**Understanding resistance in inter-specific rice cultivars to the parasitic witchweed *Striga***

**A thesis submitted by**

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**Declaration**

No portion of the work referred to in this thesis has been submitted in support of another application for another degree or qualification at this or any other university or institute of learning.

**Abstract**

The root hemi-parasitic witchweeds *Striga hermonthica* and *S. asiatica* are considered the most important biotic constraint to cereal crop production in sub-Saharan Africa (SSA). These parasites infect the staple cereal crops (rice, maize, sorghum and millet) resulting in considerable yield losses. Control of these parasites is very difficult as the *Striga* seed bank is widespread and damage to the crop occurs long before the parasite emerges above ground. Resistant cultivars are considered to be an effective and affordable component of an integrated *Striga* management strategy but very few are available to farmers as sources of resistance to *Striga* are relatively scarce and little is known about the molecular genetic basis of resistance to this parasite. Rice is an economically important cereal crop in SSA that is mostly cultivated by resource-poor farmers. Both cultivated rice species, *Oryza sativa* (L.) and *Oryza glaberrima* (Steud.), are grown in Africa. To take advantage of superior traits from each species, AfricaRice Center and partners developed inter-specific rice cultivars called NERICA (NEw RICe for Africa) for rain-fed upland ecosystems. Because of their high yields, even on low nutrient soils where *Striga* spp. are prevalent, the NERICA cultivars have been widely adopted by farmers. Despite this, very little is known about their resistance to different species and ecotypes of *Striga*. The aims of this study are to determine how resistant and/or tolerant the upland NERICA cultivars are to different species and ecotypes of *Striga* under controlled environment and *Striga*-infested field conditions, to identify whether resistance is broad spectrum or specific to particular ecotypes of *Striga* and to characterize the phenotype of the resistance at a histological level. Finally using a Chromosome Segment Substitution Line (CSSL) population derived from a cross between an *O. glaberrima* cultivar MG12 (donor parent) and an *O. sativa* cultivar Caiapo (recurrent parent), the genetic basis of post-attachment resistance to *Striga* is investigated.

The NERICA rice cultivars showed different susceptibilities to both *S. hermonthica* and *S. asiatica* species under controlled environment conditions. Some cultivars showed good broad-spectrum resistance against several *Striga* ecotypes and species whilst others showed intermediate resistance or were very susceptible. In addition, some cultivars showed resistance to a particular ecotype of *Striga* but were susceptible to others. The phenotype of a resistant interaction was often characterized by necrosis at the host parasite interface and an inability of the parasite to penetrate the host root endodermis. In general, the most resistant NERICA cultivars grew better than the very susceptible cultivars although even a small number of parasites caused a reduction in above ground host biomass. There was however, genetic variation for tolerance to *Striga* (the ability to grow and yield well in the presence of *Striga*) amongst the NERICA cultivars. The NERICA cultivars were also grown in field trials at Kyela in Tanzania (under *S. asiatica* infestation) and at Mbita Point in Kenya (under *S. hermonthica* infestation) in 2010 and 2011 to determine the impact of environment on the expression of resistance. The resistance of the NERICA cultivars against *S. hermonthica* and *S. asiatica,* in the field, was broadly similar to that observed in the laboratory although there were some exceptions. These results allow us to recommend particular cultivars for *Striga*-infested regions but they also illustrate the necessity of understanding the genetic basis of resistance to different ecotypes of *Striga* for breeding of durable resistance (and pyramiding of appropriate resistance genes) in host cultivars adapted to different rice agro-ecosystems in sub-Saharan Africa.

Sixty four lines of an inter-specific CSSL population and the parent cultivars MG12 and Caiapo were phenotyped for resistance to *S. hermonthica*. MG12 showed good resistance to *S. hermonthica* whilst Caiapo was very susceptible. The CSSLs showed a range of susceptibility to the parasite, however, only two CSSLs showed the same strong resistance phenotype as MG12. Graphical genotyping and a Quantitative Trait Loci (QTL) analysis revealed a large QTL on chromosome 12 (designated STR12.1) which explained at least 80 % of the variation for resistance in the population and suggests that resistance to *S. hermonthica* (in MG12) is due to one (or a few genes) of major effect. This finding opens the way for the identification of candidate *Striga* resistance genes (through fine mapping approaches) and their transfer to farmer-preferred cultivars via marker assisted breeding.

**Abbreviations and Acronyms**

**ABA** : Absicissic Acid

**AfricaRice** : Africa Rice Center

**AFLP**  : Amplified Fragment Length Polymorphism

**AI** : Artificial Infestation

**ANOVA** : Analysis of variance

**BBSRC** : Biotechnology and Biological Sciences Research Council

**BIL** : Backcross Inbred Line

**CIAT** : International Center of Tropical Agriculture

**CSSL** : Chromosome Segment Substitution Line

**DAI** : Days After Infection

**DAS** : Days After Sowing

**DFID**  : Department oF International Development

**DH** : Doubled Haploids

**2,6-DMBQ** : 2,6-dimethoxy-benzoquinone

**FAO** : Food and Agriculture Organization of the United Nations

**GCP**  : Generation Challenge Program

**HIF** Haustorial Initiation Factor

**HR** : Hypersensitive Response

**HSD** : Honest Significant Difference

**IRD**  : Institut de Recherche pour le Developement

**IRRI** : International Rice Research Institute

***lgs***: Low germination stimulant

**MAB** : Marker Assisted Breeding

**N** : Nitrogen

**NERICA** : New RICe for Africa

**NI**  : Natural Infestation

**NIL**  : Near Isogenic line

***NS***: Number of *Striga* seedlings

**P** : Phosphorus

**PVS** : Participatory Varietal Selection

**QTL** : Quantitative Trait Loci

**RDC** : République démocratique du Congo

**RIL** : Recombinant Inbred Line

**RSNV** : *Rice Stripe Necrosis Virus*

**RYMV** : *Rice Yellow Mottle Virus*

**Sa-Kyela** : *Striga asiatica* from Kyela-Tanzania

**Sa-USA** : *Striga asiatica* form North Carolina-USA

**SG3**  : race of *S. gesnerioides* parasitic on cowpea localized in Nigeria and

Niger

**Sh-Busia** : *Striga hermonthica* from Busia-Kenya

**Sh-Kibos** : *Striga hermonthica* from Kibos-Kenya

**Sh-Kouto** : *Striga hermonthica* from Kouto-Côte d’Ivoire

**Sh-Medani** : *Striga hermonthica* from Medani-Sudan

***SNmax*** : Maximum number of above-ground *Striga* plants

**SQR** : Shanqui Red

**SSA** : sub-Saharan Africa

**SSR** : Single Sequence Repeat

**WARDA** : West Africa Rice Development Association

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# Chapter 1

# General introduction

## 

## Introduction to parasitic plants

Today the world is characterized by an increasing human population (particularly in developing countries), decreasing land availability, climate change, major pests and diseases, and many constraints or stresses that affect food and specifically cereal crop production. One of these constraints is parasitic weeds known to infect cereal crops in Africa resulting in considerable yield losses. Parasitic plants are classified as hemi-parasites or holo-parasites based on the presence or the absence of chlorophyll, respectively in their tissue (Press and Graves, 1995). Hemi-parasites such as *Striga* species are able to acquire some of their own carbon from photosynthesis once they appear above ground whilst holo-parasites such as O*robanche* species do not photosynthesize and depend on their host carbon source (Steward and Press, 1990; Cechin and Press, 1993a, 1993b; Parker and Riches, 1993; Press and Graves, 1995; Estabrook and Yoder, 1998). Both *Striga* and *Orobanche* species cannot survive in the absence of their host and are termed obligate parasites (e.g. Estabrook and Yoder, 1998). However, some hemi-parasitic plants e.g. *Rhinanthus* species can grow and survive without being connected (through attachment) to their hosts and are known as facultative parasites (Gibson and Watkinson, 1989; Estabrook and Yoder, 1998).

The root hemi-parasitic weed *Striga* causes severe damage to crops in Africa, particularly in sub-Saharan African (SSA) regions (Mohamed *et al.*, 2006). Berner *et al.* (1995) estimated that *Striga* infestation in cereal crops causes losses in yield estimated to be worth some 7 billion US dollars annually. The parasite affects several millions of African people (Sauerborn, 1991) with the potential to devastate more than half of arable land under cereal production (Parker, 1991; Lagoke *et al.*, 1991). Problems with parasitic *Striga* species are becoming more important especially in resource poor farming systems where soil fertility is very low; they have been reported in multiple cereal crops (Dugje *et al.*, 2006) such as sorghum and maize (Gurney *et al.*, 2003; Amusan *et al.*, 2008) millet (Wilson *et al.*, 2000, 2004) and rice (Johnson *et al.*, 1997, 2000; Rodenburg and Johnson, 2009; Rodenburg *et al.*, 2010).

More than 40 species constitute the genus *Striga* which has recently been placed in the *Orobanchaceae* family (formerly known as the *Scrophulariaceae*) (Olmstead *et al.*, 2001). Among them only 22 species are widespread. Native to Africa, *Striga* species are mostly found in SSA and 11 parasitize agricultural crops (Berner *et al.*, 1995). *Striga hermonthica* (Del.) Benth., *S. asiatica* (L) Kuntze, *S. aspera* and *S. gesnerioides* (Willd) are the most economically important witch weeds in the semi-arid to sub-humid tropics (Mohamed *et al.*, 2006). *Striga hermonthica* and *S. asiatica* infect sorghum, maize, pearl and finger millets and upland rice (Ejeta *et al.*, 1992; Parker et Riches, 1993; Johnson *et al.*, 1997; Haussmann *et al.*, 2000; Ejeta, 2007; Amusan *et al.*, 2008; Scholes and Press, 2008). *Striga gesnerioides* attacks crops like cowpea in West Africa and tobacco and sweet potato in India causing approximately 30% loss of yield.

Figure 1.1 shows the distribution of *Striga* species in Africa. *Striga* is extremely widespread with heavy infestations from West (e.g. Senegal, Mali, Niger and Nigeria) to East Africa (e.g. Sudan, Ethiopia, Kenya and Tanzania) and throughout SSA (Ejeta, 2007). *Striga* species are particularly problematic in fields where soils are poor in nutrients belonging mostly to farmers with low incomes. They infect several million hectares of cereal crops and their effects are often very severe (Fig 1.2)(Ejeta, 2007). According to the Food and Agricultural Organization of the United Nations (FAO) approximately 300 million people and 100 million hectares of farmland are affected by *Striga*. The yield loss in cereals due to *Striga* in SSA is more than 40% and the extent of its damages and losses varies with parasite species, ecotypes, infestation level and virulence (Ejeta, 2007). *Striga* is very difficult to control as the life cycle of the parasite is tightly coordinated with that of its host, the parasite damages the crop very shortly after attachment to the host roots and relatively little is known about host resistance to the parasite.

## The life cycle of *Striga*

The life cycle of *Striga* is linked with that of its host and can be divided into different stages involving seed dormancy, preconditioning and seed germination, radicle growth, haustorial differentiation and attachment to the host, growth of the parasite, metabolic interactions with the host and flowering (Fig 1.3) (Scholes and Press, 2008).

*Striga* spp. produce a lot of very small seeds (greater than 10,000 seeds per plant) approximately 0.2 mm to 0.5 mm long and weighing 4 to 7 µg each. Seed dispersal occurs via agricultural practices, transportation by water or insect and animal activities (Kuijt, 1969). Once *Striga* seeds have been produced they can stay viable for at least 14 years and they constitute a very large and durable seed bank in the soil (Parker and Riches, 1993).Before seeds can germinate they need to condition i.e. to be exposed to favourable environmental conditions (wet soils in the rainy season and warm moist

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Figure 1.1 Geographic distribution and prevalence of *Striga* species in Africa (from Ejeta, 2007).

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Figure 1.2 Rice field in Kenya infested by *Striga hermonthica.*

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Figure 1.3 The life cycle of *Striga.* (From Scholes and Press, 2008).

conditions) (Fig 1.3a).

The germination of *Striga* seeds takes place in response to stimulants present in root exudates of host or some non-host plants (Keyes *et al.*, 2001; Yoder, 2001). The stimulants produced by the host root are chemically diverse and include dihydrosorgoleone, sesquiterpene lactones and strigolactones, of which the latter are known to be the most active in inducing germination (Chang and Lynn, 1986; Fischer *et al.*, 1989; Bouwmeester *et al.*, 2003; Humphrey and Beale, 2006). Strigolactones have been isolated from roots of cotton (non-host), maize, millet (strigol), sorghum (sorgolactone) and red clover (orobanchol). The chemical structures of some of the germination stimulants are shown in Figure 1.4. Recently, Matusova *et al.* (2005) and Bouwmeester *et al.* (2007) reported that strigolactones are synthesised via the carotenoid biosynthetic pathway. In addition, strigolactones are not only the germination stimulants for parasitic plants but they act as a signal for mycorrhizal branching, a process necessary for infection of roots, and they also play a role in inhibiting branching in shoots (Umehara *et al.*, 2008; Gomez-Roldan *et al.*, 2008). In addition to the stimulants mentioned above some hormones like abscisic acid, kinetin, giberellic acid and ethylene can act as substitute germination stimulants. Products similar to strigol have been synthesized and among them, GR-24 is the most used, especially in laboratories to stimulate and assess the germination of *Striga* seeds (Wigchert and Zwanenburg, 1999).

Once they germinate, *Striga* radicles elongate (Fig 1.3b)and in response to host-derived Haustorial Initiation Factors (HIF) form the parasitic structure known as the haustorium (Fig 1.3c) (Parker and Riches, 1993; Estabrook and Yoder, 1998). HIFs differ from the germination stimulants and are usually quinones (e.g. 2,6-dimethoxy-benzoquinone (2,6-DMBQ), phenolic substances and cytokinins (Lynn and Chang, 1990). The haustorium is a parasitic organ which penetrates into the cortex and endodermis. The portion of the parasite within the root is often termed the endophyte (Fig 1.3d). Once the parasite crosses the endodermis, parasite cells fuse with the host xylem elements establishing xylem-xylem continuity (Fig 1.3e) allowing the transfer of the nutrients, some amino-acids and water from host to parasite (Press *et al.*, 1987a, 1987b; Parker and Riches, 1993; Pageau *et al.*, 2003). The parasite starts to grow and a few days later, cotyledon leaves are formed, followed by leaf pairs (Fig 1.3f). The *Striga* shoot grows up through the soil and emerges above the ground. It then flowers, sets seeds within 6 weeks and produces more than 5,000-100,000 tiny seeds per plant

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Figure 1.4 Chemical structures of representative strigolactones (from Umehara *et al.*, 2008).

