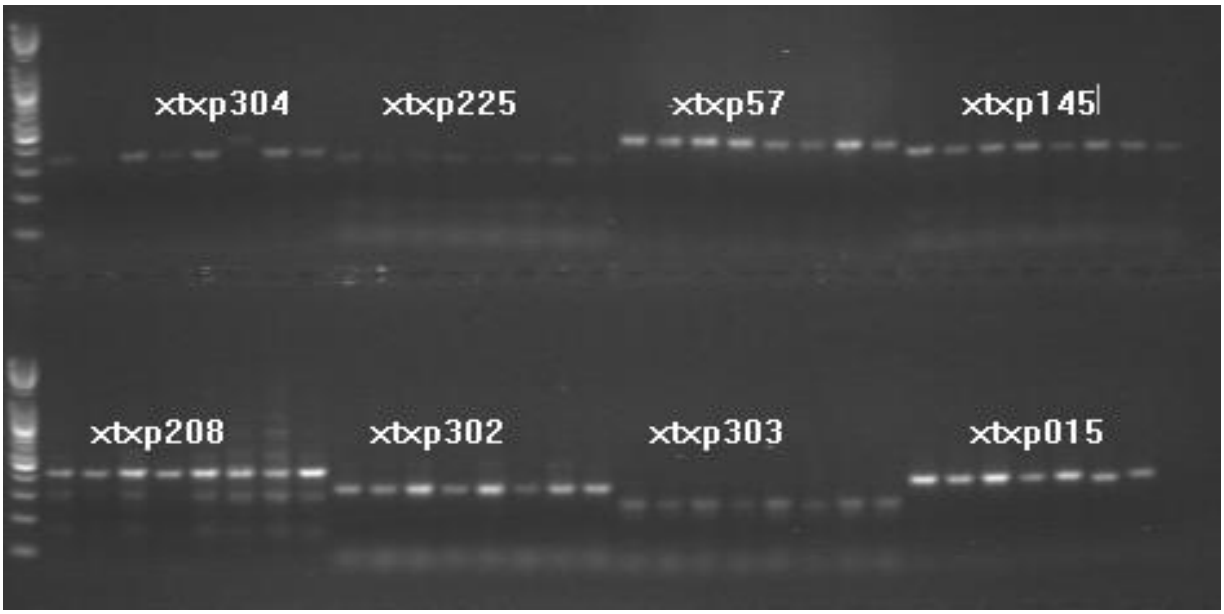


Figure 9. A pie chart showing Nanodrop readings of 288 samples of BC_4F_1

The agarose gel electrophoresis and Nanodrop reading showed that the extracted DNA was suited to be used for PCR and genotyping therefore, the extracted genomic DNA was amplified using a PCR .The PCR products were separated through capillary electrophoresis and Gene Mapper software V4.0 was used for DNA sizing and quality allele calling.



2%agarose gel run for 30 min at 110 V

Figure 10 . Agarose gel image of amplification of some of the markers used in foreground selection.

The gel image in Figure 10 shows that amplification was successful for all the markers except Xtxp 225. The best amplified markers were Xtxp 208, Xtxp 302, Xtxp 015, Xtxp 057 and Xtxp 145. Markers Xtxp 304 and Xtxp 303 did not amplify very well.

3.5.2 Foreground selection

Among the 72 samples at BC_3F_1 genotyped only two plants showed the Striga resistance QTL (Table 7). Out of the 83 samples ($BC_3F_3/BC_3S_{2)}$ collected from the plants in the field trials at Kibos and Alupe, no QTL was identified. In the BC_4F_1 and BC_3F_2/BC_3S_1 generations that were analysed no QTL were observed as the plants with QTL in BC_3F_1 were selfed and not

backcrossed to the next generation, therefore their progenies were not among the samples genotyped in BC_4F_1.

Table 7. Results for foreground selection for BC_3F_1 and BC3F2 (BC_3S_1)

PLANT ID	SBI-01 QTL A				SBH-02 QTL B						SBH-06 QTL I				SBH-05 QTL J1				SBH-05 QTL J2			
	Xtxp 208		Xtxp 302		Xtxp 050		Xtxp 201		Xtxp 304		Xtxp 145		Xtxp 057		Xtxp 065		Xtxp 303		Xtxp 225		Xtxp 015	
	N13	Ochull	N13	Ochull	N13	Ochull	N13	Ochull		Ochull	N13	Ochull	N13	Ochull	N13	Ochull	N13	Ochull	N13	Ochull	N13	Ochull
BC3F1/L33/p25	260	257	237	196					323	231		232				151	150		183	187	217	219
BC3F2/L33p25p7	260		237		317		202		324		260		260		150		169					
BC3F2/L33p25p13	260			197		315	207	202	324	232			266		150	152	169	171	208	188	236	238
BC3F1/L33/p27							183		323	231	262	232					150				217	219
BC3F2/L33p27p16	260			197		315	207	202	324	232			266		152		171	169	208	188	236	238

KEY

Introgressed QTLs for BC3F1
Fixed QTLs for BC3F2
Heterozygous QTLs for BC3F2

From the 207 genotyped plants for BC_3F_1 and BC_3F_2, only five plants had Striga resistance QTL introgressed (Table 7), two plants from BC_3F_1 (L33p25 and L33/p27) and 3 plants in BC_3F_2 (L33/p25/p7, BC_3F_2 L33/p25/p13 and BC_3F_2 L33/p27/p16). BC_3F_1 L33p25 had QTL A flanked by xtxp 208 and xtxp 302, QTL B flanked by xtxp 50, xtxp 201 and xtxp 304, QTL J1 flanked by xtxp65 and xtxp 303 and QTL J2 introgressed, plant L33p27 had QTL J2 introgressed and in BC_3F_2 plant L33/p25/p7 had fixed QTL at A, B, I and J1, plant L33/p25/p13 had J1 and J2 in heterozygous state and plant L33/p27/p16 had J2 in heterozygous state.

3.6 Discussion

The extracted DNA was of high quality and quantity as the Nanodrop readings indicated, many samples had pure DNA of optical density (OD) at A260/280 ranging from 1.8 to 2.0. Samples that had absorbance ratio of less than 1.6 indicate the presence of co-purified contaminants like

protein and absorbance ratio of more than 2.0 indicated contamination with RNA, the absorbance ratio used was A260/280nm (Thermo Fisher Scientific Inc, 2008).

DNA estimation by 0.8% (w/v) agarose gel was good as the gel showed some degraded/sheared DNA and some bands also indicated presence of high molecular weight DNA. Markers Xtxp 225 did not show amplification on 2% (w/v) agarose gel, and few alleles were called on GeneMapper software V4.0. Other markers that did not amplify at first were Xtxp 145, Xtxp 057 and Xtxp 015, but after PCR optimization (PCR profile was changed), the markers showed amplification except for Xtxp 225.

The numbers of targeted QTL in the foreground were 4 out of the possible 5 QTL for Striga resistance from N13 donor. The expected QTL are found on Chromosome A /linkage group 1, Chromosome J1/ linkage group 5, Chromosome B /linkage group 2, Chromosome I /linkage group 6 and Chromosome J2/ linkage group 5 and each of these QTL accounts for 12 -30% of the total variation observed for Striga resistance (Haussmann et al., 2004, Kim et al., 2004). The SSRs markers used were polymorphic hence they could distinguish between the alleles of donor parent (N13) and susceptible parent (Ochuti).

According to the results (table 7) five plants were identified with striga resistance QTL introgressed 2 plants in BC_3F_1 L33/p25 had QTL from chromosome A, B, J1 and J2 while L33/p27 had chromosome J2 and 3 plants in BC_3F_2 L33/p25/p7 had four fixed QTL in chromosomes A, B, I and J1, L33/p25/p13 had QTL on chromosome J1 and J2 introgressed in heterozygous state and L33/p27/p16 had chromosome J2 introgressed in heterozygous state. From these results, Chromosome A /linkage group 1 and Chromosome B /linkage group 2 were present in two plants out of the five plants, Chromosome I /linkage group 6 had one plant out of the five plants, Chromosome J1/ linkage group 5 had three plants out of the five plants and

Chromosome J2/ linkage group 5 had the four plants out of the five plants. Chromosome J2/ linkage group 5 had the least flanking distance (20cM) between the two SSR markers, while the other QTL were flanked by markers whom flanking distance ranged from 40-50cM, hence explaining their least frequency in this data set. These results are in correspondence to the findings of (Semagn et al., 2006 c) where more QTL were identified when the flanking distance was 5-20cM. These foreground selection result for marker assisted backcrossing were also similar to the result got by Neeraja et al., (2007) and Alam et al.,(2012) from their study on introgressing various resistant genes in rice for different abiotic stresses.

The ideal number of plants to genotype to capture 5 QTL would have been 1024 plants per generation (Hospital and Charcosset, 1997), in this study there was a shortage in number of seeds available, hence the reason why small number of plants were planted and sampled for analysis in the laboratory. There are several reasons why few plants were identified with Striga resistant QTL, the number of plants per population genotyped was below the ideal size to capture the 5 QTL and the flanking distances between the markers used and QTL were too large, hence recombination could have occurred during any of the backcrosses or selfing activities and this would have led to the loss of the intended QTL or partial introgression of the QTL in the majority of the progenies of each population. In this study the flanking marker distance ranged from 20-50cM while the ideal size would be 5-20cM (Semagn et al., 2006 c). Use of at least a pair of flanking markers is preferred for use to a single perfect marker which is rarely available. In addition the use of third marker further reduces the chances of loss of the favourable allele at the target QTL due to multiple recombination events (Hash and Senthilvel, 2008).

3.7 Conclusion

From the study, good quality and quantity DNA was obtained and amplified with PCR

A total of 584 plants were genotyped and foreground selection was performed for all them (table 4). Foreground selection was carried out using 11 polymorphic SSR markers. Out of the genotyped plants, 5 plants were found to contain Striga resistance QTL, the other plants no Striga QTL were identified with the used 11 SSR markers, this would be due to failure of backcrossing or the markers were not tightly linked the QTL or recombination took place. The five plants confirmed to have the Striga resistance QTL will be selfed and further selection done.

CHAPTER 4
EVALUATION OF STRIGA RESISTANCE IN BACKCROSSES WITH INTROGRESSED RESISTANCE QTL IN WESTERN KENYA

4.0 ABSTRACT

Field trials were conducted in Western Kenya in Alupe in Busia county and Kibos in Kisumu county. The trials were sown in May 2010 and October 2010 with replications. The back cross lines namely BC_3S_1/L33 and BC_3S_1/L87 with introgressed striga resistant QTL along with their parental lines namely donor line N13 and recurrent parent Ochuti were evaluated in the field during May to October 2010 and October to March 2011 seasons, at both locations. According to the Area Under Striga Progressive Curve total score (AUSPC total), genotype BC_3S_1/L87/H1evaluated at Kibos during May to October 2010 season had the least AUSPC value, while genotype BC_3S_1/L87/H5 evaluated at Alupe during May to October 2010 season had the highest AUSPC value. The backcross lines BC_3S_1/L33 and BC_3S_1/L 87 had lower Striga scores throughout the two seasons in both locations than the susceptible parental line Ochuti, confirming that the Striga resistance QTL had successfully been introgressed into Ochuti.

4.1 INTRODUCTION

Successful screening for Striga resistance in the field is hampered by various factors, including heterogeneity of natural field infestations, large environmental effects on Striga emergence and complex interactions between host, parasite and environment. Various methodologies have been suggested for effective screening. Haussmann et al., (2000a) summarized improved field testing methodologies, which included consideration of many variables such as field inoculation with Striga seeds, use of appropriate experimental design, plot layout and large number of replications. Equally important in scoring for Striga resistance is the inclusion of susceptible and resistant checks at regular intervals, evaluating in adjacent infested and un- infested plots and using selection indices such as Striga count, Striga vigour, grain yield and host plant damage

score. Field inoculation with Striga has also been recommended for effective field screening (Kim, 1991, Efron, 1993, Kim and Adetimirin, 1994, Berner et al., 1996b). However variation in the number of emerged Striga plants between plots of the same host cultivar can still be considerable despite careful field inoculation (Vasudeva Rao, 1985, Efron, 1993, Haussmann et al., 1999). This can be due to micro variability of soil fertility and variation in the natural base level of Striga within the experimental area. Differences may also be caused by local occurrence of natural Striga antagonists like *Fusarium oxysporum (*Haussmann et al., 1999).

The objective of this study was to evaluate the performance of the backcrosses under artificial Striga inoculation under field conditions. The yield performance of the backcrosses was used to select for Striga resistant and tolerant plants.

4.2 MATERIALS AND METHODS

4.2.1 FIELD TRIALS AT ALUPE AND KIBOS

On station trials were established at Alupe and Kibos which are Striga infested hot spot areas in Kenya, and past experiments for defining Striga resistance have been carried out there (Haussmann et al., 2000a,b, 2004, 2001a,b, Omanya et al., 2004). These experimental sites are in agro-ecological zone III (FAO 1996). Soils found in Kibos are retroentric, planosol, sandy loam, while those at Alupe are orthic, ferrosol, and part petroferric with orthic acrisols (Appendix III). Kibos is located at 00°04´ latitude and 34 ° 48´E longitudes and has an elevation of 1214 masl , while Alupe is located at 00°29´latitude and 34 ° 08´E longitudes with an altitude of 1189 masl (Haussmann et al., 2004).

The backcrosses were sown in randomized complete block design (RCBD) with three replications during May to October 2010 season and four replications for October 2010 to March 2011 season. The plots consisted of four rows with spacing of 30cm between plants and 75 cm

between rows. Approximately 3000 Striga seeds were artificially applied in each hill in all the plots. The Striga inoculum was prepared by mixing 10g of Striga seeds with 5kg of fine sand. The planting holes were infested with one table spoon scoop of Striga seed and sand mixture which consisted of approximately 3000 Striga seeds (IITA, 1997). Data was collected from the plants on the inner two rows, outer rows and outer plants from the two mid rows were avoided to eliminate border effect. After harvesting seeds were bulked per row.