(Parker and Riches, 1993; Pageau *et al.*, 2003; Stewart and Press, 1990; Webb and Smith, 1996) (Fig 1.3g). Finally, after 10 to 16 weeks, the parasitic weed *Striga* completes its life cycle (Musselman, 1980).

## Host responses to S*triga* infection

Host responses to *Striga* infection are characterized by effects on host growth and yield which can be moderate to severe depending on host plant, genotype, nutrition, infection and attachment time (Graves *et al.*, 1989; Frost *et al.*, 1997; Arnaud *et al.*, 1999; Gurney *et al.*, 1999; Rodenburg *et al.*, 2006a, 2006b; Cissoko *et al.*, 2011).

The parasitic weed *Striga* has a severe effect on host plant growth and development within days of attachment and small parasite biomass can cause considerablestunting of the host plant (due to a reduction in internode elongation), chlorosis and brown streaks on leaves. In addition the stems of the host are very thin in comparison to infected plants. Overall there is a reduction of shoot biomass, compared to root biomass which is much less affected by the parasite (Riches *et al.*, 1996; Gurney *et al.*, 1999; Cissoko *et al.*, 2011). Some of the effects, for example the stunting, are observed shortly after attachment.

The fact that some of the alterations to plant growth occur shortly after attachment when the parasite biomass is very small has led to the suggestion that *Striga* produces a toxin (Musselman, 1980) but as yet there is no evidence for this. It has also been hypothesised that the developmental changes occur through altered plant growth regulator signalling and metabolism (Taylor *et al.*, 1996; Ackroyd and Graves, 1997; Frost *et al.*, 1997).

It is clear that the early effects on host development in response to *Striga* infection are not just due to competition for host resources as parasites are very small. However, once the parasite begins to grow it is an effective sink for host carbon, nitrogen and inorganic compounds and this will also lower growth and yield of the plant (Frost *et al.*, 1997; Gurney *et al.*, 1999; Gurney *et al.*, 2000). Many studies, on maize (Gurney *et al.,* 1995; Taylor *et al.,* 1996), millet (Graves *et al.,* 1990), sorghum (Cechin and Press, 1993b; Gurney et *al.,* 1995; Frost *et al.,* 1997) and on rice ( Cechin and Press, 1994a; Rodenburg *et al.,* 2008), revealed that photosynthesis is lower in infected plants compared to uninfected plants and this again contributes to host biomass reduction (Gurney *et al.*, 2000).

When *Striga* is underground all of its carbon needs are supplied by the host plant (Parker and Riches, 1993), but when it grows above the ground, it is able to photosynthesize to some degree but still obtains approximately 30% of its carbon from the host and all of its nitrogen requirements (Gurney *et al.*, 1995). The reduction of the photosynthesis rate in infected plants is a result of stomatal closure, possibly linked with the increase in absicisic acid (ABA) production or concentration in the host plants (Cechin and Press, 1994a, 1994b; Gurney *et al.*, 1995; Taylor *et al.*, 1996; Frost *et al.*, 1997). It is also possible that this ABA accumulation can explain the change which appears in the distribution of biomass partitioning especially in the root and shoot ratio of infected plants but further research is needed to demonstrate this. Because *Striga* affects host growth and photosynthesis so soon after attachment, the best improvement in yield occurs if control methods either prevent attachment or destroy *Striga* soon after their attachment to the host roots.

## *Striga* control strategies

To date, many control strategies against *Striga* have been established and used, but unfortunately they have had little effect due to their high costs, unavailability and/or inapplicability for resource-poor African farmers. These control strategies are of various types: agronomic, chemical, biological and genetic and have been summarised in detail in many reviews (Elzein and Kroschel, 2008; Hearne, 2009; Rodenburg *et al.*, 2010).

Agronomic practices include hand weeding, crop rotation with trap and catch crops, soil fertilization, intercropping systems (Cechin and Press, 1993a, 1993b; Parker and Riches, 1993; Omanya *et al.*, 2001; Kanampiu *et al.*, 2002a, 2002b; Khan *et al.*, 2002; Showemimo *et al.*, 2002). Some of these strategies are commonly used by farmers, but success is not notable due to continuing seed accumulation in the seed bank over the years, underground development of the parasite and unavailability of financial resources. Using hand weeding, the farmers can reduce *Striga* seedproduction when they do it before flowering, but this method is time consuming and needs to be repeated several times during many crop seasons before its value can be noticed (Parker and Riches, 1993).

One option to reduce the impact of *Striga* in farmer’s fields is to improve soil fertility as *Striga* is more prevalent in nutrient deficit areas. The increase of nitrogen concentration in the soil can decrease the number of parasite attachments on host plant roots as shown in the studies of Riches *et al.* (2005) and Adagba *et al.* (2002) for rice, of Showemimo *et al.* (2002) and Cechin and Press (1993b) on sorghum and of Kamara *et al.* (2009) and Ahonsi *et al.* (2002) on maize. Although this is a practical method, it remains difficult for African farmers to carry out since they have only limited finances and access to fertilizers.

To control *Striga* infestation and or to decrease the *Striga* soil seed bank,crop rotations can be used with trap and catch crops. Trap crops are non-host plants (like soybean, cotton, groundnut and pigeon pea) that induce parasite seed germination by producing root exudates but are not infected or parasitized by them. Riches *et al.* (2005) showed that rice which rotates with pigeon pea in fields infested with *S. asiatica* in Tanzania is less infected and has a good yield. Catch crops are host plants which stimulate seed germination and are infected, so additional hand pulling is needed to eliminate the parasite before its flowering time. This is not very attractive for farmers who must apply two methods or double their efforts in order to achieve a reduction of the impact of *Striga.* An example of a catch crop is *Sorghum sudanense* L. used to control *S. hermonthica* (Oswald *et al.*, 1997).

Among the strategies to control *Striga* aimed at preventing the attachment and proliferation of the parasite seeds, intercropping remains one of the most successful. Indeed, Oswald *et al.* (2002) showed a significant reduction of emerged *Striga* when maize is intercropped with sweet potato and cowpea in Kenya. The last two crops are called false hosts as they cause *Striga* seeds to germinate but are unable to support their attachment. They create a suicidal germination of *Striga* seeds in the vicinity of intercrop roots and add also nitrogen to soil. More recently, *Desmodium uncinatum* is being used as an intercrop legume with maize and sorghum and also reduces *Striga* emergence, especially *S. hermonthica* (Khan *et al.*, 2002; Khan *et al.*, 2007). The legume root exudates stimulate parasite seed germination but can also cause inhibition of parasite development (Hooper *et al.*, 2010). The basis of this phenomenon is an allelopathic compound which is thought to inhibit the development of the haustorium (Khan *et al.*, 2002; Hooper *et al.*, 2010). This method is very popular among poor African farmers in some areas, since it is very effective in reducing the level of seeds in infested fields.

Herbicides and artificial germination stimulants, which, although used as a means of control against *Striga* often remain out of reach for poor farmers (Ransom, 2000). Although, 2,4-Dichlorophenoxyacetic acid (2,4-D) which is used as an herbicide applied directly and repeatedly on the parasite, has shown good results (Carsky *et al.*, 1994; Paré *et al.*, 1996), the fact that most of the development of the parasite takes place underground, reduced its effectiveness. A more successful strategy is to use herbicide-resistant maize coated with the herbicide imazapyr which causes a considerable reduction in the attachment of *Striga* on roots (Kanampiu *et al.*, 2002b). In addition, analogues of germination stimulants can be applied to soil to control *Striga* proliferation as they induce germination of *Striga* seeds that then die because of the absence of host roots. This is the case with ethylene which has been used occasionally to reduce *Striga* levels (reduction of soil seed bank) but is not a feasible control method for widespread use. Ethylene is very expensive and like most of the methods mentioned above, does not meet the expectations of subsistence farmers who would like a more suitable strategy which can allow them both to fight effectively against *Striga* species/ecotypes as well as to improve their crop production. Therefore, a suitable, durable and cheap control option for poor farmers seems to be the use of resistant and tolerant cultivars.

## Can resistant and tolerant cultivars play a role in controlling *Striga* and improving crop yield?

Research obtained for some specific crops gives hope that the use of resistant and tolerant cultivars could be an effective *Striga* control method particularly if used in combination with other strategies. The early damage caused soon after parasite attachment and particularly the low incomes of African farmers are the most important aspects to be considered. Resistant cultivars are defined as those that support few or no *Striga* attachments when parasitized (Doggett, 1988; Ejeta *et al.*, 1992; Parker and Riches, 1993). Many studies have been carried out to identify resistant crop cultivars and their reactions to *Striga* infection. Some host plants produce low amounts of germination stimulants in their root exudates and this reduces *Striga* germination resulting in decreased parasite attachment (Vogler *et al.*, 1996). This is the case for SAR-1, a *S. asiatica* resistant sorghum line in the study of Lane and Bailey (1992). Low germination stimulant (*lgs*) gene for resistance to *Striga* species has been found in a population of recombinant inbred lines (RILs) derived from *Striga* resistant sorghum cultivar SRN39 and the susceptible cultivar Shanqui Red (SQR) (Ejeta, 2007). Recently, Satish *et al.* (2012) have tagged and mapped molecular markers associated with this *Striga* resistance gene which will accelerate breeding activities for the development of *Striga*-resistant cultivars and also help the fine mapping and identification of the gene responsible. However, despite the fact that *lgs* production can reduced parasite germination prior to attachment to the host roots system, only few parasite attachments are needed to have a large effect on plant growth and yield as Gurney *et al.* (1999) point out.

Other types of resistance occur post-attachment. A study by Mohamed *et al.* (2003) showed that some resistant sorghum cultivars exhibit a hypersensitive response (HR), when infected by some ecotypes of *S. asiatica*. The HR is characterized by the development of necrotic tissue around the parasite attachment site which is thought to prevent host root cortex invasion. Among them, P47121 (a wild relative of sorghum), and Framida and Dobbs (sorghum cultivars) are resistant to one ecotype of *S. asiatica*. Many research studies have shown different levels of resistance to *Striga* species after attachment. In the case of N13, a sorghum cultivar, *S. asiatica* penetrates the root cortex but cannot go beyond it (Maiti *et al.*, 1984). At Sheffield University we have screened N13 and E36-1 (both parents of a RIL (Recombinant Inbred Lines) sorghum mapping population) for post-attachment resistance against different ecotypes of *S. hermonthica* and *S. asiatica* (Fig 1.5). In our study, N13 supported less *Striga* dry biomass than E36-1. N13 showed high levels of resistant against all the *Striga* species and ecotypes tested. N13 also significantly reduced *S. hermonthica* seed reproduction in the field as demonstrated by the study of Rodenburg *et al.* (2006b).

Further fine mapping of the genes responsible for this resistance and the development of markers linked to the resistance loci are required to aid breeding efforts using N13 as the donor parent. Recombinant inbred lines derived from the resistant sorghum cultivars N13 and IS9830 as a donor parent in the genetic background of a susceptible sorghum cultivar E-36 have been used to identify 5 common Quantitative Trait Loci (QTL) responsible for field resistance to *S. hermonthica* in two season trials at two locations in Kenya and Mali (Haussmann *et al.*, 2004). These QTL are thought to represent robust, durable resistance QTL as the authors validated the QTL by using 2 different mapping populations in different environments across two seasons. This study again suggests that N13 and IS9830 are suitable candidates for use in Marker Assisted Breeding (MAB) programmes.

Gurney *et al.* (2006) demonstrated that rice germplasm also contains important sources of post-attachment resistance to *S. hermonthica* and *S. asiatica*, after five years of laboratory screening of many rice cultivars. One *O. sativa* ssp. japonica cultivar

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Figure 1.5 Susceptibility of the sorghum cultivars N13 (black bars) and E36-1 (grey bars) to ecotypes of *Striga hermonthica* (from Kibos-Kenya and Côte d’Ivoire) and *S. asiatica* (from Ethiopia and USA) (Boisnard, Cissoko and Scholes, unpublished data).

Nipponbare, exhibited a higher level of post-attachment resistance to one particular ecotype of *S. hermonthica* from Kibos in Kenya when compared with an *O. sativa* ssp. indica cultivar Kasalath, but the two cultivars were both more susceptibleto *S. asiatica* ecotypes. The cultivar IAC165 (*O. sativa* ssp. japonica) was very susceptible to all *Striga* species tested. In the resistance response of Nipponbare to the *S. hermonthica* ecotype from Kibos (Kenya) parasites attached and invaded the cortex normally but were unable to cross the endodermis and therefore could not make the xylem-xylem connections (Fig 1.6). By mapping post-attachment resistance in a backcross inbred line (BILs) population, derived from a cross between Nipponbare (a resistant cultivar) and Kasalath (a susceptible cultivar), the authors identified 5 major *Striga* resistance QTL on chromosomes 4, 5, 6, 8 and 12. One QTL was conferred by a Kasalath allele (on chromosome 4) whilst the 4 others were linked to Nipponbare alleles. Interestingly, a study by Swarbrick *et al.* (2009) using a BIL population derived from a cross between Koshikahari (*O. sativa* ssp. indica) (susceptible to *S. hermonthica*) and Kasalath (which contained some resistance to *S. hermonthica* witnessed previously by a *Striga* resistance QTL on chromosome 4) identified three *Striga* resistance QTL. In this population the QTL with greatest effect explaining more than 16% of the phenotypic variation for resistance was a QTL on chromosome 4 (Kasalath allele). The location of this QTL overlapped with that of the previous study of Gurney *et al.* (2006) confirming that this major resistance QTL was not dependent on the genetic background of the mapping population used. Further studies are needed to fine map this chromosomal region to identify the gene(s) involved.

Studies so far have shown different reactions or sources of resistance to *Striga* infection depending on host varieties, *Striga* species and ecotypes. These results have opened the way for exploration of the mechanisms by which this resistance response occurs at the genetic and molecular levels as described above. However, although resistance to *Striga* clearly exists very few cereal cultivars show complete immunity to the parasite. One of the reasons for that is the large genetic diversity of the parasite seed banks. A recent study by Huang *et al.* (2012) has shown variability in loci affecting the ability of individual parasites from one ecotype of *S. hermonthica* to infect different rice cultivars. Individual *Striga* attachments were harvested from 3 different rice cultivars (with different resistance to this ecotype of *Striga*) and DNA extracted from each parasite. Using amplified fragment length polymorphism (AFLP) molecular markers, they identified a subset of AFLP loci that only occurred in those parasites that had the

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Figure 1.6 (A) The root systems of susceptible (IAC165 and Kasalath) and resistant

(Nipponbare) rice cultivars 25 days after infection. (B) Transverse sections of embedded

tissue of resistant cultivar (Nipponbare) (i) 3, (ii) 9 and (iii) 21 days after infection. The scale bar represents 0.1 mm. Hc, host root cortex; He, host endodermis; P, parasitehaustorium; En, endophyte (internal part of haustorium) (Adapted from Gurney *et al.*, 2006).

ability to attach to the most resistant cultivar. These loci represented candidate ‘virulence loci’. As explained previously, even a few parasites can cause substantial loss of yield thus more knowledge about parasite genetic diversity and the genetics of host parasite specificity is required.

Many studies have shown that some cereal cultivars are more tolerant to *Striga* infection and grow and yield better than sensitive cultivars. By definition tolerant cultivars are those that possess the ability to display minimal impairment of growth or grain yield when infected with *Striga*. A sensitive cultivar is one which is affected badly (poor growth and yield) in the presence of that same amount of infection as that on the tolerant cultivar (Rodenburg *et al.*, 2006a; Rodenburg and Bastiaans, 2011). Two sorghum lines P9405 and P9406, named Hakaki and Wahi respectively, exhibited good levels of grain yield when infected in the field by *S. hermonthica* and *S. asiatica* compared to their uninfected controls (Mbwaga *et al.*, 2001) indicating that they were quite tolerant to infection. In maize, some varieties (inbred lines and hybrids) are tolerant and show low numbers of emerged parasites when infested by *S. hermonthica* (Berner *et al.*, 1995; Kim, 1995; Gurney *et al.*, 2002). Kaewchumnong and Price (2008) conducted a study to evaluate different rice cultivars for resistance and tolerance to *S. hermonthica* (collected from a sorghum host) and they also used 115 F6 rice RILs derived from a cross between Azucena and Bala to map *Striga* tolerance QTL. In this pot experiment they found four cultivars with a high number of emerged *Striga*. Among them, Bala and IR64 suffered a lot of damage due to early emergence of *Striga* and were very susceptible. A mapping experiment using a RIL population derived from a cross between Azucena (partially tolerant) and Bala (susceptible) revealed several QTL for tolerance/resistance distributed along the genome, of which the major QTL for *Striga* tolerance was on chromosome 1. In addition, Kaewchumnong and Price (2008) also noted that two *Striga-*tolerance QTL regions coincided with the four confirmed QTL found by Gurney *et al.* (2006) for post-attachment resistance to *S. hermonthica* in Nipponbare and Kasalath RILs population suggesting that both mechanisms of resistance and tolerance may be affected by similar quantitative gene(s). Cereal cultivars that combine both tolerance and good resistance would have a major impact on controlling *Striga* by reducing the *Striga* seed bank and would also dramatically improve yield (Rodenburg and Bastiaans, 2011). Thus from a farmers point of view both resistance and tolerance are highly desirable characteristics in cultivars. A major aim of my study is to identify post-attachment resistance and tolerance to different rice cultivars and to identify a mapping population to find QTL for post-attachment resistance to different *Striga* species and ecotypes in order to select the most stable QTL to be used in MAB programmes. This work will involve detailed laboratory studies but results and promising cultivars will also be tested in the field in Tanzania and Kenya.

## Rice: A model system for molecular studies of resistance and a natural host for *Striga*

As well as being the most important staple food for more than half of the world’s population, rice is an excellent model cereal crop for molecular genetic studies as it has a small genome size (430 Mb) compared to that of the other cereal crops such as sorghum (818 Mb), maize (2500Mb) and wheat (16000 Mb) (Cohen, 1997). There is also good synteny between rice and other cereals (Gale and Devos, 1998; Devos, 2005) suggesting that results from rice could be applied to the other cereals. Since 2002, the complete genome sequence for both subspecies of *Oryza sativa* (japonica and indica) have been available (Goff *et al.*, 2002; Shimamoto and Kyozuka, 2002; Yu *et al.*, 2002; Garris *et al.*, 2005) and recently the sequence of the Africa rice species *O. glaberrima* has been released (Sakai *et al.*, 2011). Rice is a good model for comparative mapping in *Gramineae* species with the availability of high density molecular linkage maps and mapping populations (McCouch *et al.*, 1988; Yano and Sasaki, 1997) which facilitate the identification and characterisation of QTL for advanced rice breeding prospects. All the above mentioned benefits, together with microarray technology for transcript profiling (Rensink and Buell, 2005) and the existence of rice mutant germsplam such as *Tos 17* insertion mutants (Sallaud *et al.*, 2004), make rice a suitable model cereal for molecular genetic studies.

Rice is an economically important cereal crop for human food consumption. It supplies staple food in Africa and has started to replace more traditional crops like sorghum and maize; rice consumption is increasing faster than that of the other cereal crops (IRRI). The annual production of rice in SSA is close to 17 million metric tons according to FAO which represents 54% of population needs, and the remaining 46% is imported from Asia (WARDA, 2007, 2008). In sub-Saharan Africa, rice occupies 10% of total land under cereals (Ohnuki-Tierney, 1993). Eighty percent of rice in the world is cultivated by resource-limited farmers (IYR, 2004). Rice grows in diverse ecosystems depending on the availability of a water system. Five environments are used to characterize rice ecologies in Africa. These are irrigated rice with water control, deep-water rice, mangrove swamp rice and rain-fed upland rice and lowland rice, the last two being the most important cultivated areas (Windmeijer *et al.*, 1994).

Asian rice (*Oryza sativa* L.) originated in South and Southeast Asia from *O. rufipogon*,is now cultivated worldwide. African rice (*O. glaberrima* Steud.) was domesticated in West Africa from *O. barthii*. These two types of rice are the two cultivated rice species in the world and are members of a group of more than 20 grass species included in the genus *Oryza* of the family *Poaceae.* The cultivated rice species are characterized by the same AA genome shared with the following wild species distributed throughout the tropics of Asia (*O. rufipogon* and *O. nivara*), Africa *(O. longistaminata* and *O. barthii)*, South and Central America *(O. glumaepatula)*, and Australia *(O. meridionalis)* (Morishima, 1998; Doi *et al.*, 2008) (Fig 1.7).

In Africa, a lot of research has been carried out on the interaction between *Striga* and cereals including rice. As discussed previously *Striga* species attack rice making this crop a natural host of *Striga*. *S. hermonthica* and *S. asiatica* are considered the most prevalent species parasitizing rice in free-draining upland areas whilst *S. aspera* infects rice in hydromorphic unimproved lowlands (Rodenburg *et al.*, 2010). In a recent study, the latter authors expose actual and future problems of parasitic weeds in general and particularly *Striga* in rice. They provide an overview of important different aspects of the interactions between parasite and its host by browsing the parasite biology, ecology and distribution in African rice farming systems and focus on efficiency of up to date existing control options recommended against parasitic weeds. Management, research and development issues and prospects of parasitic weeds in rice in Africa are also being explored in their study.

For example, evidence of parasitic weed *Striga* problems inflicting severe yield losses in rice in SSA has been reported for *S. aspera* in Nigeria, West Africa (Dugje *et al.*, 2006), *S. asiatica* in Tanzania, East Africa (Mbwaga and Riches, 2006) and Madagascar (Elliot *et al.*, 1993). Several studies have been conducted and shown variation in resistance responses to *Striga* parasitism in the cultivars of the two cultivated rice species *O. sativa* and *O. glaberrima.* Riches *et al.* (1996) and Johnson *et al.* (2000) showed that resistance to *S. hermonthica* and *S. aspera* was observed in African rice cultivars IG10, Makassa, ACC102196 and also in Asian rice cultivars IR49255-B-B-5-2 and IR47255-B-B-5-4 (Johnson *et al.*, 2000). In addition, Nipponbare and IR64, cultivars of *O. sativa* ssp. japonica and indica, respectively and

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Figure 1.7Geographic distribution of AA genome species in the genus *Oryza* represented in Asia by *O. sativa*, *O. rufipogon* and *O. nivara*, in Africa by *O. glaberrima*, *O. barthii*  and *O. longistaminata*, in Australiaby *O. meridionali*s and in South and Central America by *O. glumaepatula*. Wild AA genome rice species share genetic information with cultivated rice species. The top and bottom red lines represent Northern and southern limits of *O. sativa* cultivation. (From Ashikari and Matsuoka, 2006).

an *glaberrima* cultivar CG14 exhibited good levels of resistance to *S. hermonthica* (Johnson *et al.*, 1997, 2000; Gurney *et al.*, 2006; Kaewchumnong and Price, 2008). In many studies, African rice species appears to offer better sources of resistance to *Striga* parasitism than Asian rice species as demonstrated by Johnson *et al.* (1997, 2000) and, Kaewchumnong and Price (2008).

Several QTL for resistance and tolerance to *Striga* species and ecotypes have been identified as discussed in section 1.4 with different rice mapping populations derived from intra-specific crosses of *O. sativa* species (Gurney *et al.*, 2006; Kaewchumnong and Price, 2008; Swarbrick *et al.*, 2009). To date, no studies have been conducted to map *Striga* resistance/tolerance QTL in interspecific crosses between *O. sativa* and *O.* *glaberrima* species.

## Development, characteristics and performance of New Rice for Africa (NERICA) cultivars

The African rice species (*O. glaberrima*) possesses several useful and important traits such as weed competiveness (Sarla and Swamy, 2005), drought tolerance (Dingkuhn *et al.*, 1998), resistance to biotic (Silue and Notteghem, 1991; Ndjiondjop *et* *al.*, 1999) and abiotic stresses and have the potential to cope with poor management conditions. All these genetic attributes have been combined with the high-yield potential of Asian rice species to generate a series of new rice cultivars called the New Rice for Africa (NERICA) cultivars.

Despite the high hybrid sterility barrier and recombination restriction between *O*. *glaberrima* (tolerant to African environments but with a low yield potential) and *O*. *sativa* (not well adapted to African environments but with a high yield potential) (Ghesquière *et al.*, 1997; Jones *et al.*, 1997a; Lorieux *et al.*, 2000), several hundred interspecific lines derived from the crosses between the cultivars WAB56-104, WAB56-50 and WAB181-18 (*O*. *sativa* ssp. japonica) as the recurrent parent and CG14 (*O*. *glaberrima*) as a donor parent, were developed at the Africa Rice Center (ex-WARDA) using backcrossing and doubled haploid breeding (Jones *et al.*, 1996; Jones *et al.*, 1997a, 1997b, 1997c). Some lines combined and demonstrated very desirable characteristics from each parent, such as early maturity (75-100 days), wide droopy leaves that confer weed competitiveness, high levels of drought tolerance, resistance to African rice gall midge, blast, and rice yellow mottle virus, adaptation to low input conditions, responsiveness to fertilizer, high yield potential associated with large panicles and a large number of grains per panicle, increased panicle branching, good protein content and grain quality (Jones *et al.*, 1997a, 1997b, 1997c; Kroschel and Muller Stover, 2004; Somado *et al.*, 2008).

Between 2000 and 2005, the best 18 lines were characterized and designated suitable for SSA upland rice ecologies and were named NERICA by WARDA’s Variety Nomination Committee followed by their pedigree number and were ranged as follows: NERICA 1 to 11 derived from WAB56-104 x CG14, NERICA 12 to 14 from WAB56-50 x CG14 and NERICA 15 to 18 from WAB181-18 x CG14 (Table 1.1). Furthermore NERICA rice cultivars from crosses between *O. glaberrima* and *O*. *sativa* ssp. indica were also produced for irrigated and rain-fed lowland environments (Sie *et al.*, 2008). Several upland NERICA cultivars have been grown and adopted by farmers in SSA and they covered estimated upland areas of 200,000 hectares in West, Central, East and Southern Africa in 2005 (Fig 1.8). For example, among the countries under upland NERICA rice cultivars production, Côte d’Ivoire, Guinea and Uganda have more than 10,000 hectares whilst Congo (Brazzaville), Congo (RDC), Kenya, Mali, Nigeria and Togo between 5,000 and 10,000 ha and less than 5,000 for the others (Somado *et al.*, 2008).

Because of their high yields even on low nutrient soils, resistance and tolerance to several abiotic stresses, the NERICA cultivars provide hope for resource-poor upland African farmers, even moreaffected by the food crisis when the price of rice increased dramatically.In addition, the introduction of the high yielding NERICA cultivars has had a good impact on rice consumption and production in SSA where both have increased by more than 6% per annum (Kormawa *et al.*, 2005). However, with the increasing take up and growth of the NERICA cultivars in rain-fed upland areas where *Striga* infestation is high (Fig 1.9) problems with *Striga* infection is beginning to emerge and information about resistance and tolerance to *Striga* in these new cultivars is lacking. Thus, many upland rice farmers, after years of growing some of these new rice cultivars, are beginning to report that the parasitic weed *Striga* appears to be one of the major constraints that they are facing in their fields. To date, very little is known about the resistance or tolerance to different species and ecotypes of *Striga* of the parental lines or of the NERICA cultivars themselves. An aim of my study was to identify sources of resistance and tolerance in the NERICA rice cultivars to different species and ecotypes of *Striga* and to understand the molecular genetic nature of their

Table 1.1 Pedigree of 18 NERICA rice cultivars derived from donor parent CG14 and recurrent parents WAB56-104, WAB56-50 and W181-18.