4.2.2 DATA COLLECTION AND STATISTICAL ANALYSIS

Data for the following traits were recorded, days after planting to Striga emergence, days to 50 % anthesis of sorghum, sorghum plant height from the ground to the tip of the panicle, number of tillers per plot, number of stem lodged plants, seedling vigour score, severity score, stand after thinning, number of Striga forming flowers and capsules, panicle weight and 100 seeds weight. Grain yield was recorded after harvesting and Striga infestation count was scored at two week intervals from day 42 after sowing to day 99 after sowing. Scale of 1-5 was used for scoring, 1 for very resistant, 2 for fairly resistant, 3 average, 4 below average and 5 for very susceptible according Haussmann, (2001b). The Area under Striga Progress Curve (AUSPC) was calculated in order to provide a quantitative measure of Striga infestation over the entire season according to Rodenburg et al., (2005) and this was done by summing the product of Striga plant counts and the number of days between observations.

The field data was subjected to general analysis of variance (ANOVA) using Genstat ® 12^{th} edition. Fixed model based on a randomized complete block design (RCBD) was used

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

Where

Y_{ij} = the observation in the i^{th} treatment of the j^{th} block

μ = the general mean

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

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

ϵ_{ij} = the experimental error

Karl Pearson's correlations using Proc corr in SAS ® was done for all the locations and seasons' data. Means were compared using Bonferroni test at $\alpha = 0.05$ significance level.

Broad sense heritability was calculated according to (Nyquist, 1991, Falconer and Mackay, 1996). Heritability in the study was determined on an entry –mean basis (Hallauer and Miranda 1981) defined as

$$H^2 = \delta^2_g / \delta^2_p$$

Where, δ^2_g is the genotypic variance and δ^2_p is the phenotypic variance as shown in above equation (Falconer and Mackay, 1996).

All the recorded data from the field was transformed into natural logarithms (log(X+c), where X is the original individual observation and c = 1.0. While for scores (Seedling vigour score and severity scores) square root transformation was done $((X+c)^{1/2}$, where , X is the original observation and c = 0.5. Transformations were done to meet the ANOVA assumptions as outlined by Sokal and Rohlf (1995) and Rodenburg, (2005).

Area Under the above ground Striga Number Progress Curve (ASNPC) was calculated as outlined by (Haussmann et al., 2000b). Haussmann et al., (2000b) used ASNPC formula used by Shaner and Finney (1977) to calculate "area under the disease progress curve" (AUDPC).

$$\sum_{i=0}^{n-1} = \left\lceil \frac{Yi+Y(i+1)}{2} \right\rceil (t\ (i+1).$$

Where,

n = the number of *Striga* assessment dates.

Y_i = the *Striga* count at the i^{th} assessment date.

t_i = the days after planting. at the i^{th} assessment date.

t_0 = the days after planting to *Striga* emergence minus 1.

Y_0 = is 0.

AUSPC was calculated by summing the product of Striga plant counts and the number of days between observations. AUSPC has shown to be the most discriminative, objective and complete measure for identification of both tolerance and resistance mechanisms (Rodenburg et al., 2005, Omanya et al., 2004).

4.3 RESULTS

4.3.1 Evaluation of Striga resistance in Kibos during May to October 2010 season

The traits that were highly significantly different (Table 8) included AUSPC 2, plant height, panicle weight, stand after thinning and tillers per plot. Heritability was high for the following traits AUSPC 2, stand after thinning, panicle weight, AUSPC total and plant height.

AUSPC 2 showed significant differences ($P \leq 0.05$) among genotypes and also had the highest heritability and the lowest CV therefore, AUSPC 2 was the best Striga measure in this season according to Table 8.

According to AUSNP total BC_3S_1L87/4H1 had the least number of emerged Striga throughout the entire season, while BC_3S_1L33/4H3 had the highest number of emerged Striga, closely followed by BC_3S_1L33/4H1. BC_3S_1L33/4H3 had high yield even though it was attacked more by Striga and hence had some level of tolerance. Panicle weight for N13 was significantly different from Ochuti and that of all backcrosses. All backcrosses (BC_3S_1/Lines 87 and BC_3S_1/L33) except BC_3S_1L87/4H4 had higher grain yield than Ochuti. The broad- sense heritability for panicle weight was high (0.8).

The following traits AUSPC 1, AUSPC 3, AUSPC 4, AUSPC total, Striga flowering, Striga capsule formation, 100 seed weight and days to 50% anthesis had low broad –sense heritability and did not show any significant differences ($P \leq 0.05$).

Table 8. The reaction of 8 backcross generations to *Striga* at Kibos during May 2010 to October 2010

Identity	Days to 50 % anthesis of sorghum	Number of tillers per plot	Plant height (cm)	Seedling vigour score	Stand after thinning	*Striga* capsule formation	*Striga* flowering	100 seed weight (g)	Panicle weight (g)	AUSNP 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total
$BC_3S_1L33/4H1$	4.51	3.1	5.49	1.47	4.43	2.79	3.31	3.48	6.93	2.93	4.71	5.51	6.11	6.72
$BC_3S_1L33/4H2$	4.58	3.37	5.44	1.78	4.4	2.66	3.33	3.47	7.06	2.16	4.08	4.76	5.79	6.29
$BC_3S_1L33/4H3$	4.52	3.4	5.5	1.63	4.46	2.31	3.15	3.6	7.05	1.93	4.69	5.61	6.14	6.77
$BC_3S_1L87/4H1$	4.58	3.63	5.51	1.73	4.12	1.25	2.52	3.38	6.97	0.69	1.95	3.5	5.31	5.6
$BC_3S_1L87/4H2$	4.56	3.61	5.43	1.87	4.36	1.44	2.49	3.47	6.92	1.25	4.03	4.49	5.22	5.83
$BC_3S_1L87/4H3$	4.55	3.49	5.29	1.86	4.38	1.58	2.3	3.65	6.81	1.03	2.84	3.49	5.17	5.72
$BC_3S_1L87/4H4$	4.55	3.39	5.45	1.72	4.43	1.77	3.16	3.45	6.84	0.9	4.32	5.12	5.77	6.35
$BC_3S_1L87/4H5$	5.28	3.44	5.5	1.51	4.44	2.77	3.45	3.56	6.92	1.03	3.66	4.51	5.88	6.23
OCHUTI	4.56	3.24	5.43	1.78	4.4	1.67	2.68	2.02	6.85	2.58	2.69	4.28	5.46	5.72
N13	4.56	2.52	4.53	1.08	2.88	1.67	2.45	0.64	4.2	0	0	4	5.07	5.48
Mean	4.63	3.32	5.36	1.64	4.23	1.99	2.88	3.07	6.65	1.45	3.3	4.53	5.59	6.07
l.s.d	NS	0.48	0.24	NS	0.32	NS	NS	NS	0.38	NS	1.42	NS	NS	NS
Cv (%)	2.5	5.9	2.2	7.7	1.7	43.1	22.3	16.7	3.3	11.3	6.5	6.1	10.8	8.6
Heritability (H^2)	0.3	0.4	0.6	0.4	0.9	0.1	0.1	0.4	0.8	0.5	0.9	0.5	0.5	0.6
F	NS	*	*	NS	*	NS	NS	NS	*	NS	*	NS	NS	NS

* Significant at P ≤ 0.05. NS Not Significant at 5 % level

4.3.2 Evaluation of Striga resistance in Alupe during May to October 2010 season

From Table 9, the traits that had significant differences among the genotypes were all Striga count measures (AUSPC), Striga flowering, Striga capsule formation, grain weight, panicle weight, 100 seed weight, stand after thinning, plant height and number of tillers per plot. All the traits in Table 9 gave high broad-sense heritability values. All the Striga counts in this trial were significant and can be used to differentiate the genotypes. AUSPC 2 and AUSPC 3 were the best measure as they had low CV value and the highest broad -sense heritability. There was a significant difference between N13 and the other genotypes according to AUSPC total. $BC_3S_1L87/4H4$ and $BC_3S_1L87/4H2$ had the least number of emerged Striga throughout the season, while $BC_3S_1L87/4H5$ had the most and the highest yield. It is likely therefore, that $BC_3S_1L87/4H2$ has resistant Striga QTL introgressed. AUSPC total had high broad - sense heritability and CV was low too.

Table 9. The reaction of 8 back cross generations to *Striga* at Alupe during May 2010 to October 2010

Entry	Number of tillers per plot	Number of stem lodged plants	Plant height (cm)	Stand after thinning	100 seed weight (g)	Panicle weight (g)	Grain weight (g)	*Striga* capsule formation	*Striga* flowering	AUSNP 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total
$BC_3S_1L33/4H1$	2.12	1.29	5.51	4.43	3.5	6.93	6.81	1.56	1.33	3.33	5.75	7.47	8.28	8.73
$BC_3S_1L33/4H2$	2.15	0.88	5.45	4.43	3.53	7.06	6.83	0.96	0.98	3.72	5.1	7.07	8.15	8.52
$BC_3S_1L33/4H3$	2.17	1.76	5.54	4.42	3.7	7.06	6.9	1.5	1.85	4.62	6.25	7.42	8.33	8.78
$BC_3S_1L87/4H1$	2.55	1.61	5.5	4.17	3.42	6.97	6.78	1.25	1.55	3.55	5.24	6.87	7.98	8.32
$BC_3S_1L87/4H2$	1.87	1.61	5.43	4.39	3.46	6.94	6.72	1.2	1.52	2.97	4.89	6.61	7.96	8.25
$BC_3S_1L87/4H3$	2.49	0.9	5.28	4.44	3.67	6.8	6.66	2.31	2.52	4.62	5.93	6.98	7.93	8.38
$BC_3S_1L87/4H4$	2.42	1.67	5.46	4.41	3.47	6.84	6.68	1.19	1.32	2.79	3.87	6.87	7.93	8.28
$BC_3S_1L87/4H5$	1.53	1.65	5.48	4.42	3.59	7.1	6.93	1.48	1.6	4.9	6.24	7.58	8.55	8.96
OCHUTI	2.16	1.83	5.47	4.4	2.37	6.85	6.68	1.77	2.12	4.18	5.81	7.36	8.37	8.76
N13	0	0	4.54	2.94	1.34	2.89	2.67	0	0	0	0	2	4.27	4.3
Mean	1.95	1.32	5.37	4.24	3.21	6.54	6.36	1.32	1.48	3.47	4.91	6.62	7.77	8.13
l.s.d	0.99	NS	0.26	0.26	1.24	1.32	1.24	1.01	1.28	2.06	2.05	1.41	1.93	1.94
Cv (%)	3.3	6.3	1.9	1.7	11.8	6.3	5.9	31.1	39.8	34.5	22	12.4	11	11.4
F	*	NS	*	*	*	*	*	*	*	*	*	*	*	*
Heritability (H^2)	0.8	0.7	0.9	0.9	0.8	0.9	0.9	0.8	0.7	0.8	0.9	0.9	0.8	0.8

* Significant at P ≤ 0.05. NS Not Significant at 5 %

4.3.3 Evaluation of Striga resistance in Kibos during October 2010 to March 2011season

Results from Table 10 shows that grain weight, AUSPC 4 and days to 50% of sorghum anthesis were significantly different among the genotypes. The broad - sense heritability was high for days to 50% of sorghum anthesis, grain weight, AUSPC 3, AUSPC 4, AUSPC total, Striga flowering, and Striga capsule formation.

AUSPC 4 showed significant differences among the genotypes and the measure is highly inherited. AUSPC 4 had a low CV and hence it was well measured in this trial. AUSPC total showed that BC_3S_2/L33/4/H1 supported the highest number of emerged Striga throughout the season while BC_3S_2/L87/4/H1 had the least number of Striga emerged. BC_3S_2/L33/4/H1 was more susceptible than Ochuti while, BC_3S_2/L87/4/H1 was more tolerant than Ochuti (Table 10).

Table 10. The reaction of 8 back cross generations to *Striga* at Kibos during October 2010 to March 2011

Identity	Days to 50 % anthesis of sorghum	Host Plant damage/ severity	Number of stem lodged plants	Seedling vigour score	Stand after thinning	*Striga* capsule formation	*Striga* flowering	100 seed weight (g)	Grain weight (g)	AUSNP 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total
$BC_3S_2L33/4H1$	4.47	1.27	3.48	1.1	4.16	3.1	3.13	1.28	7.12	0.68	3.7	5.51	6.41	6.81
$BC_3S_2L33/4H2$	4.53	1.41	3.11	1.4	4.14	2.9	2.83	1.26	6.95	2.03	3.68	5.23	6.29	6.65
$BC_3S_2L33/4H3$	4.5	1.32	3.16	1.06	4.16	3.07	2.75	1.32	7.09	0	3.34	4.82	5.94	6.28
$BC_3S_2L87/4H1$	4.51	1.37	3.2	1.1	4.09	2.41	2.36	1.26	7.34	0	2.07	4.36	5.6	5.9
$BC_3S_2L87/4H2$	4.46	1.27	2.98	1.34	4.15	2.25	1.72	1.32	7.35	1.2	3.02	4.23	5.35	5.72
$BC_3S_2L87/4H3$	4.47	1.27	3.22	1.22	4.16	2.87	2.71	1.3	7.36	1.2	3.45	5.07	6.05	6.44
$BC_3S_2L87/4H4$	4.48	1.32	3.46	1.06	4.16	2.67	2.37	1.28	7.48	0	1.97	4.62	5.76	6.06
$BC_3S_2L87/4H5$	4.47	1.36	3.32	1.06	4.16	2.88	2.68	1.33	7.3	0.77	3.38	5.26	6.21	6.63
Ochuti	4.49	1.41	3.05	1.16	4.16	2.83	2.57	1.27	7.28	1.2	3.31	4.84	5.97	6.32
N13	4.34	1.37	2.98	1.24	4.04	1.72	1.77	1.33	5.6	0.52	1.88	3.71	4.44	4.95
Mean	4.47	1.34	3.2	1.17	4.14	2.67	2.49	1.3	7.09	0.76	2.98	4.77	5.8	6.17
l.s.d	0.05	NS	NS	NS	NS	NS	NS	NS	0.68	NS	NS	NS	1.02	NS
Cv (%)	0.8	5.4	1.3	8.1	1.7	6.8	8.8	5.5	6.6	33.1	18.7	5.4	3.2	3.6
F	*	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS
Heritability (H^2)	0.9	0.5	0.4	0.4	0.6	0.7	0.7	0.4	0.9	0.6	0.5	0.7	0.7	0.7

* Significantly different at 5 % level. NS not significantly different at 5 % level

4.3.4 Evaluation of Striga resistance in Alupe during October 2010 to March 2011season

Results from Table 11 shows that tillers per plot, plant height and seedling vigour score had significant differences. According to AUSPC total score BC_3S_2/L87/4/H3 had the highest number of emerged Striga throughout the season, while BC_3S_2/L87/4/H1 had the least number of emerged Striga therefore, BC_3S_2/L87/4/H3 was more susceptible than Ochuti while BC_3S_2/L87/4/H1 was less susceptible. AUSPC total score did not show significant differences in this trial and had low broad– sense heritability (0.4).