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| **Cultivar** | **Pedigree** | **Backcross** |
| NERICA 1  NERICA 2  NERICA 3  NERICA 4  NERICA 5  NERICA 6  NERICA 7  NERICA 8  NERICA 9  NERICA 10  NERICA 11 | WAB450-I-B-P-38-HB  WAB450-11-1-P31-1-HB  WAB450-I-B-P-28-HB  WAB450-I-B-P-91-HB  WAB450-11-1-1-P31-HB  WAB450-I-B-P-160-HB  WAB450-I-B-P-20-HB  WAB450-1-BL1-136-HB  WAB450-B-136-HB  WAB450-11-1-1-P41-HB  WAB450-16-2-BL2-DV1 | WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104  WAB56-104/CG14//WAB56-104 |
| NERICA 12  NERICA 13  NERICA 14 | WAB880-1-38-20-17-P1-HB  WAB880-1-38-20-28-P1-HB  WAB880-1-32-1-2-P1-HB | WAB56-50/CG14//WAB56-50  WAB56-50/CG14//WAB56-50  WAB56-50/CG14//WAB56-50 |
| NERICA 15  NERICA 16  NERICA 17  NERICA 18 | WAB881-10-37-18-3-P1-HB  WAB881-10-37-18-9-P1-HB WAB881-10-37-18-13-P1-HB  WAB881-10-37-18-12-P3-HB | CG14/WAB181-18//WAB181-18  CG14/WAB181-18//WAB181-18  CG14/WAB181-18//WAB181-18  CG14/WAB181-18//WAB181-18 |

NERICAs originating from different parental crosses are indicated by different colours (Adapted from Somado *et al.*, 2008).

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Figure 1.8 Area under NERICA rice cultivation in Africa in 2005 (From Somado *et al.*, 2008).

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Figure 1.9 Distribution of NERICA rice cultivars in 2006 (in green) and areas of *Striga* infestation in Africa (red symbol) (Adapted from Somado *et al.*, 2008 and Ejeta, 2007).

defense mechanisms, as their *O. glaberrima* parentsrepresent a valuable source of genetic variation that may contain novel resistance genes against *Striga*.

## Aims and objectives of thesis

In sub-Saharan Africa (SSA), *Striga* is one of the major constraints to crop production as it severely depresses yield in areas where the majority of producers are resource-poor farmers. *Striga* resistance and tolerance are complex traits controlled by the interaction of many genes probably involving several physiological and morphological mechanisms. One of the approaches to overcome this constraint is to use resistant/tolerant cultivars to better control *Striga*.

Thus the aims of my study are to (1) determine how resistant and/or tolerant the NERICA cultivars are to different species and ecotypes of *Striga* in controlled environment and field studies, to determine the phenotypes of resistance reactions and (2) to investigate the molecular genetic basis of post-attachment resistance in rice to *Striga* species using a Chromosome Segment Substitution Line (CSSL) population derived from introgression of the *O. glaberrima* genome into a tropical *O. sativa* spp. *japonica* background.

The main objectives are:

1. To determine whether different rice cultivars particularly the NERICA rice cultivars and their parents show post-attachment resistance and tolerance to different ecotypes and species of *Striga* in laboratory conditions.
2. To characterise at a microscopic level any resistant phenotypes observed.
3. To determine the impact of environment on resistance and the performance of the NERICA rice cultivars under *Striga*-infested field conditions in Africa (Tanzania and Kenya).
4. To identify QTL for post-attachment resistance to a specific ecotype of *Striga hermonthica* species using a rice CSSL population. This will allow the identification of QTL that are specific to individual host cultivar × ecotype interactions.

# Chapter 2

# New Rice for Africa (NERICA) cultivars exhibit different levels of post-attachment resistance against the parasitic weeds *Striga hermonthica* and *Striga asiatica*

Data from this chapter have been published in Cissoko *et al.* (2011), *New Phytologist* 192: 952-963.

1. **Introduction**

Rice is an economically important cereal crop in Sub-Saharan Africa (SSA) ([Balasubramanian *et al.*, 2007](#_ENREF_1)) and is mostly cultivated by resource-poor farmers ([Nwanze *et al.*, 2006](#_ENREF_18)). Both cultivated rice species, *Oryza sativa* (L.) and *Oryza glaberrima* (Steud), are grown in Africa. *Oryza glaberrima* germplasm, domesticated in West Africa, possesses useful genetic traits such as weed competitiveness and good levels of resilience against abiotic and biotic stresses ([Sarla and Swamy, 2005](#_ENREF_26)), but generally has a low yield potential and unfavourable agronomic characteristics such as grain shattering and lodging ([Koffi, 1980](#_ENREF_16)). *Oryza sativa*, originating from Asia, is most appreciated for its high yield and grain quality. To take advantage of superior traits from both species, Africa Rice Center and partners developed inter-specific rice cultivars for rain-fed upland ecosystems called NERICA (NEw RICe for Africa) using backcrossing coupled with double haploid breeding ([Jones *et al.*, 1997a](#_ENREF_11), [1997b](#_ENREF_12), [1997c](#_ENREF_13)). These cultivars are currently distributed across Africa and are popular among subsistence rice farmers ([Diagne, 2006](#_ENREF_5); [Kijima *et al.*, 2006](#_ENREF_15)). Despite the introduction of improved cultivars like the NERICAs, the average yield obtained in rain-fed upland rice in SSA is only around 1 ton per ha due to biophysical production constraints including biotic stresses and the low capacity of resource-poor rice farmers to use inputs ([Balasubramanian *et al.*, 2007](#_ENREF_1)). Weed-inflicted yield losses in rain-fed upland rice (despite control efforts) are estimated at 16%, equivalent to an estimated annual loss of US $ 418 M for African economies (Rodenburg and Johnson, 2009). An increasingly important group of weeds in rice are the parasitic weeds of which *Striga* spp. are the most prominent ([Rodenburg *et al.*, 2010](#_ENREF_25)).

*Striga* *hermonthica* (Del.) Benth. and *Striga* *asiatica* (L.) Kuntze are the most economically important root parasitic weeds in Africa (e.g. [Mohamed *et al.*, 2006](#_ENREF_17); [Parker, 2009](#_ENREF_20)). Infection with *Striga* leads to stunting and loss of grain yield in upland rice ([Johnson *et al.*, 1997](#_ENREF_10)). As obligate hemi-parasites, they only germinate in the presence of host-derived stimulants called strigolactones ([Bouwmeester *et al.*, 2003](#_ENREF_2); [Yoneyama *et al.*, 2010](#_ENREF_29)). Each germinated *Striga* seedling forms a radicle which, in response to host-derived haustorial initiation factors, forms an organ called the haustorium. Upon contact with a host root, the haustorium develops a wedge shaped group of cells that penetrates the host root cortex and endodermis to establish parasite-host xylem-xylem connections. This allows transfer of water, carbohydrates and nutrients from host to parasite ([Parker and Riches, 1993](#_ENREF_21); [Press and Graves, 1995](#_ENREF_22)). Once attached to the host root, the parasite grows towards the soil surface, emerges above ground and flowers to produce many tiny seeds which can remain viable in the soil for many years ([Parker and Riches, 1993](#_ENREF_21)). *Striga* infection distorts host plant growth and development very early after parasite attachment, although the severity of these effects depend on many factors such as nitrogen availability and host genotype (tolerance), *Striga* species and ecotype (determining virulence) and infection time and level ([Cechin and Press, 1993](#_ENREF_3)a; [Gurney *et al.*, 1999](#_ENREF_6)). Because *Striga* impacts the host so soon after attachment, effective control methods should either prevent *Striga* attachment or itsdevelopment beyond attachment (Scholes and Press, 2008). Both can be achieved through the use of improved cultivars. Host genotypes producing low amounts or less effective types of germination stimulants (Jamil *et al.*, 2011) prevent parasite attachment while genotypes with post-attachment resistance prevent parasite development ([e.g. Gurney *et al.*, 2006](#_ENREF_7)). The use of such resistant cultivars is commonly considered an effective and affordable component of an integrated *Striga* control strategy (e.g. [Haussmann *et al.*, 2000](#_ENREF_9); [Rodenburg *et al.*, 2005](#_ENREF_23); Yoder and Scholes, 2010).

Previous studies on rice showed that cultivars exhibiting resistance (i.e. with only a few emerged parasites), could be an effective *Striga* control method ([Harahap *et al.*, 1993](#_ENREF_8); [Johnson *et al.*, 1997](#_ENREF_10)). Gurney *et al.* ([2006](#_ENREF_7)) demonstrated that rice germplasm contains sources of post-attachment resistance to *S. hermonthica.* For instance, Nipponbare, an *O. sativa japonica* lowland cultivar, exhibits a high resistance response particularly to *S. hermonthica* where parasites failed to make xylem-xylem connections after attachment to the host. Apart from the cultivars and resistance mechanisms identified in these studies, few resistant rice cultivars have been found that are well adapted to upland ecosystems ([Rodenburg *et al.*,](#_ENREF_23) 2010), and a good understanding of the molecular genetic basis of host post-attachment resistance to *Striga* is still lacking (Yoder and Scholes, 2010).

Despite the wide distribution of NERICA cultivars in *Striga*-infested regions in Africa, very little is known about their susceptibility to different species and ecotypes of *Striga*, information which is critical to the selection of the most appropriate cultivar for particular regions or agro-ecological zones.

Thus, the aims of this chapter are to determine whether the upland NERICA rice cultivars possess post-attachment resistance to different species and ecotypes of *Striga* by addressing the following questions:

1. Which NERICA rice cultivars exhibit post-attachment resistance to *Striga hermonthica* and *S. asiatica*?
2. How broad spectrum or specific is the resistance to particular species or ecotypes of *Striga*?
3. What is the phenotype of post attachment resistance?
4. Does resistance to *Striga* (i.e. few attachments) substantially improve the growth of the cultivar?
5. Do the NERICA cultivars show genetic variation for tolerance to *Striga*?
6. **Material and Methods**
7. **Plant materials**

Eighteen upland NERICA cultivars were developed by AfricaRice from three series of crosses between the *O. glaberrima* cultivar CG14 and one of three *O. sativa* ssp. japonica cultivars WAB56-104 (NERICA cultivars 1 to 11), WAB56-50 (12-14) or WAB181-18 (15-18) (Jones *et al.*, 1997a). The NERICA cultivars 1-18 and their parents were all evaluated in the present study. Rice seeds were provided by the Genetic Resources Unit of AfricaRice. The rice cultivars Nipponbare, Kasalath, Koshihikari and IAC165 (Gurney *et al.*, 2006) were used as reference cultivars in this study. The origin and description of the different *S. hermonthica* and *S. asiatica* seed populations (ecotypes) used in this study are shown in Table 2.1.

1. **Growth and infection of rice plants with *Striga***

Rice seeds were germinated between blocks of moistened horticultural rockwool (growdan®Vital, UK) for 6 days after which a single rice seedling was transferred to a root observation chamber (rhizotron) as described previously by Gurney *et al.* (2006). Each rhizotron consisted of a 25 × 25 × 2 cm Perspex container packed with vermiculite (Sinclair, UK) onto which a 100 µm polyester mesh was placed (Plastok Group, Birkenhead, UK). Roots of the rice seedling grew down the mesh, and openings at the top and bottom of the rhizotron allowed for shoot growth and water drainage, respectively. Rhizotrons were covered with foil to prevent light from reaching the roots. Rhizotrons were supplied with 25 ml of 40% Long Ashton nutrient solution (Hewitt, 1966) containing 2 mM ammonium nitrate four times each day via an automatic watering system.

*Striga* seeds were surfaced sterilised in 10% bleach, thoroughly washed and then incubated (pre-conditioned) on moistened glass-fibre filter paper (Whatman®) in Petri dishes for 12-15 days at 30 °C prior to use (Gurney *et al.*, 2006). Eighteen hours before infection of the rice seedlings 1 ml of 0.1 ppm solution of GR24 (an artificial germination stimulant) was added to Petri dishes containing pre-conditioned *Striga* seeds to trigger germination. The germination percentage of each *Striga* ecotype was checked before infection. Sixteen days after sowing, rice plants were infected with 12 mg of pre-germinated *Striga* seeds by aligning them along the roots using a paint brush (Gurney *et al.,* 2006). Infection of rice roots with pre-germinated *Striga* seeds is

Table 2.1 *Striga* species and ecotypes used to infect the NERICA cultivars.

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| ***Striga* species** | **Ecotype name** | **Details** | **Latitude: Longitude** |
| *S. hermonthica* | Sh-Kibos | Collected from maize (H511) growing at the Kenyan Agricultural Research Institute (KARI), Kibos, Kisumu, Kenya in 1997. | 0°02’ 20” S : 34°47’ 57” E |
| *S. hermonthica* | Sh-Busia | Collected from maize growing in a farmer field near Busia, Kenya in 2009 | 0° 28’ N : 34° 05’ E |
| *S. hermonthica* | Sh-Medani | Collected from sorghum growing near Wad Medani, Sudan in 2006 | 14° 24′ N : 33° 31′ E |
| *S. hermonthica* | Sh-Kouto | Collected from rice (NERICA 1) growing in Kouto near Korhogo, Côte d’Ivoire in 2009 | 09° 24' 23'' N : 05° 31' 37'' W |
| *S. asiatica* | Sa-USA | Collected from maize growing in North Carolina USA in 1989. Seeds have bulked on maize (WH 505) st the University of Sheffield. | North/South Carolina |
| *S. asiatica* | Sa-Kyela | Collected from rice (cv ‘Supa India’ a.k.a. ‘Kilombero’) growing in Kyela near Mbeya, Tanzania in 2009 | 9°37′ 30″ S : 33  52′ 30″ E |

essential when quantifying post-attachment resistance, as it ensures synchronous attachment of the parasites to the roots and eliminates any differences that may occur due to variation in the production of germination stimulants by the different rice cultivars (Jamil *et al.*, 2011). Control, uninfected plants were treated in a similar manner but without the infection step. In every experiment, a minimum of 4 replicates were evaluated for each cultivar × treatment combination.

The 18 NERICA cultivars, their parents and IAC165, Nipponbare, Koshihikari and Kasalath were assessed for their resistance against *S. hermonthica* (ecotype Sh-Kibos), and *S. asiatica* (ecotype Sa-USA) (Table 2.1). Plants were grown in a temperature-controlled glasshouse environment with day/night temperatures of 28 °C and 24 °C respectively and 60% relative humidity. Plants were screened during the summer months (June-August 2009) when irradiance averages were greater than 400 µmol m-2 s-1 at plant height. If irradiance fell below 200 µmol m-2 s-1, supplementary lighting switched on automatically. In order to determine whether resistance was specific to a particular ecotype or species of *Striga*, selected cultivars (WAB56-104, CG14, NERICAs 1, 7, 9 and 10) were also infected with 4 different ecotypes of *S. hermonthica* and 2 ecotypes of *S. asiatica* (Table 2.1).

1. **Quantification of post-attachment resistance and the effect of *Striga* on host biomass**

Post-attachment resistance was quantified 21 days after infection (DAI) of the roots. Prior to harvest, the root system of each rhizotron was photographed using a Canon EOS 300D digital camera. *Striga* seedlings growing on the roots of each infected plant were then harvested, placed in Petri dishes and photographed using a Canon EOS 300D digital camera. The number and length of *Striga* seedlings from each rice plant was determined from the Petri dish photographs using Image-Pro® (Media Cybernetics). *Striga* plants were then dried at 48 oC for 2 days and dry biomass per host plant determined. In order to assess the effect of *Striga* on growth and partitioning of host biomass the number of tillers on control and infected plants was recorded 21 DAI and plants were harvested and separated into roots, stem (culm and leaf sheath) and leaves. Plant material was dried for one week at 48 °C and dry biomass was determined thereafter. The effect of *Striga* on the host was quantified by expressing the above-ground biomass of infected plants as a percentage of uninfected control biomass.

1. **The phenotype of resistance**

The phenotype of resistance was investigated by photographing parasites developing on the root systems of each cultivar at different stages after infection using a Leica MZFLIII stereo microscope and Diagnostic Instruments camera (Model 7.4), or by cutting small sections of root (with attached parasite) and mounting on a glass slide in water. The root tissue was then observed using an Olympus BX51 microscope (Olympus Optical Ltd, London, UK) using differential interference contrast (DIC) microscopy (Nomarski) and photographed using a digital camera (Olympus DP11; Olympus Optical Ltd). In order to examine the stage of parasite development on the host root, small sections of tissue were taken at intervals after infection and fixed and embedded in Technovit solution according to the manufacturer’s instructions. Small sections of root tissue plus parasite were placed into an eppendorf tube containing Carnoy’s fixative (4:1, 100% EtOH: acetic acid) and vacuum infiltrated for 20 min. Samples were incubated in Carnoy’s fixative overnight and then washed with 2 × 100% EtOH for 2 hours each. Samples were transferred to 100% Technovit solution for 15 min and then transferred into fresh solution for 3 days. Samples were transferred into moulds and Technovit solution and hardener (1:2) added. As the Technovit resin became viscous, samples were positioned in the correct orientation for sectioning. The resin blocks were covered with foil and baked in an oven at 37 °C for 30-60 min. The resin blocks were mounted onto histoblocs, trimmed and sectioned using a microtome (Leica RM 2145). Sections (3-5 µm thick) were transferred to microscope slides (polysine slides; SLS, Nottingham, UK). Sections were stained with Toluidine Blue O in 100 mM phosphate buffer, pH 7.0, dried on a hot plate at 65 oC for 30 min and mounted with Depex (BDH). Sections were observed and photographed using the Olympus BX51 microscope and camera.

1. **Statistical analyses**

The statistical package R (version 2.8.1) was used for ANOVA and Pearson’s product-moment correlation analyses. Data for *Striga* dry biomass and number of *Striga* seedlings were log-transformed to meet assumptions of ANOVA. Tukey’s honest significant difference test was then performed to calculate the corresponding critical honest significant difference (HSD) and establish the different groups.

1. **Results**
2. **How resistant are the NERICA rice cultivars to *S. hermonthica* and *S. asiatica?***

Figure 2.1 shows the mean biomass of *S. hermonthica* (Sh-Kibos) and *S. asiatica* (Sa-USA) attached to the rootsof all 18 NERICA cultivars, their parents and two susceptible (IAC165 and Koshihikari) and two resistant (Nipponbare and Kasalath) cultivars, 21 days after infection (DAI). The NERICA cultivars and their parents exhibited a range of susceptibility to the two *Striga* species and are ranked from most susceptible to most resistant to the *S. hermonthica* ecotype from Kibos, Kenya. Interestingly, the cultivar ranking of resistance against this ecotype of *S. hermonthica* was quite similar to that of resistance against the ecotype of *S. asiatica* tested in this experiment (Fig 2.1).

Some NERICA cultivars, e.g. NERICAs 7, 8, 9, 11 and 14 were very susceptible to *S. hermonthica* supporting between 100 and 150 well developed parasites per host root system (Fig 2.1 and 2.2a). NERICAs 6, 15, 16, 18 and WAB56-104 were also susceptible, supporting between 40-100 parasites, although the parasites were, on average, smaller (Fig 2.2a). The remaining NERICA cultivars and CG14, WAB56-50 and WAB181-18 exhibited good levels of post-attachment resistance to the ecotypes of the two *Striga* species (Fig 2.1 and 2.2). NERICAs 1 and 10 exhibited the greatest resistance to both *S. hermonthica* and *S. asiatica* and were more resistant than their *O.* *glaberrima* parent (CG14) and Nipponbare (Fig 2.1, 2.2 and 2.3 d - f). These cultivars had very few successful attachments at 21 DAI and the parasites were very small; less than 3 mm (Sh-Kibos) and 1 mm (Sa-USA) in size (Fig 2.2a and 2.2b). In contrast, NERICAs 9 and 7 (Fig 2.3a and 2.3b) were more susceptible than their *O. sativa* parent WAB56-104, (Fig 2.3c) and were as susceptible as IAC165 (Fig 2.1). *Striga hermonthica* parasites on the most susceptible cultivars grew rapidly and were relatively large. The relationship between the increase in size of *S. asiatica* attachments and increasing susceptibility was not as clear (Fig 2.2.). This may have been due to the fact that there were more *S. asiatica* attachments on susceptible cultivars (than *S. hermonthica* attachments) and that the available carbon supply from the host may have limited their size.

Although the number of parasites undergoing a resistance response varied on the different NERICA cultivars, the visible phenotype of the resistance reaction to

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Figure 2.1 Evaluation of post-attachment resistance of New Rice for Africa (NERICA) cultivars to *Striga hermonthica* (Sh-kibos, grey bars) and *S. asiatica* (Sa-USA, black bars) ecotypes. One-way ANOVA showed that the genotype effect is highly significant (*P* < 0.0001) for both *Striga* ecotypes. Tukey’s honestly significant differences (HSDs, *P* < 0.05) are represented for each *Striga* ecotype. Data are presented as means ± SE.

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Figure 2.2 The relationship between the number and length of *Striga hermonthica* (Sh-Kibos) (a) and *S. asiatica* (Sa-USA) (b) seedlings attached to the roots of the New Rice for Africa (NERICA) cultivars. Data are presented as means ± SE. The genotypes used for further analyses of *Striga* resistance are indicated as white symbols and are named. Pearson’s product-moment correlation probability and coefficient are indicated for each *Striga* ecotype.

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Figure 2.3 *Striga hermonthica* (Sh-Kibos) growing on the roots of selected New Rice for Africa (NERICA) cultivars (in rhizotrons) 21 days after infection. NERICAs 7 and 9 are very susceptible, showing many *S. hermonthica* attachments (a, b). Fewer parasites attached to WAB56-104 (c), and CG14, NERICAs 1 and 10 exhibited good levels of resistance to the parasite with only few viable attachments (d-f).

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Figure 2.4 The phenotype of resistance in the New Rice for Africa (NERICA) cultivars to *Striga hermonthica* (Sh-Kibos). (a, b) The phenotype of resistance against both *S. hermonthica* (Sh-Kibos) and *S.asiatica* (Sa-USA) is associated with intense necrosis at the site of attachment. (c-f) Transverse sections through the root and parasite attachment in compatible and incompatible interactions 6 days after infection with pre-germinated *S. hermonthica* (Sh-Kibos) seeds. (c) In the compatible interaction (NERICA 7), the parasite has penetrated the cortex and endodermis, and is beginning to form parasite-host xylem connections. (d, e) In many incompatible interactions (e.g. CG14, NERICAs 1 and 10), the parasite penetrates the cortex, but is unable to traverse the endodermis and form a connection with the xylem of vessels of the host. (f) In some interactions, the parasite is able to penetrate the endodermis and establish a few connections to the vascular system. However, this is associated with the deposition of dense staining material and the parasites remain small (e.g. NERICA 10).

*S. hermonthica* and *S. asiatica* was similar (Fig 2.4a and 2.4b). *Striga hermonthica* and *S. asiatica* attached to the root systems of the NERICA cultivars within 2-3 days of inoculation in both susceptible and resistant interactions. However by day 7 parasites that had elicited a host resistance response were clearly dying as haustoria failed to increase in size and the host root exhibited an intense necrosis at the site of attachment (Fig 2.4a and 2.4b). In susceptible interactions *S. hermonthica* (e.g. NERICAs 7 and 9) the parasites penetrated through the cortex and endodermis, and by day 6-7, had begun to form connections with the host xylem resulting in differentiation of the haustorium (Fig 2.4c) and emergence of the shoot. In resistant interactions (e.g. CG14, NERICAs 1 and 10), transverse sections through the rice root at the site of attachment revealed two phenotypes. In the most frequently observed phenotype, the parasite penetrated the cortex, but was unable to traverse the endodermis to form host-parasite xylem continuity. The parasites grew around the vascular cylinder and sometimes exited the root again (Fig 2.4d and 2.4e). In some cases, the parasite was able to penetrate the endodermis, although this took longer than in the more susceptible interactions. A few connections to the vascular system were visible but they were often associated with the deposition of dense staining material and resulting parasites remained small and grew slowly (Fig 2.4e).

1. **How broad-spectrum is the resistance found in the NERICA cultivars?**

The NERICA cultivars showed a wide range of resistance levels to an ecotype of *S. hermonthica* (Sh-Kibos) and an ecotype of *S. asiatica* (Sa-USA) (Fig 2.1). In order to determine whether the resistance exhibited by some of the NERICA cultivars was broad-spectrum or specific to the ecotype of *Striga* used, the two most resistant (NERICAs 1 and 10) and two most susceptible (NERICAs 7 and 9) cultivars (together with their parental cultivars CG14 and WAB56-104) were infected with four different ecotypes of *S. hermonthica* and two ecotypes of *S. asiatica* (Table 2.1).

Figure 2.5 shows the amount of *Striga* biomass, number of *Striga* seedlings and the average length of *Striga* seedlings of each *Striga* ecotype on the different rice cultivars. The rice cultivars showed the same pattern of resistance to three of the four *S. hermonthica* ecotypes (Sh-Kibos, Sh-Busia and Sh-Medani) and to the two *S. asiatica* ecotypes (Sa-USA and Sa-Kyela) despite the fact that they were collected from different

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Figure 2.5 Evaluation of post-attachment resistance of New Rice for Africa (NERICA) 1, 7, 9 and 10 and their parents to a range of *Striga hermonthica* and *S. asiatica* ecotypes. *Striga* dry biomass (a), number of *Striga* seedlings (b) and average length of *Striga* seedlings (c) were measured after harvesting at 21 days after infection. Data shown for the Sh-Kibos and Sa-USA ecotypes are also shown in Figures 1 and 2. Data presented are means ± SE. For the three traits, genotype, ecotype and genotype x ecotype effects were highly significant (two-way ANOVA, *P* < 0.001). The significance of a genotype effect calculated by one-way ANOVA for each *Striga* ecotype and trait combination is shown as: \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001. The letters above each bar indicate the different significance groups after Tukey’s pairwise comparison.

host species and from different regions of Africa (Table 2.1). Essentially, NERICAs 7 and 9 were very susceptible with in excess of 100 well developed *Striga* seedlings per root system in the case of the *S. hermonthica* ecotypes (Fig 2.5b and 2.5c). WAB56-104 was also susceptible and supported between 50-70 well developed parasites. CG14, NERICAs 1 and 10 exhibited high levels of resistance to both *S. hermonthica* and *S. asiatica* ecotypes (Fig 2.5 a-c). As observed in the previous screen of all 18 NERICA cultivars, the parasites of the *S. asiatica* ecotypes were smaller than those of *S. hermonthica*, both in weight and length. However, although the pattern of resistance of the rice cultivars to the *S. asiatica* ecotype from Kyela was similar to that observed for all other *Striga* ecotypes, the total number of attachments was significantly lower when compared to the ecotype from the USA (Fig 2.5b), suggesting that the Kyela population was less virulent on the cultivars used in this study.

The interaction between the rice cultivars and *S. hermonthica* collected from NERICA 1 growing in Kouto, Côte d’Ivoire (Sh-Kouto) differed in several respects from the interaction observed with the other *S. hermonthica* ecotypes. NERICAs 7 and 9 and WAB56-104 were susceptible to Sh-Kouto. The total number of attachments on the root systems was similar to that observed for the other *S. hermonthica* ecotypes (Fig 2.5b) but the average size of the parasites was smaller (Fig 2.5c). NERICA 10 was slightly more susceptible to this ecotype than to the other *S. hermonthica* ecotypes, but CG14 remained very resistant to this ecotype. NERICA 1, which exhibited high levels of resistance to all other *Striga* ecotypes was as susceptible to the Sh-Kouto ecotype as NERICAs 7 and 9 and WAB56-104.