Table 11. The reaction of 8 backcross generations to *Striga* at Alupe during October 2010 to March 2011

Identity	Number of stem lodged plants	Number of tillers per plot	Plant height (cm)	Seedling vigour score	Stand after thinning	*Striga* capsule formation	*Striga* flowering	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total
$BC_3S_2L33/4H1$	3.15	3.75	4.85	1.93	4.56	2.78	2.95	0.52	3.39	5.7	7.05	7.3
$BC_3S_2L33/4H2$	2.89	3.76	4.83	2.05	4.45	3.01	3.2	1.04	3.97	6.14	7.49	7.76
$BC_3S_2L33/4H3$	3.38	3.72	4.97	1.87	4.58	3.44	3.75	1.35	4.15	6.55	8	8.23
$BC_3S_2L87/4H1$	3.14	3.93	4.91	1.93	4.59	2.42	2.33	0	2.5	5.16	6.6	6.82
$BC_3S_2L87/4H2$	3.07	3.64	4.79	1.99	4.55	2.99	2.68	0.52	2.99	5.7	6.98	7.25
$BC_3S_2L87/4H3$	3.29	4.08	4.95	1.71	4.55	3.34	3.58	0.68	3.9	6.49	8.32	8.54
$BC_3S_2L87/4H4$	3.49	3.85	5.05	1.47	4.62	2.83	2.94	0.52	4.22	6	7.15	7.47
$BC_3S_2L87/4H5$	3.29	3.81	4.97	1.85	4.56	2.6	2.67	0.52	2.73	5.99	7.18	7.47
OCHUTI	3.38	3.78	4.93	1.62	4.59	3.11	3.02	0.68	3.41	5.76	7.61	7.79
N13	2.89	3.1	4.48	2.12	4.45	2.31	2.46	0	3.16	4.52	5.16	5.46
Mean	3.2	3.74	4.87	1.85	4.55	2.88	2.96	0.58	3.44	5.8	7.15	7.41
l.s.d	NS	0.33	0.31	0.34	NS	NS	NS	NS	NS	NS	NS	NS
Cv (%)	1.5	5.3	1.9	9	1.9	3	5.4	54.5	11.3	5	5.2	5
F	NS	*	*	*	NS	NS	NS	NS	NS	NS	NS	NS
Heritability (H^2)	0.5	0.8	0.7	0.8	0.6	0.3	0.3	0.4	0.3	0.3	0.5	0.4

* Significant at P ≤ 0.05. NS Not Significant at 5 % level

4.3.5 Combined Analysis of variance (ANOVA)

4.3.5.1 May to October 2010 Season

With combined ANOVA analysis (Table 12) the traits that had significant differences due to environment were all AUSNPs scores, Striga capsule formation, Striga flowering and number of tillers per plot. These traits were influenced by the environment, therefore the genotype reactions were masked by the effects of the environment. The following traits did not show any significant differences ($P \leq 0.05$) among the genotypes plant height, stand after thinning and seedling vigour score.

Traits that showed significant differences ($P \leq 0.05$) among genotypes were AUSPC 1, AUSPC 2, AUSPC 3, AUSPC 4 and AUSPC total, number of tillers per plot, panicle weight, plant height, stand after thinning and 100 seeds weight. In these traits the genotypic variance was significant. This indicates that the genetic effects were expressed despite environment influence or interaction.

Genotype by Environment interactions (GxE) showed significant difference ($P \leq 0.05$) for the following traits AUSPC 2, AUSPC 3, AUSPC 4 and AUSPC total. This indicates that the GxE variance influenced the expression of Striga resistance in the specific locations and not across the two locations. The number of AUSPC 2, tillers per plot, plant height, stand after thinning, Striga capsule formation and Striga flowering did not show significant GxE interactions at ($P \leq 0.05$) suggesting that the genotypes performance of these traits was predictable in Alupe and Kibos.

4.3.5.2 October 2010 to March 2011 season

The results from table 12 shows combined ANOVA results for October 2010 to March 2011 season. The traits that had significant environment variances were AUSNP 3, AUSNP 4, AUSNP total, Striga capsule formation, plant height, seedling vigour score, stand after thinning and number of tillers per plot. This means these traits were influenced by the environment. Their expression was less likely to be heritable since the environmental variance was greater than the genotypic variance. Striga flowering, AUSPC 1 and AUSPC 2 did not show any significant environmental variance and are less likely to be influenced by the environment. This means that the Striga scores using AUSPC 1 and AUSPC 2 are likely to be consistent in both locations. The trait that did not show significant genotypic differences ($P \leq 0.05$) was plant height meaning that the trait is likely to be highly heritable and less influenced by the environment.

GxE showed significant difference ($P \leq 0.05$) for number of tillers per plot only during October to March 2011 season. The following traits did not show significant GxE interaction during October 2010 to March 2011 season all AUSPC counts, Striga capsule formation, Striga flowering, seedling vigour score, stand after thinning and plant height. This suggests that the genotype performed predictably in both locations.

Table 12. Genotype x Environment reactions of 8 back-cross generations in two locations during May 2010 to October 2010 and October 2010 to March 2011

Trait	Environment		Env*rep		Genotype		GxE		Heritability (H^2)	
	F Probability		0.05 significant level		F Probability		F Probability			
	May 2010 to October 2010	October 2010 to March 2011	May 2010 to October 2010	October 2010 to March 2011	May 2010 to October 2010	October 2010 to March 2011	May 2010 to October 2010	October 2010 to March 2011	May 2010 to October 2010	October 2010 to March 2011
AUSPC1	61.05*	0.62NS	10.23*	0.82NS	6.16*	1.32NS	2.37NS	1.12NS	0.8	0.6
AUSPC 2	38.90*	4.26NS	6.05*	2.30NS	14.28*	2.16NS	2.72*	1.39NS	0.9	0.7
AUSPC 3	65.91*	21.38*	3.72*	0.75NS	5.64*	2.25NS	4.26*	0.42NS	0.7	0.8
AUSPC 4	71.37*	36.58*	5.52*	0.86NS	2.86*	3.71*	2.29*	0.60NS	0.7	0.9
AUSPC Total	63.39*	30.49*	5.63*	0.93NS	3.74*	3.45NS	2.51*	0.62NS	0.7	0.9
***Striga* Capsule formation**	6.70*	18.98*	4.51*	0.52NS	1.10NS	0.96NS	0.99NS	0.39NS	0.6	0.7
***Striga* flowering**	29.62*	4.36NS	3.80*	0.37NS	0.71NS	1.29NS	1.24NS	0.37NS	0.5	0.8
Number of tillers per plot	28.09*	16.70*	0.21NS	0.30*	1.57*	0.19*	0.41NS	0.31*	0.9	0.5
Plant height (cm)	0.01NS	6.95*	0.12*	0.04NS	0.53*	0.17*	0.001NS	0.01NS	0.9	0.9
Stand after thinning	0.003NS	3.42*	0.05NS	0.04NS	1.35*	0.01*	0.002NS	0.01NS	0.9	0.8
Seedling vigour score	0.12NS	9.21*	0.09NS	0.18*	0.25NS	0.16*	0.02NS	0.06NS	0.9	0.8

* Significant at $P \leq 0.05$. NS Not Significant at 5 % level

4.3.6 Phenotypic Correlations results

4.3.6.1 Correlation for evaluation of Striga resistance in Kibos during May to October 2010 season

The traits that had positively significantly correlation differences ($P \leq 0.001$) were Striga capsule formation and Striga flowering (0.922), plant height and stand after thinning (0.775), panicle weight and plant height (0.853), 100 seeds weight and stand after thinning (0.720), 100 seeds weight and plant height (0.724) and 100 seeds weight and panicle weight (0.692), AUSPC 4 and Striga capsule formation (0.393) and AUSPC3 and days to 50 % anthesis (0.406) showed significant difference ($P \leq 0.05$). Days to 50 % flowering and Striga flowering and Striga capsule formation (-0.394 and -0.425 respectively) had negative significant correlations ($P \leq 0.05$) (Table 13)

Table 13. The correlation summary of 8back cross generations reaction to *Striga* at Kibos during May 2010 to October 2010

	Striga Flowering	*Striga* Capsule formation	Stand after thinning	Seedling Vigour Score	Number of Tillers Plot	Plant Height	Days to 50%Flowering	Panicle weight	100 seeds weight	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC total
***Striga* Flowering**	1													
***Striga* Capsule formation**	0.922***	1												
Stand after thinning	0.223	0.156	1											
Seedling Vigour Score	-0.166	-0.226	0.006	1										
Number of Tillers Plot	-0.491	-0.502	0.481	0.098	1									
Plant Height	0.194	0.151	0.775***	-0.126	0.406*	1								
Days to 50% Flowering	-0.394*	-0.425*	-0.212	0.291	0.276	-0.319	1							
Panicle weight	0.299	0.306	0.078	0.033	0.264	0.853***	-0.436*	1						
100 seeds weight	0.067	0.023	0.720***	0.122	0.285	0.724***	-0.314	0.692***	1					
AUSPC 1	0.354	0.303	0.183	-0.173	-0.135	0.147	-0.196	-0.057	0.22	1				
AUSPC 2	0.096	0.157	-0.152	0.069	0.119	-0.117	0.358	-0.275	-0.11	0.458	1			
AUSPC 3	0.056	0.182	-0.285	0.086	0.181	-0.161	0.406*	-0.202	-0.086	0.033	0.707***	1		
AUSPC 4	0.365	0.393*	-0.04	0.126	-0.044	-0.119	0.114	-0.188	0.043	0.244	0.464*	0.730***	1	
AUSPC total	0.3	0.327	-0.13	0.11	0.041	-0.138	0.246	-0.228	-0.006	0.279	0.689***	0.888***	0.948***	1

*** - very significant

** - 99% significant

* - 95% significant

4.3.6.2 Correlation for evaluation of Striga resistance in Alupe during May to October 2010 season

Results from table 14 shows there was significant correlation among several traits. Positive significant correlation at ($P \leq 0.001$) was exhibited by AUSPC 1 with Striga flowering (0.691), AUSPC 2 with Striga flowering (0.661), AUSPC 3 with Striga flowering (0.662), AUSPC 4 with Striga flowering (0.976) and AUSPC total with Striga flowering (0.683). AUSPC 1 with Striga capsule formation (0.704), AUSPC 2 with Striga capsule formation (0.735), AUSPC 3 with Striga capsule formation (0.755), AUSPC 4 with Striga capsule formation (0.941) and AUSPC total with Striga capsule formation (0.749). AUSPC 1 with yield (0.521), AUSPC 2 with yield (0.539), AUSPC 3 with yield (0.649), AUSPC 4 with yield (0.697), AUSPC total with yield (0.680) and Striga capsule formation with Striga flowering (0.845).

Traits with negative correlation with significance difference ($P \leq 0.001$) were plant height and seedling vigour (-0.759), panicle weight and seedling vigour (-0.704), and grain weight and seedling vigour (-0.724).