1. **The impact of *Striga* on the biomass of susceptible and resistant NERICA cultivars**

The tiller number and dry biomass of the infected NERICA cultivars was compared to that of the uninfected control plants to evaluate the impact of *Striga* infection on the growth of the host. A large variation in the biomass dry weights of the uninfected control plants was observed among the cultivars (Table 2.2). The dry biomass of the control plants ranged from 1.57g for NERICA 4 to 3.44g for NERICA 9. Infection of the NERICA cultivars with either *S. hermonthica* (Sh-Kibos) or *S. asiatica* (Sa-USA) altered the partitioning of biomass to the roots, stems and leaves in comparison to their uninfected controls (Table 2.2). The most noticeable difference was in the above-ground

Table 2.2 The effect of *Striga* on biomass and tiller production of *Striga*-infected

NERICA cultivars compared to uninfected controls.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cultivar** | **Ecotype** | **Total Dry**  **Biomass (g)** | **Biomass of *Striga*-infected**  **plants as a % of the control** | | | | | | **Number**  **of Tillers** | | |
| **Root** | | **Stem** | | **Leaf** | |
| NERICA 1 | Control | 1.77 +/- 0.21 a |  | - | | - | | - | | 4.0 +/- 0.00 a |  | |
|  | Sh-Kibos | 1.30 +/- 0.07 ab |  | -3 | | -36 | | -26 | | 3.0 +/- 0.45 a |  | |
|  | Sa-USA | 0.94 +/- 0.08 b |  | -43 | | -50 | | -45 | | 3.2 +/- 0.37 a |  | |
| NERICA 2 | Control | 1.67 +/- 0.10 a |  | - | | - | | - | | 4.0 +/- 0.00 a |  | |
|  | Sh-Kibos | 1.12 +/- 0.20 ab |  | 2 | | -43 | | -37 | | 3.0 +/- 0.32 b |  | |
|  | Sa-USA | 0.71 +/- 0.14 b |  | -55 | | -61 | | -56 | | 2.5 +/- 0.29 b |  | |
| NERICA 3 | Control | 1.88 +/- 0.26 a |  | - | | - | | - | | 4.3 +/- 0.33 a |  | |
|  | Sh-Kibos | 1.11 +/- 0.15 b |  | -20 | | -46 | | -45 | | 2.8 +/- 0.37 b |  | |
|  | Sa-USA | 0.97 +/- 0.06 b |  | -41 | | -50 | | -50 | | 3.2 +/- 0.20 ab |  | |
| NERICA 4 | Control | 1.57 +/- 0.27 a |  | - | | - | | - | | 4.3 +/- 0.25 a |  | |
|  | Sh-Kibos | 1.09 +/- 0.08 ab |  | 10 | | -42 | | -34 | | 2.5 +/- 0.29 b |  | |
|  | Sa-USA | 0.84 +/- 0.11 b |  | -37 | | -52 | | -45 | | 3.0 +/- 0.45 ab |  | |
| NERICA 5 | Control | 1.95 +/- 0.06a |  | - | | - | | - | | 4.8 +/- 0.25 a |  | |
|  | Sh-Kibos | 1.02 +/- 0.20b |  | -29 | | -53 | | -49 | | 2.8 +/- 0.25 b |  | |
|  | Sa-USA | 1.00 +/- 0.07b |  | -41 | | -52 | | -49 | | 3.6 +/- 0.24 b |  | |
| NERICA 6 | Control | 2.16 +/- 0.11a |  | - | | - | | - | | 3.8 +/- 0.25 a |  | |
|  | Sh-Kibos | 0.72 +/- 0.10b |  | -34 | | -74 | | -72 | | 2.4 +/- 0.40 b |  | |
|  | Sa-USA | 0.70 +/- 0.09b |  | -53 | | -70 | | -70 | | 1.3 +/- 0.25 b |  | |
| NERICA 7 | Control | 2.41 +/- 0.45a |  | - | | - | | - | | 3.3 +/- 0.33 a |  | |
|  | Sh-Kibos | 0.91 +/- 0.10b |  | -38 | | -74 | | -60 | | 2.3 +/- 0.25 a |  | |
|  | Sa-USA | 0.81 +/- 0.26b |  | -52 | | -73 | | -66 | | 2.0 +/- 0.00 a |  | |
| NERICA 8 | Control | 3.20 +/- 0.16a |  | - | | - | | - | | 6.5 +/- 0.50 a |  | |
|  | Sh-Kibos | 1.03 +/- 0.11b |  | -36 | | -79 | | -68 | | 3.4 +/- 0.24 b |  | |
|  | Sa-USA | 1.15 +/- 0.21b |  | -45 | | -70 | | -65 | | 3.3 +/- 0.33 b |  | |
| NERICA 9 | Control | 3.44 +/- 0.27a |  | - | | - | | - | | 7.0 +/- 0.41 a |  | |
|  | Sh-Kibos | 1.12 +/- 0.06b |  | -37 | | -81 | | -65 | | 4.0 +/- 0.00 b |  | |
|  | Sa-USA | 0.82 +/- 0.08b |  | -66 | | -81 | | -75 | | 2.6 +/- 0.24 c |  | |
| NERICA 10 | Control | 1.76 +/- 0.17a |  | - | | - | | - | | 4.0 +/- 0.00 a |  | |
|  | Sh-Kibos | 1.26 +/- 0.12ab |  | -10 | | -36 | | -28 | | 3.0 +/- 0.00 b |  | |
|  | Sa-USA | 1.08 +/- 0.11b |  | -30 | | -42 | | -39 | | 3.2 +/- 0.37 ab |  | |
| NERICA 11 | Control | 3.43 +/- 0.31 a |  | - | | - | | - | | 7.0 +/- 0.32 a |  | |
|  | Sh-Kibos | 1.25 +/- 0.18 b |  | -38 | | -75 | | -63 | | 3.6 +/- 0.24 b |  | |
|  | Sa-USA | 1.06 +/- 0.08 b |  | -62 | | -74 | | -68 | | 3.2 +/- 0.20 b |  | |
| NERICA 12 | Control | 2.45 +/- 0.12 a |  | - | | - | | - | | 5.6 +/- 0.40 a |  | |
|  | Sh-Kibos | 1.43 +/- 0.14 b |  | -25 | | -55 | | -35 | | 3.2 +/- 0.20 b |  | |
|  | Sa-USA | 0.78 +/- 0.12 c |  | -60 | | -73 | | -66 | | 3.2 +/- 0.37 b |  | |
| NERICA 13 | Control | 2.66 +/- 0.15 a |  | - | | - | | - | | 5.5 +/- 0.29 a |  | |
|  | Sh-Kibos | 1.12 +/- 0.04 b |  | -35 | | -71 | | -53 | | 2.6 +/- 0.24 c |  | |
|  | Sa-USA | 1.12 +/- 0.09 b |  | -46 | | -63 | | -57 | | 4.2 +/- 0.20 b |  | |
| NERICA 14 | Control | 2.63 +/- 0.14 a |  | - | | - | | - | | 4.6 +/- 0.24 a |  | |
|  | Sh-Kibos | 1.08 +/- 0.13 b |  | -34 | | -71 | | -59 | | 3.0 +/- 0.00 b |  | |
|  | Sa-USA | 1.04 +/- 0.22 b |  | -46 | | -68 | | -60 | | 2.6 +/- 0.24 b |  | |
| NERICA 15 | Control | 1.68 +/- 0.16 a |  | - | | - | | - | | 3.3 +/- 0.25 a |  | |
|  | Sh-Kibos | 0.48 +/- 0.06 b |  | -37 | | -85 | | -71 | | 1.6 +/- 0.24 b |  | |
|  | Sa-USA | 0.59 +/- 0.01 b |  | -44 | | -72 | | -65 | | 1.0 +/- 0.00 b |  | |
| NERICA 16 | Control | 1.89 +/- 0.21 a |  | - | | - | | - | | 3.5 +/- 0.29 a |  | |
|  | Sh-Kibos | 0.54 +/- 0.09 b |  | -50 | | -82 | | -70 | | 1.5 +/- 0.29 b |  | |
|  | Sa-USA | 0.58 +/- 0.03 b |  | -60 | | -75 | | -68 | | 1.0 +/- 0.00 b |  | |
| NERICA 17 | Control | 2.93 +/- 0.29 a |  | - | | - | | - | | 6.4 +/- 0.60 a |  | |
|  | Sh-Kibos | 1.66 +/- 0.18 b |  | -20 | | -56 | | -41 | | 3.2 +/- 0.20 b |  | |
|  | Sa-USA | 1.62 +/- 0.28 b |  | -30 | | -53 | | -44 | | 3.6 +/- 0.24 b |  | |
| NERICA 18 | Control | 1.78 +/- 0.20 a |  | - | | - | | - | | 3.5 +/- 0.29 a |  | |
|  | Sh-Kibos | 0.52 +/- 0.05 b |  | -47 | | -86 | | -67 | | 1.6 +/- 0.24 b |  | |
|  | Sa-USA | 0.62 +/- 0.04 b |  | -48 | | -72 | | -65 | | 1.0 +/- 0.00 b |  | |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cultivar** | **Ecotype** | **Total Dry**  **Biomass (g)** | **Biomass of *Striga*-infected**  **plants as a % of the control** | | | | | | **Number**  **of Tillers** | |
| **Root** | | **Stem** | | **Leaf** | |
| CG14 | Control | 2.69 +/- 0.20 a |  | - | | - | | - | | 8.0 +/- 0.77 a | |  |
|  | Sh-Kibos | 1.83 +/- 0.18 b |  | -6 | | -46 | | -31 | | 5.4 +/- 0.75 a | |  |
|  | Sa-USA | 2.37 +/- 0.22 ab |  | 17 | | -24 | | -14 | | 5.6 +/- 0.75 a | |  |
| WAB181-18 | Control | 1.96 +/- 0.10 a |  | - | | - | | - | | 5.8 +/- 0.37 a | |  |
|  | Sh-Kibos | 1.17 +/- 0.05 b |  | 4 | | -58 | | -41 | | 3.8 +/- 0.20 b | |  |
|  | Sa-USA | 1.58 +/- 0.17 ab |  | 27 | | -32 | | -23 | | 4.2 +/- 0.20 b | |  |
| WAB56-104 | Control | 1.98 +/- 0.17 a |  | - | | - | | - | | 4.0 +/- 0.00 a | |  |
|  | Sh-Kibos | 0.91 +/- 0.13 b |  | -33 | | -64 | | -53 | | 2.8 +/- 0.20 b | |  |
|  | Sa-USA | 1.00 +/- 0.21 b |  | -43 | | -53 | | -49 | | 2.5 +/- 0.50 b | |  |
| WAB56-50 | Control | 2.37 +/- 0.15 a |  | - | | - | | - | | 4.5 +/- 0.29 a | |  |
|  | Sh-Kibos | 1.31 +/- 0.10 b |  | -12 | | -62 | | -39 | | 3.0 +/- 0.00 b | |  |
|  | Sa-USA | 1.11 +/- 0.14 b |  | -40 | | -62 | | -49 | | 2.4 +/- 0.40 b | |  |

Data presented for total dry biomass and numbers of tillers are means ± S.E. For the two traits genotype, ecotype and genotype × ecotype effects were highly significant (two-way ANOVA, *P* < 0.001). The significance of a treatment effect was calculated by a one-way ANOVA for each genotype and the letters next to each mean value indicate the different significance groups after Tukey’s pairwise comparisons (*P* < 0.05).

biomass, where partitioning of dry matter to the stems and leaves was severely reduced by *Striga* infection (Table 2.2). In the two most susceptible cultivars, NERICAs 7 and 9, the stem biomass of infected plants was 74% and 81% less than the control plants, respectively (when infected with either *S. hermonthica* or *S. asiatica*) and leaf biomass was between 60 - 66% (NERICA 7) and 65 - 75% (NERICA 9) less than control plants (Table 2.2). In the two most resistant cultivars, NERICAs 1 and 10, the effect of the parasite on stem and leaf biomass was less severe but still significant (Table 2.2).

Figure 2.6 shows the relationship between the percentage reduction in total host biomass of infected plants (compared to control plants) and the amount of parasite biomass on the roots. There was a significant, linear relationship between the effect of *S. hermonthica* (Sh-Kibos) on host biomass and the amount of parasite biomass on the roots i.e. the most susceptible NERICA cultivars were generally the most badly affected whilst the most resistant NERICA cultivars were the least affected (Fig 2.6, Table 2.2). This negative relationship between host and parasite biomass was less obvious when plants were infected with *S. asiatica* (Sa-USA), as different cultivars were affected to different extents for a similar amount of parasite biomass on the roots. For example, CG14 and NERICA 12 had similar levels of infection with 1.52 mg and 1.34 mg of *Striga* biomass, respectively, yet the percentage reduction in host biomass (relative to their controls) was 31% and 82% respectively, suggesting a cultivar difference in tolerance to the deleterious effects of infection by *S. asiatica*. Infection of the NERICA cultivars with either *S. hermonthica* or *S. asiatica* also significantly suppressed tillering when compared to their uninfected controls (Table 2.2).

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|  |

Figure 2.6 Relationship between the percentage loss in total host biomass of infected plants compared with control plants and the amount of parasite biomass on the roots of *Striga hermonthica* (Sh-Kibos) (a) and *S. asiatica* (Sa-USA) (b). Data are presented as means ± SE.

1. **Discussion**

This study has shown, for the first time, that the 18 upland NERICA cultivars exhibit different levels of post-attachment resistance against different *Striga* species and ecotypes, ranging from extremely susceptible (e.g. NERICAs 7, 8, 9, 11 and 14) to highly resistant (e.g. CG14, NERICAs 1, 2, 3, 5 and 10). Interestingly, the resistance levels of the different NERICA cultivars against the ecotype of *S. hermonthica* (Sh-Kibos) was similar to the resistance observed against the ecotype of *S. asiatica* (Sa-USA), although the biomass and size of *S. asiatica* seedlings attached to the NERICA cultivars was lower than that observed for *S. hermonthica*. This likely reflects the different morphology, structure and rate of development of the two *Striga* species rather than an inherent difference in virulence. In addition, there were more *S. asiatica* parasites on the susceptible NERICA cultivars when compared to *S. hermonthica*, which may have led to a greater level of intra-specific competition for host nutrients, contributing to the smaller size of *S. asiatica* parasites. Although none of the NERICA cultivars (or their parents) exhibited complete resistance to the ecotypes of *Striga* used in this study, the strongest resistance was associated with very few, small *Striga* seedlings. The most resistant cultivars e.g. CG14 and NERICAs 1 and 10, were more resistant than Nipponbare, which is known to exhibit very strong post-attachment resistance to *S. hermonthica* (Gurney *et al.*, 2006; Kaewchumnong and Price, 2008).