Table 14. The correlation summary of 8 back cross generations reaction to *Striga* at Alupe during May 2010 to October 2010

	Striga Flowering	Striga Capsule formation	Number of lodged plants	Stand after thinning	Seedling Vigour Score	Number of Tillers per Plot	Plant Height	Days to 50% flowering	Panicle weight	100 seeds weight	Grain Weight	Yield (Kg/M^2)	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC total
Striga Flowering	1																
Striga Capsule formation	0.845***	1															
Number of stem lodged plants	-0.057	-0.035	1														
Stand after thinning	0.417*	0.470*	0.480**	1													
Seedling Vigour Score	-0.154	-0.277	-0.217	-0.459*	1												
Number of Tillers Plot	0.012	0.018	0.274*	0.006	-0.028	1											
Plant Height	0.2	0.235	0.612***	0.824***	-0.759***	0.15	1										
Days to 50% Flowering	-0.433*	-0.508*	0.26	-0.285	0.741***	0.267*	-0.460*	1									
Panicle weight	0.367*	0.343	-0.145	0.746***	-0.704***	0.075	0.857***	0.267	1								
100 seeds weight	0.450*	0.581**	0.114	0.775***	-0.562*	0.041	0.683***	0.258	0.760***	1							
Grain Weight	0.375*	0.362	0.405**	0.766***	-0.724***	0.097	0.856***	0.3**	0.987***	0.764***	1						
Yield (Kg/M^2)	0.3**	0.272**	0.405**	0.829***	-0.138	0.51***	0.888***	0.3*	-0.414**	0.595***	1***	1					
AUSPC 1	0.691***	0.704***	-0.126	0.569**	-0.38	0.28	0.532**	-0.035	0.645***	0.595***	0.649***	0.521*	1				
AUSPC 2	0.661***	0.735***	-0.153	0.623***	-0.356*	0.282	0.607***	-0.019	0.708***	0.641***	0.697***	0.539*	0.955***	1			
AUSPC 3	0.662***	0.755***	0.048	0.619***	-0.321	0.263	0.599***	0.039	0.263***	0.640***	0.691***	0.649***	0.903***	0.987***	1		
AUSPC 4	0.976***	0.941***	0.127	0.455*	-0.06	0.247	0.222	0.099	0.421*	0.462*	0.433*	0.697***	0.723***	0.717***	0.726***	1	
AUSPC total	0.683***	0.749***	0.07	0.618***	-0.353	0.281	0.593***	0.068	0.703***	0.639***	0.693***	0.680**	0.962***	0.999***	0.903***	0.737***	1

***- very significant, **- 99% significant, *- 95% significant

4.3.6.3 Correlations for evaluation of Striga resistance in Kibos during October to March 2011 season

The traits with significant positive correlation (P≤0.001) are AUSPC 2, 3, 4 with total Striga flowering (0.099, 0.045, 0.034 and 0.043 respectively), Striga flowering with Striga capsule formation (0.150), days to 50 % anthesis with plant height (0.610), plant height with panicle weight (0.741), grain weight with plant height (0.726) and grain weight with panicle weight (0.939). The significant correlations (P≤0.05) were AUSPC 3 and total with severity score (0.431 and 0.486 respectively), severity score with Striga flowering and Striga capsule formation (0.003, 0.390 respectively), plant height with stand after thinning (0.444), panicle weight and stand after thinning (0.501). Negative correlation was exhibited by 100 seeds weight with days to 50 % anthesis (-0.320) significant differences (P≤0.001). There was no significant correlation observed between yield and AUSPC measures (table 15). The genotypes had tolerance during the first Striga count, but susceptibility increased as the Striga infestation increased, thus negative correlation in the other Striga counts.

Table 15. The correlation summary of 8 back cross generations reaction to *Striga* at Kibos during October 2010 to March 2011

	Striga Capsule formation	*Striga* Flowering	Stand after thinning	Seedling Vigour Score	Severity	Number of Tillers Plot	Plant Height	Days to 50% Flowering	Panicle weight	100 seeds weight	Grain Weight	Yield (Kg/M^2)	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC total
Striga Capsule formation	1																
***Striga* Flowering**	0.150***	1															
Stand after thinning	0.085	0.123	1														
Seedling Vigour Score	-0.075	0.122	-0.021	1													
Severity	0.003*	0.390*	-0.213	-0.016	1												
Number of Tillers Plot	0.003***	-0.168	0.021	-0.207	0.252	1											
Plant Height	0.152	0.135	0.444**	-0.229	-0.224	-0.153	1										
Days to 50% Flowering	0.006	0.201	0.267	-0.095	0.064	-0.251	0.610***	1									
Panicle weight	-0.009	-0.076	0.501**	-0.112	-0.2	-0.017	0.741***	0.291	1								
100 seeds weight		-0.094	0.187	-0.093	-0.12	0.016	-0.109	-0.320*	-0.063	1							
Grain Weight	-0.047	-0.131	0.474*	-0.085	-0.121	0.036	0.726***	0.277	0.939***	-0.062	1						
Yield (Kg/M^2)	-0.047	-0.066	0.474**	-0.085	-0.122	0.365*	0.726***	0.277	0.939***	-0.062	1***	1					
AUSPC 1	0.078	0.027	0.102	0.119	-0.028	0.538	0.125	-0.046	-0.017	0.169	0.997*	0.001	1				
AUSPC 2	0.099***	0.745***	0.052	0.032	0.209	0.039	0.123	-0.07	-0.05	0.009	-0.089	-0.089	0.346*	1			
AUSPC 3	0.045***	0.882***	0.08	0.078	0.431**	-0.043	0.15	0.172	-0.04	-0.106	-0.097	-0.097	0.212	0.841***	1		
AUSPC 4	0.034***	0.96***	0.097	0.139	0.512	-0.094	0.162	0.296	-0.015	-0.013	-0.062	-0.062	0.157	0.658***	0.939***	1	
AUSPC total	0.043***	0.923***	0.093	0.123	0.486**	-0.074	0.162	0.249	-0.024	-0.116	-0.074	-0.074	0.198	0.747***	0.975***	0.992***	1

***- very significant, **- 99% significant, *- 95% significant

4.3.6.4 Correlations for evaluation of Striga resistance in Alupe during October to March 2011 season

Results showed that there were significant positive correlations (P≤0.001) among the trait studied (Table 16). Striga flowering and Striga capsule formation (0.882), panicle weight and plant height (0.706), grain weight and plant height (0.712) and grain weight and panicle weight (0.990) were all positively correlated. There were also positive significant correlations (P≤0.05) between AUSPC 2,3,4 and total with Striga flowering (0.613, 0.909,0.889 and 0.906 respectively), plant height and number of stem lodged plant (0.556), number of stem lodged plants and Striga capsule formation (0.508), number of stem lodged plants and Striga flowering (0.570), number of stem lodged plants and stand after thinning (0.530). Yield and AUSPC 1 (0.150) had positive significant correlation exhibiting presence of tolerance, but susceptibility increased as AUSPC 4 and total (-0.085 and -0.04 respectively) had negative correlation. Negative correlation with significant differences (P≤0.05) are seedling vigour score and Striga flowering (-0.362), seedling vigour score and stand after thinning (-0.529), number of tillers per plot and seedling vigour score (-0.325) and plant height and seedling vigour score (-0.404).

Table 16. The correlation summary of 8 back cross generations reaction to *Striga* at Alupe during October 2010 to March 2011

	Striga Capsule formation	*Striga* Flowering	Stand after thinning	Seedling Vigour Score	Number of Tillers Plot	Plant Height	Stem lodged plants	Panicle weight	100 seeds weight	Grain Weight	Yield (Kg/M^2)	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC total
Striga Capsule formation	1															
Striga Flowering	0.882***	1														
Stand after thinning	0.134	0.156	1													
Seedling Vigour Score	-0.3	-0.362*	-0.529**	1												
Number of Tillers Plot	-0.225	-0.195	0.279	-0.325*	1											
Plant Height	0.424	0.360*	0.172	-0.404*	0.072	1										
Stem lodged plants	0.508**	0.570**	0.530**	-0.603***	-0.04	0.556**	1									
Panicle weight	0.239	0.168	-0.11	-0.087	0.045	0.706***	0.155	1								
100 seeds weight	0.047	-0.016	-0.231	-0.067	-0.033	0.152	-0.037	0.340***	1							
Grain Weight	0.252	0.18	-0.11	-0.091	0.02	0.712***	0.151	0.990***	0.348*	1						
Yield (Kg/M^2)	0.282	0.208	-0.125	-0.128	-0.02	0.721***	0.195	0.990***	0.348*	1***	1					
AUSPC 1	-0.014	-0.135	-0.2	-0.098	-0.447	-0.104	-0.307	0.157	-0.049	0.15	0.150*	1				
AUSPC 2	0.4	0.613*	-0.168	-0.405	0.02	0.582	0.226	0.285	0.212	0.317*	0.317	0.476	1			
AUSPC 3	0.783**	0.909***	0.25	-0.664	0.466	0.941***	0.692*	0.209	0.09	0.217	0.217	-0.053	0.705*	1		
AUSPC 4	0.807*	0.889**	0.486	-0.739	0.616	0.869**	0.654*	-0.091	-0.084	-0.085	-0.085	-0.236	0.512	0.943***	1	
AUSPC total	0.809*	0.906***	0.419	-0.729	0.574	0.899***	0.665*	-0.047	-0.06	-0.04	-0.04	-0.172	0.588	0.973***	0.995***	1

***- very significant, **- 99% significant, *- 95% significant

4.3.7 AUSPC TOTAL

AUSPC totals was used to rank genotypes in both location for the trials performed from May 2010 to March 2011.

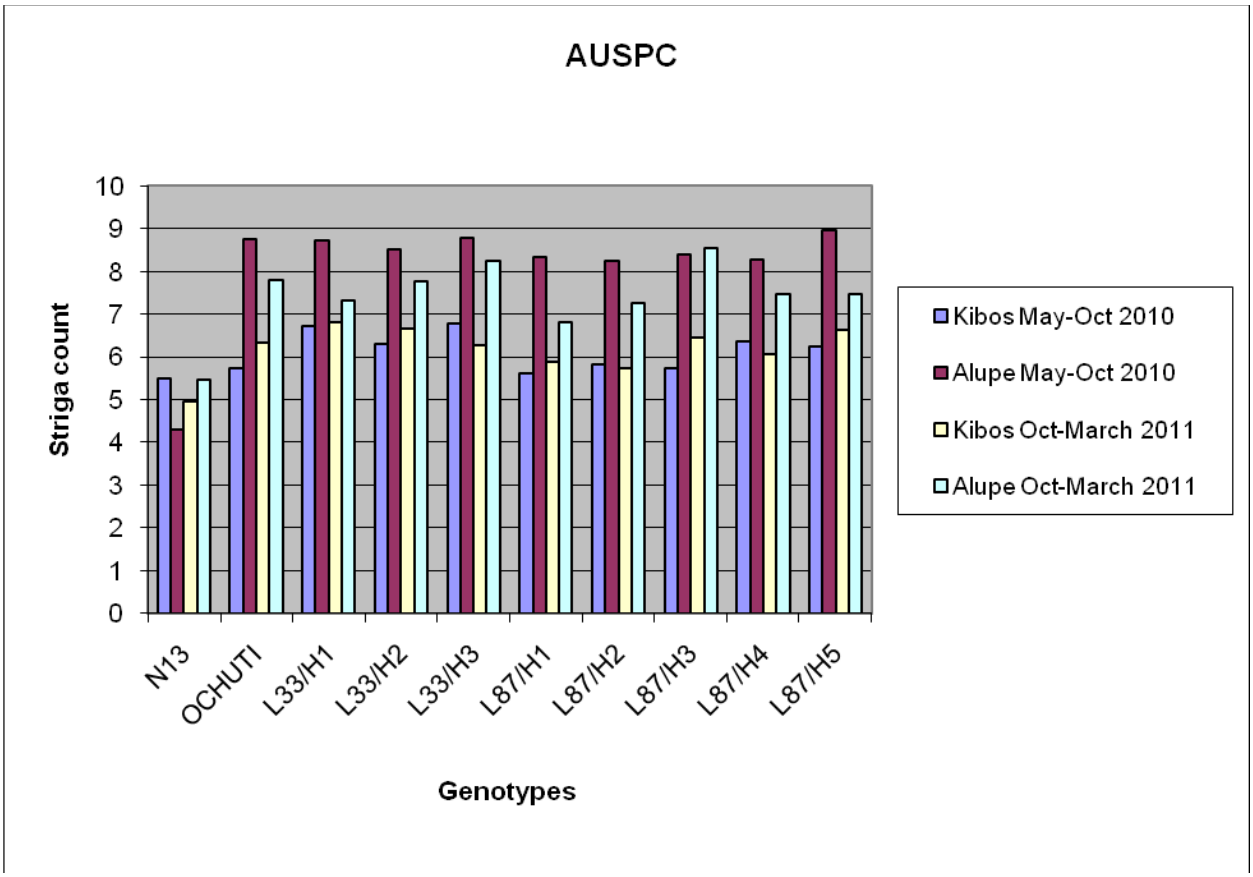


Figure 11. AUSPC total for the genotypes under *Striga* infestation for May 2010 to March 2011.

According to area under Striga progressive curve (AUSPC) total (figure 11) Kibos May to October 2010 had the least emerged Striga followed by Kibos October to March 2011 then Alupe October to March 2011 and Alupe May to October 2010 had the highest. L87/H1 Kibos May to October 2010 had the least AUSPC, while L87/H5 Alupe May to October 2010 had the highest AUSPC.

4.4 DISCUSSION

Low values for Area under Striga Progressive Curve (AUSPC) indicates resistance to Striga whereas high values indicate susceptibility to Striga according to (Haussmann et al., 2001a). According to this study the plants were less susceptible to Striga for the first two counts of Striga at 42 days and 56 days after planting, whereas, susceptibility increased with increased infestation.

There were no significant differences between genotypes in AUSPC on the two trials conducted at Kibos, while at Alupe the trials showed significant difference. The number of emerged Striga was more in Alupe compared to Kibos. This results are in correspondence with Haussmann et al., (2001b) where higher number of emerged Striga where shown in Alupe than in Kibos. These variations could be due to differences in Striga pathogenicity in the two locations. In addition Alupe might be having more natural Striga infestation than Kibos, thus the problem of heterogeneity of natural field infestation in African soils (Haussmann et al., 2001a,b,c,).

It is also important to note that irrigation was applied in Kibos when the rains failed, but no irrigation was applied in Alupe. This may had an effect on complex interactions between host, parasite and environment affecting the parasite's establishment and reproduction (Haussmann et al 2000b). According to Shank, (1996) Striga thrives on low rainfall or moisture stress as continuous wet periods are unfavourable to Striga development. Odhiambo and Ariga, (2004) reported low Striga infestation during the long rains in Karura, Kenya. This was attributed to high soil moisture content for an extended period, causing Striga seeds to undergo wet dormancy. Leaching of crop exudates is possible following heavy showers and this may have reduced Striga germination later in the season as reported by Gbèhounou et al., (2004).

According to Area under Striga Progressive Curve (AUSPC) evaluations done at Kibos during May to October 2010 season had the least emerged Striga followed by October to March 2011 season

conducted at Kibos. The trials conducted at Kibos in both seasons had the least AUSPC values, whereas those at Alupe had the highest AUSPC values. Striga emergence was delayed in all the trials, indicating that the environment influenced Striga germination and therefore AUSPC values.

All AUSPC values were highly positively correlated to each other. AUSPC values were also highly correlated to Striga flowering and Striga capsule formation, plant height, stem lodged plants, severity score, 100 seed weight, grain weight, yield and stand after thinning. All AUSPC values had high broad – sense heritability values meaning that these counts are not likely to be affected by the environment.

Susceptible sorghum plants are likely to have the highest number of flowering Striga plants, while resistant and less susceptible plants should sustain low number of flowering striga. Plants that flowered late had less number of Striga flowering, and plants that flowered early had a higher number of Striga.

The number of flowering Striga plant had high positive correlations with all AUSPC values again indicating that in plots where most Striga flowered, those genotypes were susceptible to Striga. Plants that had high number of flowered Striga had the highest number of emerged Striga and the highest number of Striga capsules. In plots where there was high number of flowering Striga there was also a high number of emerged Striga and a high number of Striga capsules. Striga flowering and Striga capsule formation were positively significantly correlated, hence increase in Striga flowering resulted in increase in Striga capsule formation. There was negative correlation between Striga capsule formation and days to 50 % anthesis meaning that plants that flowered late had less Striga capsules formed.

According to the results, the tall plants were more susceptible, this explains why plants with high means for plant height had high means for area under Striga progressive curve.

The high the number of tillers the more the number of emerged Striga, this was likely because the more the tillers the more the host exudates are produced and the more the roots are available for the parasite to attach to as suggested by Odhiambo and Ransom, (1995). Babewi and Abdulaziz (1983) reported increased growth of Striga with increasing maize plant density.

Plants that flowered late had less number of emerged Striga. Landraces with a longer growing season were shown to support a high number of emerged Striga, because of longer period of exposure to the Striga seeds (Talleyrand et al., 1991, Kureh et al., 1999). This may explain why Ochuti had a high number of emerged Striga.

Low grain yield was more strongly associated with high severity score, areas under Striga progressive curve (AUSPC) with number of Striga plants flowering and forming capsule as would be expected (Kim et al., 1997).

Table 12 shows that the two environments and seasons were different. There were significant differences in all traits in the May 2010 to October 2010 season than in the October 2010 to March 2011 season in both locations. This probably means there are differences in natural infestation of Striga, pathogenicity, soil and climatic conditions. Environmental factors also affected the way the genotypes reacted to Striga.

The results in Table 12 shows that the genotypes were significantly different in the reaction to Striga infestation, meaning the genotypes BC_3S_1/L33 and BC_3S_1/L87 reacted differently to the Striga in each of the two environments. The GxE interaction for all AUSPC was significant. The reaction of the genotypes to Striga was influenced by the climatic conditions and the environment of each location and the performance of a genotype in one location were not predictable in the other location. Genotypes that had high Striga resistance scores in one environment did not perform the same way in another environment and season.

Line 87 and 33 performed well than Ochuti and were comparable to N13. Plants with high value of severity score had poor yield compared to those with less values of severity score.

4.5 CONCLUSION

All AUSPC were highly positively correlated to each other and with the following traits Striga flowering and Striga capsule formation, plant height, stem lodged plants, severity score, 100 seed weight, grain weight, yield and stand after thinning. All AUSPC measures had high broad-sense heritability values this means that these counts are not likely affected by the environment.

For AUSPC values, plant height, number of tillers per plot and stand after thinning, genotype variance and GxE interactions were significant in the two environments (Kibos and Alupe). The performance of genotypes and their reaction to Striga infestation was influenced by the specific environmental conditions in the specific locations. The resistance levels of the materials being evaluated was more in early stage (first AUSNP i.e. 42 days after planting) but the susceptibility increased with increasing Striga infestation. There were no significant differences observed between the resistance and susceptible parent.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Sorghum is the second most important cereal crop in East Africa as a source of food. Given that its productivity is seriously constrained by Striga, leading to a substantial loss of yield and the failure by conventional breeding methods to substantially combat the weed. There was need for using molecular methods in developing Striga resistant sorghum varieties. Molecular work was done at the BecA laboratories using 11 polymorphic SSR markers for foreground selection identified five plants with Striga resistance QTL introgressed, two plants in BC_3F_1 and three plants in BC_3F_2 having four to one QTL.

Evaluation of back cross lines namely BC_3S_1/L33 and BC_3S_1/L87 with introgressed Striga resistant QTL along with their parental lines namely donor line N13 and recurrent parent Ochuti done under field trials in Western Kenya in Alupe in Busia county and Kibos in Kisumu county showed that the Striga resistance QTL had successfully been introgressed into Ochuti. According to Area Under Striga Progressive Curve total score (AUSPC total), genotype BC_3S_1/L87/H1 evaluated at Kibos had the least AUSPC value, while genotype BC_3S_1/L87/H5 evaluated at Alupe had the highest AUSPC value. The backcross lines BC_3S_1/L33 and BC_3S_1/L 87 had lower Striga scores throughout the two seasons in both locations than the susceptible parental line Ochuti.

The study thus concluded that Marker Assisted Selection (MAS) was successfully used to introgress Striga resistance Quantitative Trait Loci (QTL) into a Kenyan adapted farmer preferred sorghum

variety, Ochuti. N13, which is an Indian durra sorghum with mechanical and antibiosis resistance mechanism to Striga, was used as a donor parent.

5.2 RECOMMENDATIONS

The identified plants with Striga resistance QTL should be selfed to fix the QTL and the foreground selection to include more markers which are closely linked to the Striga QTL (5-20cM) and more plants genotyped per generation to increase the chances of more QTL being captured. Field trials should also include Striga-free plots besides the infested plots. Gene pyramiding would be used instead of backcrossing to be able to capture all the possible five QTLs for Striga resistance. Farmers should also to be advised to practise use of integrated Striga management options to curb Striga problem.

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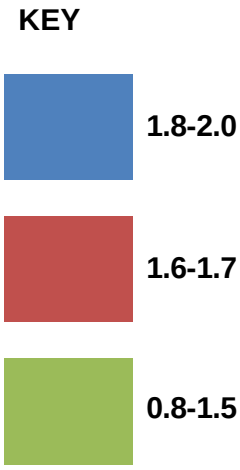
7.0 APPENDIXES

APPENDIX V. NANODROPS READING FOR BC_3F_1

Sample ID	ng/ul	260/280	260/230	DNA	TE buffer
Line 87					
1	251.91	1.6	1.3	4.0	96.0
2	318.54	1.7	1.2	3.1	96.9
3	715.57	1.9	1.6	1.4	98.6
4	222.52	1.8	1.4	4.5	95.5
5	327.04	1.9	1.6	3.1	96.9
6	384.56	1.8	1.4	2.6	97.4
7	134.77	1.8	1.2	7.4	92.6
8	348.65	1.9	1.6	2.9	97.1
9	355.7	1.9	1.6	2.8	97.2
10	405.59	1.7	1.4	2.5	97.5
11	338.35	1.9	1.6	3.0	97.0
12	286.53	1.7	1.3	3.5	96.5
13	280.26	1.8	1.3	3.6	96.4
14	222.01	1.9	2.0	4.5	95.5
15	440.78	1.9	1.8	2.3	97.7
16	126.1	1.7	1.5	7.9	92.1
17	239	1.6	1.2	4.2	95.8
18	546.83	1.8	1.9	1.8	98.2
19	146.26	1.9	2.0	6.8	93.2
20	243.28	1.9	1.9	4.1	95.9
21	800.42	2.0	1.9	1.2	98.8
22	347.97	1.8	1.9	2.9	97.1
23	435.14	1.8	1.5	2.3	97.7
24	298.73	1.8	1.2	3.3	96.7
Line33					
25	248	1.7	1.4	4.0	96.0
26	264.46	1.7	1.1	3.8	96.2
27	2.73	0.8	2.1	366.3	-266.3
28	249.78	1.8	1.3	4.0	96.0
29	174.91	1.7	1.1	5.7	94.3
30	264.71	2.0	2.3	3.8	96.2
31	245.24	1.8	1.3	4.1	95.9
32	305.39	1.9	1.9	3.3	96.7
33	127.92	1.7	1.2	7.8	92.2
34	112.49	1.8	1.3	8.9	91.1
35	160.38	1.8	1.2	6.2	93.8



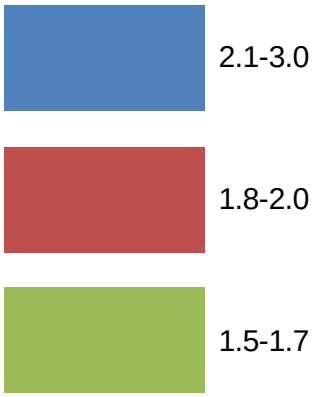
36	69.5	1.7	1.0	14.4	85.6
37	90.54	1.7	1.3	11.0	89.0
38	109.4	1.6	0.7	9.1	90.9
39	80	1.4	0.6	12.5	87.5
40	72.36	1.8	1.4	13.8	86.2
41	456.17	2.0	1.7	2.2	97.8
42	548.87	1.7	1.6	1.8	98.2
43	619.92	2.0	2.3	1.6	98.4
44	438.51	1.9	1.7	2.3	97.7
45	318.29	1.5	0.9	3.7	96.3
46	397.77	1.8	1.4	2.5	97.5
47	106.8	1.9	1.2	9.4	90.6
48	299.66	1.9	2.1	3.3	96.7
49	114.89	1.6	1.1	8.7	91.3
50	62.84	1.7	1.0	15.9	84.1
51	43.61	1.9	1.6	22.9	77.1
52	52.09	1.6	0.7	19.2	80.8
53	130.31	1.8	1.6	7.7	92.3
54	121.19	1.7	1.0	8.3	91.7
55	161.69	1.8	1.3	6.2	93.8
56	199.61	1.8	1.2	5.0	95.0
57	279.45	1.9	1.5	3.6	96.4
58	165.2	1.7	1.2	6.1	93.9
59	583.83	2.0	2.0	1.7	98.3
60	212.02	1.9	1.4	4.7	95.3
61	150.47	1.9	1.8	6.6	93.4
62	287.11	1.8	1.3	3.5	96.5
63	149.3	1.8	1.2	6.7	93.3
64	248.94	1.9	1.5	4.0	96.0
65	613.11	2.0	1.9	1.6	98.4
66	304.67	1.9	1.5	3.3	96.7
67	399.69	1.9	1.7	2.5	97.5
68	324.17	1.7	1.1	3.1	96.9
69	213.36	1.8	1.2	4.7	95.3
70	371.25	1.9	1.6	2.7	97.3
71	253.23	1.8	1.2	3.9	96.1
72	203.71	1.8	1.1	4.9	95.1

APPENDIX VI. NANODROPS READING FOR BC_4F_1

Sample ID	ng/ul	260/280	260/230	DNA	TE BUFFER
line87					
1	239.37	1.9	2.4	4.18	95.82
2	218.16	1.9	2.5	4.58	95.42
3	168.39	1.9	2.4	5.94	94.06
4	79.73	1.9	1.9	12.54	87.46
5	49.42	1.9	1.7	20.23	79.77

6	291.84	1.9	2.4	3.43	96.57
7	306.99	1.9	2.4	3.26	96.74
8	258.33	1.9	2.4	3.87	96.13
9	138.47	1.9	1.7	7.22	92.78
10	94.89	1.9	2.1	10.54	89.46
11	181.78	1.9	2.3	5.50	94.50
12	77.9	1.9	2.1	12.84	87.16
13	87.17	1.9	2.0	11.47	88.53
14	221.47	1.9	2.2	4.52	95.48
15	107.78	1.9	2.0	9.28	90.72
16	108.39	1.9	2.1	9.23	90.77
17	120.18	1.9	1.8	8.32	91.68
18	256.19	1.9	2.2	3.90	96.10
19	244.49	1.9	2.3	4.09	95.91
20	323.61	1.9	2.3	3.09	96.91
21	484.4	1.9	2.5	2.06	97.94
22	194.45	1.9	2.5	5.14	94.86
23	505.49	1.9	2.5	1.98	98.02
24	217.37	1.9	2.4	4.60	95.40
25	10.21	2.1	1.2	97.94	2.06
26	121.27	1.9	1.9	8.25	91.75
27	266.95	2.0	2.4	3.75	96.25
28	117.19	1.9	2.2	8.53	91.47
29	278.7	2.0	2.4	3.59	96.41
30	151.99	2.0	2.4	6.58	93.42
31	152.26	1.9	2.1	6.57	93.43
32	162.06	2.0	2.2	6.17	93.83
33	58.96	1.9	2.3	16.96	83.04
34	218.1	2.0	2.4	4.59	95.41
35	149.12	2.0	2.3	6.71	93.29
36	61.37	2.0	2.1	16.29	83.71
37	49.62	2.0	1.0	20.15	79.85
38	4.84	1.9	1.1	206.61	-106.61
39	114.17	1.9	2.3	8.76	91.24
40	34.84	1.9	2.0	28.70	71.30
41	160.61	2.0	2.4	6.23	93.77
42	41.35	1.9	2.3	24.18	75.82
43	134.02	1.9	2.2	7.46	92.54
44	250.15	1.9	2.4	4.00	96.00
45	233.59	1.9	2.3	4.28	95.72
46	16.55	2.1	2.5	60.42	39.58
47	86.58	1.9	2.1	11.55	88.45
48	303.18	1.9	2.4	3.30	96.70
49	30.71	1.9	1.7	32.56	67.44
50	115.47	1.9	2.3	8.66	91.34
51	348.15	1.9	2.5	2.87	97.13
52	143.98	2.0	2.4	6.95	93.05
53	302.24	1.9	2.5	3.31	96.69
54	211.41	1.9	2.4	4.73	95.27