The different groups of NERICA cultivars share a large part of their genomes between themselves and their respective *O. sativa* recurrent parent due to their breeding history (Jones *et al.*, 1997a). For example, the genetic background of NERICAs 1 to 7 is derived, predominately, from WAB56-104 with between 3.4 and 12.1% of loci coming from their donor parent CG14 (Semagn *et al.*, 2007). Thus, it might be expected that only a few genetic regions would explain the difference in susceptibility to *Striga* observed in our screen. However, we observed a wide range in susceptibility of the NERICA cultivars to *Striga* infection from those that were much more susceptible than the *O. sativa* parent, to those that exhibited greater resistance than observed in CG14. The strong resistance in some of the NERICA cultivars is probably controlled by a few loci, with the resistant allele coming from CG14. However, the larger than expected transgressive segregation toward susceptibility may be due to the introduction of susceptibility alleles from CG14 at different loci to those present in the *O. sativa* parent or, to genetic introgressions (non-parental alleles) from unknown rice parent(s) during the breeding process. The presence of non-parental alleles has been detected for about 3% of loci analysed in NERICA cultivars 1 to 7 (Semagn *et al.*, 2007).

1. **Some of the upland NERICA cultivars exhibit strong, broad spectrum post- attachment resistance against *Striga* ecotypes**

Our study indicates that the resistance in some of the NERICA cultivars is relatively broad spectrum and therefore potentially of great interest to farmers in the short term as well as for longer term studies of the genetic basis of resistance for breeding programmes. The resistance ranking of the 18 upland NERICA cultivars was similar when challenged with *S. hermonthica* (Sh-Kibos) and *S.asiatica* (Sa-USA). In addition, the pattern of susceptibility/resistance of NERICAs 7 and 9 (susceptible), 1 and 10 (resistant), CG14 (resistant) and WAB56-104 (intermediate) was largely maintained when challenged with another 3 ecotypes of *S. hermonthica* and another ecotype of *S. asiatica* collected from different host species and from different regions of Africa (Table 2.1).

However, there was one exception to this general pattern of resistance and susceptibility. The *S. hermonthica* ecotype Sh-Kouto was very virulent on NERICA 1, despite the fact that this cultivar exhibited strong post-attachment resistance to all other ecotypes tested and also produced low amounts of germination stimulants (Jamil *et al.,* 2011). This *S. hermonthica* ecotype was collected in 2008 from NERICA 1 growing in fields in the Kouto area of northern Côte d’Ivoire. NERICA 1 had been cultivated in this area for several years in succession and was highly infested with *S. hermonthica* at the time seeds were collected (Rodenburg, pers. com.). This suggests that either the *S. hermonthica* population in this area was already highly virulent against NERICA 1 before its cultivation or, more probably, that the high genetic diversity of the *Striga* seedbank had led to rapid evolution/build up of virulent genotypes from a subset of the natural seed bank population over a period of time (Huang *et al.,* 2012).

The deployment of resistant cultivars is considered to be an important and cost effective component of integrated *Striga* management programmes, and the fact that NERICA 10 and, particularly CG14 still showed good resistance to the Sh*-*Kouto ecotype suggests that the genetic basis of resistance in the NERICA cultivars is multi-layered and controlled by different loci. However, the results of this study clearly demonstrate the need to understand the genetic basis of both host resistance and the adaptation of *Striga* populations (parasite virulence) to new host resistance phenotypes. Such insights would facilitate the stacking of appropriate resistance loci in farmer preferred and *Striga* tolerant cultivars to enhance the durability and stability of defence in the long term (Scholes and Press, 2008; Rodenburg and Bastiaans, 2011).

1. **The phenotype of *Striga* resistance in the NERICA cultivars**

Different numbers of attached parasites developed successfully (compatible) or died (incompatible) on the different NERICA cultivars and their parents. Failure of parasites to develop after attachment was often associated with the appearance of host necrosis at the site of attachment within 4-6 days of placing pre-germinated seeds on the roots (Fig 2.4a). A similar resistance phenotype was observed in the *O. sativa* cultivar Nipponbare infected by *S. hermonthica* (Gurney *et al.* 2006), in some sorghum cultivars following infection by *S. asiatica* (Mohamed *et al.,* 2003) and in resistant cowpea cultivars infected by *S. gesnerioides* (Li and Timko, 2009). In the case of cowpea resistance the necrosis reaction was associated with a gene-for-gene resistance mechanism (Li and Timko, 2009). In the NERICA cultivars, their parents and Nipponbare infected with *S. hermonthica*, it is not clear whether the underlying resistance mechanisms involve gene-for-gene interactions as resistance is considered to be controlled by several QTL, although there are indications that a proportion of this quantitative resistance is controlled by a few genes of major effect (Gurney *et al.,* 2006). Analysis of changes in gene expression in the roots of Nipponbare undergoing a resistance reaction to *S. hermonthica* revealed that genes encoding resistance and hypersensitive response (HR) protein homologs, pathogenesis-related (PR) proteins and WRKY transcription factors were all up-regulated (Swarbrick *et al.,* 2008). How the alterations in the expression of defence genes and pathways relates to the genetic basis of resistance, awaits fine mapping of genes underlying *Striga* resistance QTL.

In this study transverse sections through incompatible parasite attachments, whether on very resistant or on the more susceptible cultivars, often revealed that the parasite endophyte had penetrated the host root cortex but not the endodermis. The parasite was therefore unable to form the parasite-host xylem connections that are necessary to access host nutrients and or developmental signals within the host vascular system. This phenotype was associated with failure of the parasite haustorium to differentiate fully and initiate shoot growth and it appears to be quite common in many rice cultivars including Nipponbare (Gurney *et al.,* 2006; Yoshida and Shirasu, 2009), Koshihikari (Yoshida and Shirasu 2009) and Kasalath (Scholes, unpublished data). On the most resistant NERICA cultivars (e.g. NERICAs 1 and 10), some parasites also took longer to penetrate the endodermis than in very susceptible interactions. This phenotype was characterised by fewer successful connections to the parasite xylem and the cells and xylem vessels associated with parasite ingress into the vascular cylinder had become occluded by dense staining material (Fig 2.4f). It is not clear whether this represents lignifications of the cell walls or the deposition of materials such as callose, as observed in some resistance responses to *Orobanche* species (e.g. Pérez de Luque *et al.,* 2008; Fernández-Aparicio *et al.,* 2008).

1. **Does resistance and/or tolerance to *Striga* reduce the damaging effects of the parasite on the host plant?**

Resistance lowers the number of successful attachments and consequently the reproductive output of the parasite (Rodenburg *et al.,* 2006b). However, even highly resistant cultivars usually have some attachments which will grow successfully and many studies have shown that the deleterious effects of *Striga* on plant growth, morphology and yield are complex and are not always related, in a linear manner, to the number of infections or to the amount of parasite biomass on the host roots (Graves *et al.,* 1989; Frost *et al.,* 1997; Gurney *et al.,* 1999; Rodenburg *et al.,* 2006a). Some cultivars appear to perform better than others under similar parasite loads, a phenomenon known as ‘tolerance’. Recent findings regarding the physiological expression of tolerance (Gurney *et al.*, 2002; Rodenburg *et al.,* 2008), coupled to increased understanding of the genetic mechanisms underlying this trait, could enable plant breeders to incorporate tolerance into largely resistant cultivars to improve yields further (Haussmann *et al.,* 2001a; Rodenburg *et al.,* 2005; Rodenburg and Bastiaans, 2011). In this study, partitioning of dry matter to stems and leaves (above-ground biomass) was reduced in the NERICA cultivars infected with *S. hermonthica* or *S. asiatica* compared to their uninfected controls, but the most resistant cultivars were also the least affected (Fig 2.6). The total biomass of the most resistant cultivars NERICAs 1 and 10, when infected by *Striga*, was approximately 30% (Sh-Kibos) or 40% (Sa-USA) lower than the uninfected controls compared with a 70% (Sh-Kibos) or 80% (Sa-USA) reduction in NERICAs 7 and 9. The fact that the relationship between the % reduction in host biomass of infected plants (compared to controls) was linearly related to the amount of parasite biomass on the host roots (particularly for *S. hermonthica*) suggests that the most resistant NERICA cultivars may also produce the highest grain yields in *Striga*-infested fields. Our data also indicate that there is some genetic variation for tolerance to *Striga* amongst the cultivars. For example, CG14 lost approximately 18% of above ground biomass, Nipponbare, 28%, NERICA 4, 48% and NERICA 12, 70%, (compared to uninfected plants) when parasitized by 1.2 - 1.5 mg of *S. asiatica* (Fig 6b), highlighting the need to further elucidate the physiological/molecular mechanisms underlying these differences in tolerance to similar amounts of *Striga*. At present, it is not clear whether the correlation between resistance/tolerance and % loss in above ground biomass, revealed in this study, is a good predictor of grain yield in the field. In order to test this relationship (and the expression of resistance and tolerance), the resistance performance and yield of the NERICA rice cultivars will be evaluated in field trials under *S. hermonthica* and *S. asiatica* infestation in Kenya and Tanzania, respectively (Chapter 3).

**Conclusions**

This study has shown that some of the NERICA cultivars exhibit very strong post-attachment resistance and show reduced impact on their growth to a range of *S. hermonthica* and *S. asiatica* ecotypes and that the resistance in some of the cultivars is relatively broad spectrum. Jamil *et al.* (2011) have also shown that the NERICA cultivars exhibit differences in the amounts and types of germination stimulants that they produce resulting in differences in pre-attachment resistance to *Striga*. We suggest that rice cultivars which combine low germination stimulant production and strong post-attachment resistance will perform and yield well in areas where *Striga* infestation is prevalent. However, in order to increase the durability of host defence, when faced with a genetically diverse *Striga* seed bank, it is essential to use resistant cultivars as part of an integrated control programme and further our understanding of the molecular nature of host-parasite specificity.

# Chapter 3

# Field expression of resistance against the parasitic weeds *Striga asiatica* (L.) Kuntze and *Striga hermonthica* (Del.) Benth. in NERICA rice cultivars and their *O. glaberrima* (Steud.) and *O. sativa* (L.) parents

2. **Introduction**

In sub-Saharan Africa (SSA), rice is an increasingly important cereal crop (FAO, 2008). The crop is primarily grown in rain-fed agro-ecosystems. Of the total area under rice in SSA, 33% can be characterized as rain-fed lowland and 39% as rain-fed upland, with average estimated yields of around 1 ton ha-1 (Rodenburg and Demont, 2009). The extreme low productivity in these rain-fed environments is caused by a myriad of bio-physical and socio-economic factors (Balasubramanian *et al.*, 2007). According to Waddington *et al.* (2010), major production constraints for smallholder farms in rain-fed agro-ecosystems in Africa are drought, poor soil fertility and weed competition. The weeds *Striga*, of which *Striga hermonthica* and *S. asiatica* are the most wide-spread and economically important species, are frequently observed in poorly fertile and drought-prone soils, and are among the most problematic weeds in upland rice production systems (Mohamed *et al.*, 2001; Parker, 2009; Rodenburg *et al.*, 2010).

*Striga* species negatively affect the growth and yield of the crops they infect (for a review see Parker, 2009). The extent of these negative effects is a function of the environment and the genetic make-up of the host and parasite. A number of studies have shown that genetic variation in either host resistance or parasite virulence can determine the outcome of the interaction. For example, several studies have shown that some Asian rice cultivars (e.g. IR47255-B-B-5-4, IR49255-B-B-5-2, Nipponbare and IR64) and some African rice cultivars (e.g. ACC102196, Makassa, CG14 and IG10) exhibit good resistance against some ecotypes of *S. hermonthica* and/or *S. asiatica* whilst other rice cultivars (e.g. IAC165) were susceptible to the same ecotypes (Harahap *et al.*, 1993; Johnson *et al.*, 1997; Gurney *et al.*, 2006; Kaewchumnong and Price, 2008; Swarbrick *et al.*, 2009). A number of studies have also shown that ecotypes of *S. hermonthica* or *S. asiatica* are genetically very variable (Botanga *et al.*, 2002; Huang *et al.*, 2012). This is particularly true for *S. hermonthica* which, because it is an out-breeding parasite, generates variation from season to season (Safa *et al.*, 1984). Botanga *et al.* (2002) and Huang *et al.* (2012) used amplified fragment length polymorphism (AFLP) analysis to estimate genetic variability within and among different populations of *S. hermonthica* collected from different locations in Africa and revealed great genetic differentiation among sub-populations of *Striga*. This high genetic diversity makes *Striga* management more complex as the resistance found in some cultivars may be overcome by a small subset of *Striga* individuals within the seed bank leading to the development of a virulent population over time.

Nevertheless, the use of *Striga* resistant cultivars is widely considered as one of the most suitable and effective control options for resource-poor farmers (Haussmann *et al.*, 2000; Rodenburg *et al.*, 2005). However, very few rice cultivars are known to combine resistance to *Striga* with adaptability to African upland rice growing environments (Rodenburg *et al.*, 2010). This gap can potentially be filled by the group of highly popular, inter-specific, NERICA rice cultivars which are widely distributed and adopted across Africa (Diagne *et al.*, 2006; Kijima *et al.*, 2006; Wopereis *et al.*, 2008). Recently, NERICA cultivars with pre- and post-attachment resistance against *Striga* spp. (*S. asiatica* and *S. hermonthica*) have been identified (Cissoko *et al.*, 2011, Chapter 2; Jamil *et al.*, 2011). Some of the NERICA cultivars displayed an excellent degree of post-attachment resistance to both *Striga* species and ecotypes and exhibited a range of resistance phenotypes under standard controlled conditions (Cissoko *et al.*, 2011, Chapter 2). However, despite our increasing knowledge of which rice cultivars show good resistance to *Striga* in laboratory studies, we know much less about the impact of environment on the expression of host resistance or, in other words, whether the resistance exhibited by some cultivars in the laboratory will be effective under field conditions.

The objectives of the work reported in this chapter were therefore to (1) evaluate, for the first time, the *Striga* resistance levels of the complete set of upland NERICA rice cultivars under *Striga*-infested field conditions and (2) determine if the resistance mechanisms observed in the laboratory are also expressed under field conditions. To achieve theses objectives, two seasons of field screening trials were conducted with all 18 upland NERICA cultivars and their parents, and susceptible, resistant and local cultivars (checks) in two different locations, at Kyela, Tanzania (where fields are infested with *S. asiatica*) and at Mbita Point, Kenya (under *S. hermonthica* infestation).