KEY



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58	322.19	2.0	2.4	3.10	96.90
59	286.3	2.0	2.4	3.49	96.51
60	146.83	1.9	2.2	6.81	93.19
61	139.72	1.9	2.3	7.16	92.84
62	2.92	1.9	0.5	342.47	-242.47
63	310.76	1.9	2.2	3.22	96.78
64	3.23	2.2	0.5	309.60	-209.60
65	118.48	1.9	2.1	8.44	91.56
66	5.44	2.8	1.0	183.82	-83.82
67	128.55	1.9	2.0	7.78	92.22
68	122.73	2.0	2.1	8.15	91.85
69	301.33	1.9	2.4	3.32	96.68
70	172.39	1.9	2.1	5.80	94.20
71	391.42	1.9	2.4	2.55	97.45
72	163.84	1.9	2.2	6.10	93.90
73	98.79	1.9	2.0	10.12	89.88
74	178.2	1.9	2.1	5.61	94.39
75	90.19	2.0	2.0	11.09	88.91
76	292.41	1.9	2.2	3.42	96.58
77	324.8	2.0	2.3	3.08	96.92
78	193.66	1.9	2.1	5.16	94.84
79	129.16	1.9	2.1	7.74	92.26
80	173.29	1.9	2.1	5.77	94.23
81	275.98	1.9	2.3	3.62	96.38
82	216.48	2.0	2.2	4.62	95.38
83	156.11	2.0	2.3	6.41	93.59
84	192.04	1.9	2.3	5.21	94.79
85	211.28	1.9	2.2	4.73	95.27
86	32.08	1.9	1.6	31.17	68.83
87	100.21	2.0	2.0	9.98	90.02
88	119.05	1.9	2.0	8.40	91.60
89	291.65	1.9	2.4	3.43	96.57
90	216.76	1.9	2.2	4.61	95.39
91	357.75	1.9	2.4	2.80	97.20
92	137.91	1.9	2.2	7.25	92.75
93	230.33	2.0	2.2	4.34	95.66
94	136.93	1.9	2.2	7.30	92.70
96	300.57	2.0	2.4	3.33	96.67
97	189.05	2.0	2.2	5.29	94.71
98	173.82	2.0	2.2	5.75	94.25
99	95.8	1.9	2.1	10.44	89.56
100	178.34	2.0	2.1	5.61	94.39
101	220.31	2.0	2.3	4.54	95.46
103	134.71	2.0	2.2	7.42	92.58
104	68.76	1.9	1.9	14.54	85.46
105	4.11	3.0	2.8	243.31	-143.31

106	182.4	2.0	2.1	5.48	94.52
107	254.18	2.0	2.2	3.93	96.07
108	6.69	2.9	2.3	149.48	-49.48
109	167.18	2.0	2.1	5.98	94.02
110	119.33	1.9	2.0	8.38	91.62
111	39.67	1.9	1.6	25.21	74.79
112	148.65	2.0	2.2	6.73	93.27
Line 33					
1	137.12	1.9	2.1	7.29	92.71
2	163.6	1.9	2.2	6.11	93.89
3	76.88	1.9	1.9	13.01	86.99
4	115.4	1.9	2.0	8.67	91.33
5	455.22	2.0	2.4	2.20	97.80
6	54.49	1.9	1.9	18.35	81.65
7	124.74	2.0	2.1	8.02	91.98
8	77.03	2.0	1.8	12.98	87.02
9	45.08	1.8	1.5	22.18	77.82
10	16.3	2.1	2.3	61.35	38.65
11	195.11	2.0	2.1	5.13	94.87
12	168.66	2.0	2.0	5.93	94.07
13	27.52	1.9	2.1	36.34	63.66
14	143.32	2.0	1.8	6.98	93.02
15	203.89	2.0	2.3	4.90	95.10
16	199.74	2.0	2.2	5.01	94.99
17	257.5	2.0	2.2	3.88	96.12
18	103.35	2.0	1.7	9.68	90.32
19	324.8	2.0	2.3	3.08	96.92
20	26.67	2.0	1.9	37.50	62.50
21	124.89	2.0	2.0	8.01	91.99
22	24.22	2.0	1.7	41.29	58.71
23	86.66	1.8	1.6	11.54	88.46
24	320.73	2.0	2.2	3.12	96.88
25	225.7	2.0	2.1	4.43	95.57
26	256.26	1.9	2.0	3.90	96.10
27	41.73	2.0	2.5	23.96	76.04
28	327.77	1.8	1.6	3.05	96.95
29	263.06	2.0	2.2	3.80	96.20
30	278.93	2.0	2.2	3.59	96.41
31	245.89	2.0	2.2	4.07	95.93
32	177.65	2.0	2.3	5.63	94.37
33	195.37	2.0	2.1	5.12	94.88
34	162	2.0	2.1	6.17	93.83
35	405.33	2.0	2.4	2.47	97.53
36	179.72	2.0	2.0	5.56	94.44
37	183.58	2.0	2.0	5.45	94.55
38	359.18	2.0	2.3	2.78	97.22
39	419.67	1.9	2.3	2.38	97.62
40	169.25	2.0	2.0	5.91	94.09
41	84.59	1.9	1.7	11.82	88.18

42	118.6	2.0	1.9	8.43	91.57
43	142.43	2.0	1.9	7.02	92.98
44	132.12	1.9	1.9	7.57	92.43
45	373.85	2.0	2.3	2.67	97.33
46	424.85	1.9	2.3	2.35	97.65
47	506.48	1.9	2.4	1.97	98.03
48	400.63	2.0	2.5	2.50	97.50
49	187.77	1.9	2.0	5.33	94.67
50	435.04	2.0	2.5	2.30	97.70
51	465.05	1.9	2.3	2.15	97.85
52	347.62	2.0	2.3	2.88	97.12
53	608.25	2.0	2.4	1.64	98.36
54	80.05	1.9	1.7	12.49	87.51
55	378.91	2.0	2.3	2.64	97.36
56	255.37	1.9	2.2	3.92	96.08
57	54.06	1.9	1.3	18.50	81.50
58	478.98	1.9	2.2	2.09	97.91
59	445.58	1.9	2.4	2.24	97.76
60	308.53	2.0	2.2	3.24	96.76
61	491.07	2.0	2.4	2.04	97.96
62	267.36	2.0	2.2	3.74	96.26
63	437.75	2.0	2.4	2.28	97.72
64	35.77	2.0	2.2	27.96	72.04
65	235.68	2.0	2.1	4.24	95.76
66	303.14	2.0	2.4	3.30	96.70
67	273.46	2.0	2.1	3.66	96.34
68	357.19	2.0	2.2	2.80	97.20
69	243.79	2.0	2.2	4.10	95.90
70	125.81	1.9	1.9	7.95	92.05
71	187.67	2.0	2.0	5.33	94.67
72	297.21	2.0	2.3	3.36	96.64
73	316.56	2.0	2.4	3.16	96.84
74	243.91	2.0	2.1	4.10	95.90
75	444.15	2.0	2.3	2.25	97.75
76	62.54	2.1	2.1	15.99	84.01
77	479.05	2.0	2.3	2.09	97.91
78	360.79	2.0	2.3	2.77	97.23
79	477.38	2.0	2.4	2.09	97.91
80	280.21	1.5	1.1	3.57	96.43
81	135.23	1.9	1.8	7.39	92.61
82	394.2	2.0	2.3	2.54	97.46
83	288.2	2.0	2.0	3.47	96.53
84	354.79	2.0	2.2	2.82	97.18
85	171.05	1.9	2.1	5.85	94.15
86	320	2.0	2.1	3.13	96.88
87	242.16	2.0	2.1	4.13	95.87
88	121.98	1.9	2.1	8.20	91.80
89	267.03	2.0	2.3	3.74	96.26
90	340.2	2.0	2.3	2.94	97.06

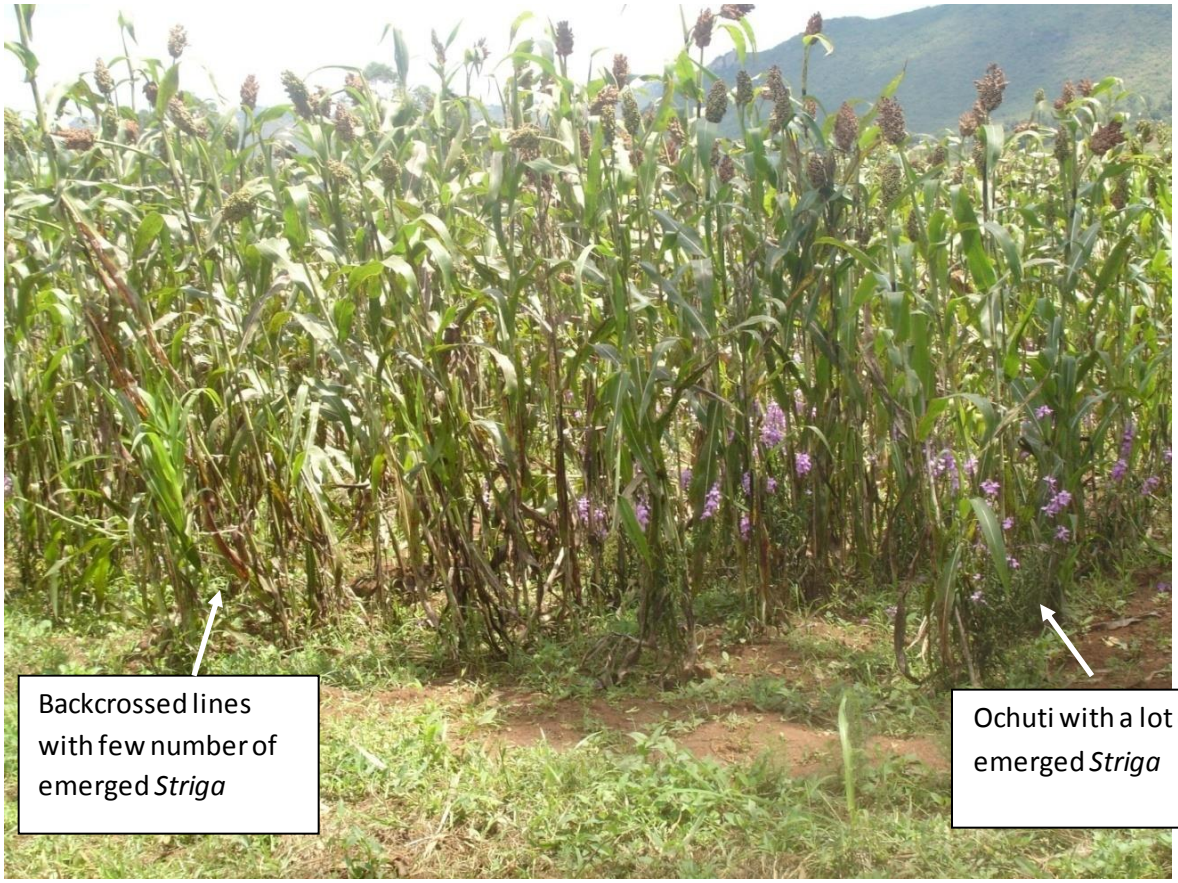
91	376.28	2.0	2.4	2.66	97.34
92	207.77	1.9	2.1	4.81	95.19
93	326.68	2.0	2.1	3.06	96.94
94	393.97	2.0	2.3	2.54	97.46
95	244.05	2.0	2.3	4.10	95.90
96	245.09	2.0	2.2	4.08	95.92
97	37.13	2.0	2.1	26.93	73.07
98	18.91	2.1	2.4	52.88	47.12
99	225.48	2.0	2.1	4.43	95.57
100	317.38	2.0	2.3	3.15	96.85
101	207.9	2.0	2.1	4.81	95.19
102	269.41	2.0	2.1	3.71	96.29
103	261.99	2.0	2.2	3.82	96.18
104	145.34	2.0	2.0	6.88	93.12
105	158.12	2.0	2.0	6.32	93.68
106	273.67	2.0	2.2	3.65	96.35
107	26.41	2.0	1.9	37.86	62.14
108	316.01	2.0	2.3	3.16	96.84
109	31.31	2.0	2.1	31.94	68.06
110	37.62	1.9	1.1	26.58	73.42
111	23.32	2.0	2.1	42.88	57.12
112	158.06	2.0	2.1	6.33	93.67
113	200.59	2.0	2.1	4.99	95.01
114	23.5	1.8	3.5	42.55	57.45
115	215.72	2.0	2.3	4.64	95.36
116	355.67	2.0	2.3	2.81	97.19
117	263.45	2.0	2.2	3.80	96.20
118	367.46	1.9	2.3	2.72	97.28
119	370.33	1.9	2.4	2.70	97.30
120	20.38	1.9	2.1	49.07	50.93
121	172.36	2.0	1.9	5.80	94.20
122	161.04	2.0	2.2	6.21	93.79
123	184.49	2.0	2.2	5.42	94.58
124	173.79	2.0	2.1	5.75	94.25
125	24.16	2.0	2.0	41.39	58.61
126	17.44	2.1	2.1	57.34	42.66
127	198.42	2.0	2.3	5.04	94.96
128	77.28	1.9	2.2	12.94	87.06
129	15.02	2.0	2.1	66.58	33.42
130	231.28	2.0	2.2	4.32	95.68
131	194.56	2.0	2.1	5.14	94.86
132	136.68	2.0	2.1	7.32	92.68
133	230.37	2.0	2.3	4.34	95.66
134	191.6	2.0	2.2	5.22	94.78
135	191.21	2.0	2.1	5.23	94.77
136	168.77	2.0	2.0	5.93	94.07
137	181.01	2.0	2.1	5.52	94.48
138	187.93	2.0	2.2	5.32	94.68
139	146.52	1.8	2.0	6.83	93.17

139	154.46	2.0	2.1	6.47	93.53
140	243.42	2.0	2.2	4.11	95.89
141	353.41	2.0	2.3	2.83	97.17
142	292.93	2.0	2.3	3.41	96.59
143	308.41	2.0	2.3	3.24	96.76
144	139.93	2.0	1.9	7.15	92.85
145	186.26	2.0	2.0	5.37	94.63
146	150.51	2.0	1.9	6.64	93.36
147	432.51	2.0	2.3	2.31	97.69
148	16.64	2.0	1.4	60.10	39.90
149	371.66	2.0	2.1	2.69	97.31
150	43.28	2.0	1.6	23.11	76.89
151	10.33	1.6	1.6	96.81	3.19
152	303.92	2.0	2.4	3.29	96.71
153	8.8	1.8	1.3	113.64	-13.64
154	39.95	1.8	1.4	25.03	74.97
155	60.15	1.9	1.6	16.63	83.37
156	40.56	1.9	1.3	24.65	75.35
157	45.4	1.5	1.3	22.03	77.97
158	12.79	2.0	1.7	78.19	21.81
159	5.87	1.7	1.6	170.36	-70.36
160	2.95	2.1	1.3	338.98	-238.98
161	116.8	2.0	1.7	8.56	91.44
162	215.53	2.0	2.1	4.64	95.36
163	274.68	2.0	2.2	3.64	96.36
164	160.06	2.0	2.1	6.25	93.75
165	59.26	1.9	1.8	16.87	83.13
166	175.36	2.0	2.0	5.70	94.30
167	156.34	1.6	1.3	6.40	93.60
168	123.51	2.0	2.1	8.10	91.90
169	78.59	1.9	1.8	12.72	87.28
170	246.59	2.0	2.2	4.06	95.94
171	247.66	2.0	2.4	4.04	95.96
172	302.53	2.0	2.4	3.31	96.69
173	250.06	2.0	2.3	4.00	96.00
174	407.07	2.0	2.4	2.46	97.54
175	239.81	2.0	2.5	4.17	95.83
176	214.25	2.0	2.3	4.67	95.33

APPENDIX VII. Summary of field information

Parameter	Season and year				
	May 2010- October 2010		October 2010-March 2011		
	On Station		On Station	On Farm	
Replication	3		4	3	
Spacing of plants	0.25-0.75 m		0.25-0.75 m	0.25-0.75 m	
Sowing date	13/5/2010 - Alupe		14/10/2010 – Alupe	14/10/2010 - Alupe	
	12/5/2010 - Kibos		13/10/2010 – Kibos	21/10/2010 - Kibos	
Harvesting date	01/10/2010 - Alupe		14/3/2011 – Alupe	12/2/2011 - Alupe	
	01/10/2010 - Kibos		9/3/2011 – Kibos	9/3/2011 - Kibos	
***Striga* infestation**	~3000 per hill		~3000 per hill	~3000 per hill	
	Kibos		Alupe		
Altitude(m)	1214			1189	
Latitude	00^{o}04' S		00^{o}29' N		
Longitude	34^{o}48' E		34^{o}08' E		
Soil type	Rentroentric planosol; sandy loam.		Orthic ferrosol, part petroferric phase with orthic acrisols		
Genotype	Origin	Race	Defense mechanism	Reference	Purpose
N13	India	Durra	Mechanical barriers and Antibiosis	Maiti *et al*, 1984	Donor parent
Ochuti	Kenya		Tolerant	Frost *et al*, 1997; Gurney *et al*, 1995	Recurrent parent
Seredo	Uganda	Caudatum	Tolerant	Haussmann *et al*, 2001a and 2001b	Farmers variety in Kibos
Wagita	East Africa	Local cultivar	Tolerant	Haussmann *et al*, 2001a	Farmers variety in Alupe

APPENDIX VIII. On station trial in Kibos for May to October 2010



A picture taken on 9th August, 2010 at Kibos on station trial May 2010 to October 2010.

APPENDIX V. Summary of agronomic traits means, ANOVA and heritability for Alupe on farm October 2010 to March 2011

Identity	Number of tillers per plot	Number of stem lodged plants	Plant height (cm)	Stand after thinning	*Striga* capsule formation	*Striga* flowering	Panicle weight (g)	Grain weight (g)	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total
BC3S2line33	4.42	2.16	5.47	5.34	1.20	1.43	8.94	8.68	1.82	2.29	3.81	5.31	5.55
BC3S2line87	4.74	1.99	5.51	5.35	1.27	2.05	9.01	8.73	1.69	2.94	4.73	6.01	6.29
N13	4.88	3.21	5.15	5.33	1.60	2.05	8.91	8.50	0.00	3.12	4.03	4.71	5.28
OCHUTI	4.49	2.48	5.49	5.31	2.06	2.42	8.81	8.58	0.69	3.60	5.38	6.51	6.83
Wagita (FV)	4.92	4.69	5.30	5.35	2.35	2.47	9.31	8.94	1.03	4.06	5.89	6.94	7.29
Grand mean	4.69	2.91	5.39	5.34	1.69	2.08	9.00	8.69	0.71	3.20	4.77	5.89	6.25
l.s.d	0.47	0.85	0.03	0.05	1.44	1.68	0.28	0.29	2.50	2.16	1.62	1.53	1.61
cv	1.9	4.9	0.3	0.5	15.8	11.8	2.2	1.8	43.9	10.1	10.6	7.4	6.9
F	NS	*	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Heritability (H^2)	0.7	0.9	0.9	0.5	0.6	0.4	0.8	0.8	0.5	0.5	0.8	0.8	0.2

* Significant at P ≤ 0.05. NS Not Significant at 5 % level

APPENDIX VI. Summary of agronomic traits means, ANOVA and heritability for Kibos On farm trial October 2010 to March 2011

Identity	Number of tillers per plot	Plant height (cm)	Seedling vigor score	Severity score	Stand after thinning	*Striga* capsule formation	*Striga* flowering	100 seed weight (g)	Panicle weight (g)	Grain weight (g)	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total
BC3S2line 33	2.87	5.31	1.00	1.58	4.44	4.34	4.63	1.02	4.13	5.60	1.45	5.70	7.61	8.22	8.71
BC3S2line 87	2.74	5.26	1.00	1.28	4.44	3.45	3.95	1.02	4.23	5.95	0.00	4.06	6.30	7.02	7.45
N13	3.26	5.12	1.47	1.63	4.35	3.75	3.96	1.37	4.24	6.06	1.72	5.04	6.60	7.01	7.66
OCHUTI	3.13	5.30	1.00	1.63	4.44	4.55	4.94	1.00	3.85	5.35	3.51	5.46	7.25	7.94	8.38
Seredo (FV)	3.01	4.88	1.00	2.08	4.44	5.35	5.44	1.08	5.06	6.87	4.39	6.75	8.35	8.90	9.43
Grand mean	3.00	5.18	1.09	1.64	4.42	4.29	4.58	1.10	4.30	5.97	2.22	5.40	7.22	7.82	8.33
l.s.d	0.55	0.16	0.08	0.30	0.12	1.31	1.51	0.10	1.05	1.53	2.05	1.34	1.37	1.33	1.35
cv	9.80	1.70	1.80	7.00	1.40	8.30	10.20	3.20	12.30	12.30	47.50	13.20	9.10	7.70	7.60
F	NS	*	*	*	NS	NS	NS	*	NS	NS	*	*	NS	NS	NS
Heritability (H^2)	0.6	0.9	0.9	0.9	0.6	0.8	0.7	0.9	0.7	0.6	0.9	0.9	0.8	0.8	0.8

* Significant at P ≤ 0.05. NS Not Significant at 5 % level

APPENDIX VII. Correlation for agronomic traits for Alupe on farm trial done in October 2010 to March 2011

	Striga Capsule formation	*Striga* Flowering	Stand after thinning	Seedling Vigor Score	Number of Tillers Plot	Plant Height	Number of stem lodged plants	Days to 50% Flowering	Panicle weight	100 seeds weight	Yield (Kg/M^2)	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC total
***Striga* Capsule formation**	1															
***Striga* Flowering**	0.859***	1														
Stand after thinning	0.104	0.22	1													
Seedling Vigor Score	0	-0.069	-0.532*	1												
Plant Height	-0.118	-0.047	0.02	-0.314	-0.541*	1										
Number of stem lodged plants	0.566*	0.543*	0.245	-0.208	0.555*	-0.431	1									
Panicle weight	0.011	-0.014	0.51	-0.254	0.063	-0.182	0.471	-0.219*	1							
100 seeds weight	-0.336	-0.324	-0.723	0.643	0.546	-0.703	-0.232	-0.677	-0.577	1						
Yield (Kg/M^2)	0.015	0.088	0.5686*	-0.416	-0.048	0.009	0.497	-0.026	0.923***	-0.659	1					
AUSPC 1	0.612	0.907*	0.071	-0.275	0.676	-0.611	0.983**	0.059	0.668	-0.064	-0.028	1				
AUSPC 2	0.668	0.939*	0.297	-0.484	0.446	-0.357	0.989**	-0.237	0.814*	-0.362	0.307	0.952*	1			
AUSPC 3	0.697	0.936*	0.349	-0.546	0.329	-0.267	0.966**	-0.332	0.836*	-0.459	0.400	0.909*	0.992*	1		
AUSPC 4	0.753	0.931*	0.365	-0.567	0.191	-0.17	0.914*	-0.415	0.815*	-0.563	0.434	0.841	0.961**	0.988***	1	
AUSPC total	0.732	0.938*	0.354	-0.555	0.26	-0.22	0.942*	-0.373	0.825*	-0.513	0.421	0.877*	0.979	0.997***	0.997***	1

*** - very significant

** - 99% significant

* - 95% significant

APPENDIX VIII. Actual AUSPC total values

AUSPC totals was used to rank genotypes in both location for the trials performed from May 2010 to March 2011.

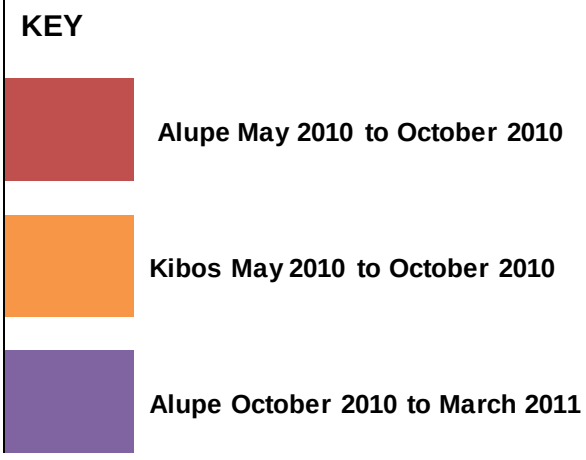
	N13	OCHUTI	L33/H1	L33/H2	L33/H3	L87/H1	L87/H2	L87/H3	L87/H4	L87/H5
Kibos May-Oct 2010	5.48	5.72	6.72	6.29	6.77	5.6	5.83	5.72	6.35	6.23
Alupe May-Oct 2010	4.30	8.76	8.73	8.52	8.78	8.32	8.25	8.38	8.28	8.96
Kibos Oct-March 2011	4.95	6.32	6.81	6.65	6.28	5.90	5.72	6.44	6.06	6.63
Alupe Oct-March 2011	5.46	7.79	7.30	7.76	8.23	6.82	7.25	8.54	7.47	7.47

APPENDIX IX. Genotype ranking using AUSPC totals

The above values were used in ranking the genotypes, according to their performances from AUSPC total values.

Genotype ranking using AUSPC totals.

Ranking	Genotype	Season	AUSPC Total
1	N13	Alupe May to October 2010	4.3
2	N13	Kibos October to March 2011	4.95
3	N13	Alupe October to March 2011	5.46
4	N13	Kibos May to October 2010	5.48
5	L87/H1	Kibos May to October 2010	5.6
6	Ochuti	Kibos May to October 2010	5.72
7	L87/H2	Kibos October to March 2011	5.72
8	L87/H3	Kibos May to October 2010	5.72
9	L87/H2	Kibos May to October 2010	5.83
10	L87/H2	Kibos May to October 2010	5.9
11	L87/H4	Kibos October to March 2011	6.06
12	L87/H5	Kibos May to October 2010	6.23
13	L33/H3	Kibos October to March 2011	6.28
14	L33/H2	Kibos May to October 2010	6.29
15	Ochuti	Kibos October to March 2011	6.32
16	L87/H4	Kibos May to October 2010	6.35
17	L87/H3	Kibos October to March 2011	6.44
18	L87/H5	Kibos October to March 2011	6.63
19	L33/H2	Kibos October to March 2011	6.65
20	L33/H1	Kibos May to October 2010	6.72



21	L33/H3	Kibos May to October 2010	6.77
22	L33/H1	Kibos October to March 2011	6.81
23	L87/H1	Alupe October to March 2011	6.82
24	L87/H2	Alupe October to March 2011	7.25
25	L33/H1	Alupe October to March 2011	7.3
26	L87/H4	Alupe October to March 2011	7.47
27	L87/H5	Alupe October to March 2011	7.47
28	L33/H2	Alupe October to March 2011	7.76
29	Ochuti	Alupe October to March 2011	7.79
30	L33/H3	Alupe October to March 2011	8.23
31	L87/H2	Alupe May to October 2010	8.25
32	L87/H4	Alupe May to October 2010	8.28
33	L87/H1	Alupe May to October 2010	8.32
34	L87/H3	Alupe May to October 2010	8.38
35	L33/H2	Alupe May to October 2010	8.52
36	L87/H3	Alupe October to March 2011	8.54
37	L33/H1	Alupe May to October 2010	8.73
38	Ochuti	Alupe May to October 2010	8.76
39	L33/H3	Alupe May to October 2010	8.78
40	L87/H5	Alupe May to October 2010	8.96

Kibos October 2010 to March 2011

APPENDIX X: FOREGROUND SELECTION RESULTS for BC_3F_1, BC_3F_2 and BC_4F_1

	Chromosome A (LG1)				Chromosome J1 (LG5)				Chromosome B (LG2)						Chromosome I (LG6)				Chromosome J2 (LG5)			
	xtxp 208 (GGA)8		xtxp 302(TGT)8		Xtxtp 065(ACC)4		Xtxp 303(GT)13		Xtxp 201(GA)36		Xtxp 050(CT)13		Xtxp 304(TCT)42		Xtxp 145(AG)22		Xtxp057(GT)21		Xtxp 225(CT)9		Xtxp015(TC)16	
Sample Name	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Ochuti		257		196		151*		152		188		314*		231*		232*		268*		187*		219
N13 Previous	260		237		149*		150		183		316*		323*		262*		261*		183*		217	
Ochuti N13 Current	249		237		150		169		202		317		324		260		260		184		236	
L87_1		257		196				152		188		314		231						187		219
L87_2										188						232				187		
L87_P2_S27		257		196		151								231								
L87_P2_S28		257		196										231				268				
L87_P2_S29		257		196		151					316			231				268				
L87_P2_S30		257		196		151					316			231				268				
L87_P2		257		196		151					316			231				268				
L87_P2_S32		257		196		151						314		231				268				
L87_P2_S33		257		196		151					316			231								
L87_P2_S34		257		196		151								231				268				
L87_P2_S35				196		151								231				268				
L87_P2		257		196		151																219

_S46																			
L87_3								188			231		232				187		
L87_4								188					232				187		
L87_5						152	183	188			231		232			183	187		
L87_6													232			183			
L87_P6 _S24			196		151						231		232						
L87_P6 _S25		257	196								231								
L87_P6 _S26		257	196		151				316		231		232		268				
L87_P6 _S36		257	196		151				316		231				268				219
L87_P6 _S37		257	196		151					314	231				268				219
L87_P6 _S47		257	196		151						231		232						
L87_P6 _S48		257	196		151				316		231								
L87_P6 _S49		257	196		151					314	231		232						
L87_P6 _S50		257	196		151						231		232						
L87_P6 _S51		257	196		151				316		231		232						
L87_P6 _S52		257	196		151						231		232						
L87_P6 _S53		257	196		151				316		231		232						219
L87_P6 _S54		257	196		151						231				268				
L87_P6 _S55			196		151					314	231				268				
L87_P6 _S56		257	196		151						231				268				
L87_P6 _S57			196		151				316		231		232						

L87_P6_S58		196	151			231		268
L87_P6_S59	257	196	151			231		
L87_P6_S60	257	196	151			231	232	268
L87_P6_S61	257	196	151			231		268
L87_P6_S62		196				231		268
L87_P6_S63		196	151			231		268
L87_P6_S64	257	196				231		268
L87_P6_S65	257	196	151			231		268
L87_P6_S66	257					231	232	268
L87_P6_S67	257	196	151			231	232	268
L87_P6_S68	257	196	151			231		268
L87_P6_S69	257	196	151			231	232	268
L87_P6_S70	257	196	151		314	231	232	268
L87_P6_S71	257	196	151			231		268
L87_P6_S72	257	196	151			231	232	268
L87_P6_S73	257	196	151	316		231	232	
L87_P6_S74	257	196	151			231	232	268
L87_P6_S75	257	196	151			231	232	268
L87_P6_S76	257	196	151			231		268
L87_P6	257	196	151			231	232	268

_S77																			
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_S78		257		196		151				316			231			268			
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L87_P6																			
_S80		257		196		151						314	231						
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_S86		257		196		151				316			231			268			
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_S88				196									231			268			
L87_P6																			
_S89						151							231			268			
L87_P6																			
_S90		257		196		151							231			268			
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_S92				196		151						314	231			268			
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L87_P6																			
_S95		257		196									231			268			
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L87_8	257		196		151			183	188		314		231		232			187		
L33_P8_S37	257				151											268				
L33_P8_S38	257				151															
L87_9	257		196			152			188		314		231		232			187	217	219
L33_P9_S35	257				151											268				
L33_P9_S36	257				151											268				
L87_10	257	237	196			152			188		314		231		232			187		219
L87_P10_S6			196								314		231			268				
L87_P10_S7			196		151					316			231		232	268				219
L87_P10_S42	257		196										231			268				
L87_P10_S43	257		196										231		232	268				219
L87_P10_S44	257		196							316			231			268				
L87_P10_S45	257		196																	219
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L87_P14_S5	257		196		151								231		232	268				
L87_P14_S8	257		196		151					316					232	268				
L87_P14_S9	257		196								314				232	268				
L87_P14_S10	257		196		151					316			231			268				
L87_P14_S11	257		196		151								231		232	268				
L87_P1	257		196		151					316			231		232	268				

4_S12 L87_P1									
4_S13 L87_P1	257	196	151			231	232	268	
4_S14 L87_P1	257	196	151		316	231		268	
4_S15 L87_P1	257	196	151		316	231	232	268	
4_S16 L87_P1	257	196	151		316		232	268	
4_S17 L87_P1	257	196	151			231	232	268	
4_S18 L87_P1	257	196	151		316	231		268	
4_S19 L87_P1	257	196	151			231	232	268	
4_S20 L87_P1	257	196	151		316	231		268	
4_S21 L87_P1	257	196	151					268	
4_S22 L87_P1	257	196	151		316	231	232	268	
4_S23 L87_P1							232		
4_S96 L87_P1	257	196	151				232	268	
4_S97 L87_P1	257	196	151		316	231	232	268	
4_S98 L87_P1	257	196	151		316	231	232	268	
4_S99 L87_P1	257	196			316	231		268	
4_S100 L87_P1	257	196	151		316	231	232	268	
4_S101 L87_P1	257	196	151		316	231	232	268	
4_S102 L87_P1		196		314		231		268	
4_S103	257	196	151			231		268	

L87_P14_S104		257		196		151					314		231						
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L87_P14_S106		257		196						316			231			268			
L87_P14_S107		257		196									231			268			
L87_P14_S112				196		151													
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L87_P20_S40		257	237	196		151				316			231			268				
L87_P20_S41		257		196		151							231			268				
L87_21		257	237	196			152		188		314		231		232			187	217	219
L87_22		257		196			152	183	188		314		231		232		183	187		219
L87_P22_S1		257		196		151							231			268				
L87_P22_S2		257											231			268				
L87_P22_S3		257		196						316					232	268				219
L87_P22_S4		257		196		151				316			231		232					
L87_P22_S108															232	268				
L87_P22_S109		257		196		151							231		232					
L87_P22_S110		257				151				316			231		232	268				

L87_P2																	
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_S38				196							314	231						
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_S36				196										232				219
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L33_P1																	
1_S156	257		196														
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1_S160	257		196									232	268				
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1_S161	257		196										268				
L33_P1																	
1_S162	257		196							231			268				
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1_S163	257		196							231							
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L33_P17_S116		257	196					231	232				219		
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L33_P17_S118		257	196	151				231	232	268					
L33_P17_S119		257	196	151				231	232	268					
L33_P17_S120		257	196	151				231	232	268					
L33_P17_S121		257	196					231	232	268					
L33_P17_S122		257	196					231		268					
L33_P17		257	196					231		268					

7_S123																		
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7_S126	257	237	196							231		232	261					
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7_S127	257	237	196							231		232		268				
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7_S128	257	237	196							231								
L33_P1																		
7_S129	257	237	196		151					231		232		268				
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7_S130	257				151					231				268				
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7_S131	257	237			151					231				268				
L33_P1																		
7_S132	257	237	196		151					231				268				
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7_S136	257		196		151					231		232		268				
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7_S140	257		196		151					231				268				
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7_S141	257		196		151					231								
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7_S142	257	237	196							231								

L33_P17_S143	257		196		151				231						
L33_P17_S144	257		196						231						
L33_P17_S145	257				151				231		268				
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L33_P19_S42	257		196		151				231		268				219
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L33_P19_S45	257		196		151				231		268				219
L33_P19_S174	257		196		151				231	232	268				
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L33_P19_S176	257		196		151				231	232					219
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0_S94	257	196							
L33_P2									
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L33_P2									
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L33_P2									
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L33_P2									
0_S106	257	196			231		268		
L33_P2									
0_S107	257	196	151		231	232			
L33_P2									
0_S108	257	196			231	232			
L33_P2									
0_S109	257	196			231		268		
L33_P2									
0_S110	257	196	151		231		268		

L33_P20_S111	257		196						231			268					
L33_P20_S112	257		196		151				231		232	268					
L33_P20_S113	257		196		151				231		232						
L33_21	257	237	196			152		188		314	231	232			187	217	219
L33_P21_S46	257	237	196		151				231			268					219
L33_P21_S47	257		196						231			268					
L33_P21_S48	257		196		151			316			231		268				
L33_P21_S49			196		151			316			231	232					219
L33_P21_S50			196						231			268					219
L33_P21_S51			196						231			268					
L33_P21_S52	257		196					316			231		268				
L33_P21_S53	257		196		151				231		232	268					219
L33_P21_S54	257		196		151			316			231	232	268				
L33_P21_S55			196					316			231		268				
L33_P21_S56	257		196		151			316			231	232					
L33_22	257		196			152		188		314	231	232			187	217	219
L33_P22_S70	257		196		151			316			231		268				219
L33_P22_S71	257		196		151			316			231	232	268				
L33_P22_S72	257		196		151			316			231		268				
L33_P22_S73	257		196					314			231		268				

L33_P22_4		257		196		151				316			231		232		268					
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L33_P22_S76		257	237	196									231		232		268					
L33_P22_S77		257		196									231		232		268					
L33_P22_S78		257		196		151							231		232		268					
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L33_27_16	260			197					207	202		315	324	232			266		208	188	236	238
L33_28	260	257		196				152	183	188		314		231		232				187	217	219
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L33_30		257		196				152		188		314		231		232				187	217	219
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L33_P31_S1	260					151					316			231		232						
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L33_P31_S3		257		196		151								231		232		268				
L33_P31_S4		257		196		151					316			231	262	232		268				219
L33_P31_S5		257		196		151									262	232		268				219
L33_P31_S6		257		196		151					316			231		232		268				
L33_P3		257		196		151					316			231	262	232		268				

1_S7 L33_P3													
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1_S9 L33_P3	257	196	151		316		231		232	268			219
1_S10 L33_P3	257	196								268			
1_S150 L33_P3	257	196	151		316		231		232	268			
1_S151 L33_P3	257	196	151		316		231		232	268			
1_S152 L33_P3	257	196	151		316		231		232	268			
1_S153 L33_P3	257	196			314		231		232	268			
1_S154 L33_P3	257	196	151		316		231		232	268			
1_S155 L33_P3					316		231		232	268			
1_S10 L33_32	257		151	188			231		232		187	217	219
2_S57 L33_P3	257	196	151		316		231			268			
2_S58 L33_P3	257	196			316		231		232	268			
2_S59 L33_P3	257	196	151				231			268			
2_S60 L33_P3	257	196	151				231		232	268			
2_S61 L33_P3	257	196			316		231		232	268			
2_S62 L33_P3	257	196	151		316		231		232				
2_S63 L33_P3					316		231		232				
2_S64	257	237 196	151		316				232				

L33_P32_S65	257		196		151			316		231	232	268					
L33_33	257		196			152				231	232			187			
L33_34	257		196		153	152			314		232			187			
L33_P34_S18	257		196							231	232	268					
L33_P34_S19	257		196							231		268				219	
L33_P34_S20	257		196		151					231	232	268					
L33_P34_S21	257		196		151			316		231	232	268				219	
L33_P34_S22	257		196		151					231	232	268					
L33_P34_S23			196							231	232						
L33_35	257		196			152					232			187	217	219	
L33_36	257		196		151	152				231	232			187	217	219	
L87_P36_S38	257																
L33_37	257	237	196			152			314					187			
L33_38	257		196			152				231				187			
L33_39	257		196			152		188	314	231	232			187			
L33_40	257								314		232			187			
L33_41	257		196			152	183		314	231	232				217	219	
L33_42	257		196					188	314	231	232			187	217	219	
L33_P42_S24	257		196							231		268					
L33_P42_S25	257		196		151			316		231		268					
L33_P42_S26	257		196		151					231		268					
L33_P42_S27	257		196		151					231		268					
L33_P42_S28	257		196							231	232	268					

L33_P4																									
2_S29			257		196								231			232		268					219		
L33_P4																									
2_S30			257		196			151					231					268					219		
L33_P4																									
2_S31			257		196								231					268							
L33_P4																									
2_S32			257		196			151					231			232		268							
L33_P4																									
2_S33			257		196			151				316			231			232							
L33_P4																									
2_S34			257		196			151				316			231			232		268					
L33_P4																									
2_S164			257		196			151				316			231			232		268					
L33_P4																									
2_S165			257		196			151				316			231					268					
L33_P4																									
2_S166			257												231			232		268					
L33_P4																									
2_S167			257		196			151				316			231			232		268					
L33_P4																									
2_S168			257		196										231			232							
L33_P4																									
2_S169			257		196			151				316			231			232							
L33_P4																									
2_S170			257		196			151				316			231			232		268					
L33_P4																									
2_S171			257		196			151				316			231			232		268					
L33_P4																									
2_S172					196										231			232		268					
L33_P4																									
2_S173					196			151				316			231			232							
Ochuti			257		196						188		314		231			232				187			
Ochuti			257		196						188		314		231			232				187			
Ochuti											188		314		231			232							
N13_Th																									
eo							150										262								
N13_Th	260					150			183					323			262				183				

eo																								
N13_Th																								
eo	260									150					183					323			262					183			
N13_Th																								
eo	260									150					183					323								183			
N13_Th																								
eo										150					183					323								183			
N13_Th																								
eo										150					183					323								183			
N13_Th																								
eo															183					323								183			
Ochuti_																								
107			257													188			314		231		232						187		
Ochuti_																								
15			257													188			314		231		232						187		
Ochuti_																								
5									196							188			314		231								187		

KEY for Foreground selection results:

Introgressed QTL for BC3F1

Fixed QTL for BC3F2 (BC3S1)

Heterozygous QTL for BC3F2 (BC3S1)

Partially introgressed QTL for BC3F1 and BC4F1