1. **Material and Methods**
2. **Plant materials**

All 18 currently available interspecific NERICA rice cultivars (NERICA 1 to 18), their *O. glaberrima* parent CG14, *O. sativa* (L.) ssp. japonica parents WAB56-104, WAB56-50 and WAB181-18, (used in Chapter 2) were grown in artificially and naturally *Striga*-infested plots at Kyela, Tanzania (under *S. asiatica* infestation) and at Mbita Point, Kenya (under *S. hermonthica* infestation). In addition to these 22 cultivars, for the trials in Kyela 2 traditional cultivars Supa India (synonym: Kilombero; a susceptible cultivar) and Mwangulu (a resistant cultivar) and a very susceptible cultivar IAC165 (*Oryza sativa* ssp. japonica) were also selected making a total of 25 cultivars. For the trials in Mbita Point, Mwangulu was replaced by IR49255-B-B-5-2 (*Oryza sativa* ssp. indica; identified in the studies of Harahap *et al.* (1993) and Johnson *et al.* (1997)). Seeds of all rice cultivars were obtained from Africa Rice Center (AfricaRice, ex-WARDA), Cotonou, Benin except for Supa India and Mwangulu which were supplied by the Agricultural District Office of Kyela. Seeds of *S. asiatica* and *S. hermonthica* used in this study were collected from plants parasitizing rice (at Kyela, Tanzania) and maize (at Mbita Point, Kenya) in farmer’ fields surrounding the experimental field sites in the previous season.

1. **Experimental sites**

The *Striga asiatica* field screening trials were conducted during the rainy seasons (January to June) of 2010 and 2011 in Mbako (9°34’54’’S - 33°47’42’’E; 500 m a.s.l.) a village approximately 15 km from Kyela, located in Kyela district, Mbeya region in southern Tanzania. The district is part of the Southern Highlands and located in the West arm of the African Rift Valley on the shores of Lake Malawi. Kyela district is a *S.* *asiatica*-infested upland rice-growing area. Cumulative rainfall measured in the field during the trials was 2275 mm in 2010 and 2474 mm in 2011 (Fig 3.1a). These data were comparable to the long-term seasonal average between 2005 and 2009 of 2275 mm (the 5-year rainfall data were obtained from the meteorological station of the Kyela District Agriculture and Livestock Development Office, 5 km from the field).

The *Striga hermonthica* field screening trials were conducted during the short rainy season of 2010 (September to February) and the long rainy season of 2011 (March to August) at the farm of the International Centre of Insect Physiology and Ecology

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Figure 3.1 Monthly cumulative rainfall data for the two field sites (a) Kyela (Tanzania) and (b) Mbita Point (Kenya). Data recorded during the rice growing single rainy seasons in 2010 and 2011 in Kyela and during short and long rainy seasons in Mbita Point are shown as bars. Monthly cumulative rainfall data for the 5 years between 2005 and 2009 are shown by red symbols connected by a line.

(*ICIPE*) at Mbita Point, located on a peninsula in Lake Victoria, Suba District, Western Kenya. The trial was laid out on a heavily *Striga*-infested field at the west-side of the peninsula (0°42’82’’ S - 34°20’53’’ E; around 1141 m a.s.l.) that was formerly under sorghum - cassava rotation. Cumulative rainfall was 281 mm in 2010 (short rain) and 615 mm in 2011 (long rain) with 1057 mm and 1067 mm long-term average between 2005 and 2009 for short and long rain, respectively (Fig 3.1b). At Mbita Point, all rainfall data were obtained from *ICIPE*’s meteorological station 500 m from the field. Following the crop’s water requirement, supplementary irrigation was applied when rainfall was insufficient.

1. **Experimental design, plot sizes and field preparation**

All field trials were laid out as a split-plot design with two *Striga* infestations (natural vs. artificial) on the plot level and 25 rice cultivars on the sub-plot level in six replications (Fig 3.2a and 3.2b). The sub-plots were randomized following a 5 × 5 lattice design. At Kyela each sub-plot, representing one cultivar × infestation treatment, measured 1.25 × 3.75 m (4.69 m2) and contained 5 rows of 15 rice planting hills (at a plant distance: 0.25 × 0.25 m) (Fig 3.2a). At Mbita Point each sub-plot measured 1.25 × 2.75 m (3.44 m2) with 5 rows of 11 rice planting hills (Fig 3.2b). Sub-plots were separated by one open row of 0.25 m to avoid neighbour effects and to allow easy access, and plots were separated by a 1.25 m alley as illustrated in Figure 3.3. Total field size, including alleys was 23.75 × 82 m (1947.5 m2) at Kyela (Fig 3.2a) and 32 × 46.25 m (1480 m2) at Mbita Point (Fig 3.2b).

In the artificially infested plots, each sub-plot received *Striga* seeds mixed with white sand. An amount of 4.25 g of *Striga* mixed with 460 g sand at Kyela and 2.067 g of *Striga* in 450 g sand at Mbita Point were used, resulting in an infestation density of 0.9 g seed m-2 at Kyela (108,000 *S. asiatica* seeds) and 0.6 g seeds m-2 at Mbita Point (43,200 *S. hermonthica* seeds). The mixture was broadcast and incorporated into the upper 5-10 cm of soil using short-handled-hoes.

In all trials, rice was direct sown at approximately 6 seeds per planting hill, and thinned to 2-3 plants per hill 25 days after sowing (DAS). To arrive at the desired plant density, in some cases gap filling was carried out by using supplemental plants from a rice nursery planted at the edge of the field at the same sowing date. From sowing onwards, each trial was regularly hand weeded (at least every 2-3 weeks) to remove all

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| Figure 3.2.a Experimental design of the rice-*Striga* field trial at Kyela, Tanzania showing each replicate plot (6 in total) with natural (white) and artificial (grey) *Striga* infestation levels. Each plot was composed of 25 sub-plots each representing one cultivar. The 25 cultivars in each plot were randomly distributed. Numbers from 1 to 18, and 56-104, 56-50 and 181-18 represent the NERICA cultivars and their *O. sativa* parents WAB56-104, WAB56-50 and WAB181-18, respectively. Supa India and Mwangulu are indicated by SI and MWA, respectively. |

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Figure 3.2.b Experimental design of the rice-Striga field trial at Mbita Point, Kenya showing each replicate plot (6 in total) with natural (white) and artificial (grey) Striga infestation levels. Each plot was composed of 25 sub-plots each representing one cultivar. The 25 cultivars in each plot were randomly distributed. Numbers from 1 to 18, and 56-104, 56-50 and 181-18 represent the NERICA cultivars and their O. sativa parents WAB56-104, WAB56-50 and WAB181-18, respectively. Supa India and IR49255-B-B-5-2 are indicated by SI and IR49, respectively.

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Figure 3.3 (a) Preparation of the field sites and (b) laying-out of the plots and sub-plots at Kyela, Tanzania in 2010. The layout of plot 5 (and sub-plots) under artificial (AI) and natural (NI) *Striga* infestation levels are shown.

weeds other than *Striga*. Fertilizer (N-P-K: 20-10-10) was applied at an equivalent rate of 50 kg ha-1 at 35 DAS to ensure reasonable rice development. Table 3.1 summarizes the experimental conditions at both field trial sites. The artificially-infested plots were maintained and re-infested in the second season in order to maintain a contrast between artificial and natural infestation levels.

1. **Experimental measurements**

Starting when *Striga* emergence was observed in 50% of the total number of sub-plots, the number of *Striga* individuals that emerged above ground in each individual sub-plot was counted regularly (weekly in Mbita Point, five times with three-week intervals in Kyela in 2010 and three times with four-week intervals in Kyela in 2011). Measurements ended at harvest. These data enabled the assessment of the maximum number of aboveground *Striga* plants (*NSmax*) following the methodology outlined by Rodenburg *et al.* (2005). At harvest emerged *Striga* plants within each sub-plot were collected, dried and weighted for determination of *Striga* biomass.

1. **Evaluation of the resistance of selected cultivars to *Striga hermonthica* (Mbita ecotype) and *S. asiatica* (Kyela ecotype) under controlled laboratory conditions**

A laboratory study was conducted with a sub-set of some of the most resistant and most susceptible NERICA cultivars and their parents in order to confirm their resistance ranking and assess their tolerance levels under standardized controlled conditions when infected by the same *Striga* ecotypes collected from the field sites. The cultivars tested were NERICAs 1, 7, 9, 10 and 17, CG14, WAB56-104, WAB56-50, WAB181-18, IAC165 (susceptible cultivar) and Supa India (cultivar commonly grown by farmers at Kyela). Rice plants were grown in rhizotrons and infected with 12.5 mg of germinated *S. hermonthica* seeds or 20 mg of germinated *S. asiatica* seeds as described in section 2.2.3 and in Cissoko *et al.* (2011). Rhizotrons were maintained in a controlled growth chamber under the conditions described in section 2.2.2 and in Cissoko *et al.* (2011). In this study, for each ecotype of *Striga*, 4 replicates of infected and uninfected control plants were established for each cultivar. The quantification of post-attachment resistance levels and the effect of *Striga* infection on the biomass of the selected cultivars were performed as described in section 2.2.3 and in Cissoko *et al.* (2011).

Table 3.1 Overview of experimental conditions of the rice-*Striga* field trials conducted at Kyela, Tanzania and at Mbita Point, Kenya in 2010 and 2011.

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| **Field** | **Kyela-Tanzania** | | **Mbita Point-Kenya** | |
| **Year** | **2010** | **2011** | **2010** | **2011** |
| **Location** | 9°37’30’’S - 33°52’30’’E | | 0°42’82’’S - 34°20’53’’E | |
| **Altitude** | 500 m | 500 m | 1141 m | 1141 m |
| **Season/Period** | Single rain/Jan-Jun | | Short rain/Sep-Feb | Long rain/Mar-Aug |
| **Cumulative Rainfall** | 2275 mm | 2474 mm | 281 mm | 615 mm |
| **Cultivars** | 24 + Mwangulu | | 24 + IR49255-B-B-5-2 | |
| **Sub-plot size**  **Plot size**  **Field size**  **Fertilization**  ***Striga*/sand**  ***Striga* infestation levels (Seeds m-2)** | 4.69 m2  143.19 m2  1947.5 m2  50 kg ha-1  4.25g/460g  108,000 | 4.69 m2  143.19 m2  1947.5 m2  50 kg ha-1  4.25g/460g  108,000 | 3.44 m2  106.94 m2  1480 m2  50 kg ha-1  2.067 g/450g  43,200 | 3.44 m2  106.94 m2  1480 m2  50 kg ha-1  2.067 g/450g  43,200 |

1. **Statistical analyses**

The statistical package Minitab (version 15) was used for ANOVA and Pearson’s product-moment correlation analyses. Prior to analyses, biomass and maximum numbers of above-ground *Striga* data were checked for homoscedasticity and normality following (Sokal and Rohlf, 1995) to assure that the assumptions of ANOVA were met. Tukey’s honest significant difference test was then performed to establish the different groups.

1. **Results**
2. **How resistant are the NERICA cultivars in the field?**

Figures 3.4 and 3.5 show the average maximum number of above ground *S.* *hermonthica* or *S. asiatica* plants (*NSmax*) for each rice cultivar in the field trials at Mbita Point, Kenya andat Kyela, Tanzania respectively, under natural and artificial *Striga* infestation. The NERICA cultivars and their parents exhibited different levels of resistance to *Striga* in the field. In Figures 3.4 and 3.5 the cultivars are ranked from the most susceptible to the most resistant based on data collected in 2011, as for both locations this was the season with the highest infection level. In this study, a greater number of *Striga* individuals attached to the susceptible rice cultivars in plots that were supplemented with additional *Striga* seeds (AI) when compared to plots with natural levels (NI) of *Striga* infestation. There was also a large difference in the number of *Striga* individuals that attached to the rice cultivars at the different locations and in different seasons (Fig 3.4 and 3.5). *Striga* infection was very low both at Kyela and Mbita Point in 2010 when compared to 2011. Although infection levels were low at Mbita Point in 2010, the rice cultivars did show similar expression of resistance to *S. hermonthica* in naturally and artificially infested plots when compared to data from 2011 (i.e. the most resistant cultivars in 2010 were also the most resistant in 2011; Fig 3.4a and 3.4b). However, at Kyela infection levels were too low in 2010 to rank cultivars with respect to resistance to *Striga* and only data from 2011 were used for this purpose.

At the Mbita Point field trial in 2011 there was a significant cultivar effect on *S. hermonthica* infection levels in artificially (*F* = 6.27, *df* = 24, *P* = 0.001) and naturally (*F* = 4.46, *df* = 23, *P* = 0.001) infested plots (Fig 3.4). The rice cultivars IAC165, WAB56-50 and NERICAs 6, 9 and 15 were very susceptible to *S. hermonthica* averaging in excess of 100 emerged *Striga* plants per sub-plot (in artificially infested plots; Fig 3.4b). These cultivars also supported a large *Striga* biomass at harvest between 154 ± 48 and 204 ± 46 g per sub-plot depending on the cultivar (Fig 3.6d). NERICAs 7, 11, 14, 16 and 18 were also considered susceptible as the numbers of emerged *Striga* plants per sub-plot ranged between 69 ± 10 and 99 ± 43, with a biomass at harvest of between 147 ± 56 and 83 ± 19 g per sub-plot (Fig 3.6d). CG14, IR49255-B-B-5-2, NERICAs 1-5, 12, 13 and 17 exhibited a strong resistance response with less than 10 emerged *Striga* plants per sub-plot associated with a dry biomass of less than 15 ± 2 g. The remaining NERICA cultivars (WAB56-104, WAB181-18 and NERICA 8)

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Figure 3.4 The maximum number of emerged *Striga hermonthica* plants per cultivar under (a) natural and (b) artificial *Striga* infestation at Mbita Point, Kenya. Data for 2010 are shown as dashed grey bars and for 2011 as light grey bars. The cultivars were ranked from the most susceptible to the most resistant based on data from 2011. Data are presented as means ± SE of 6 replicates. For 2011 data, means with the same letter do not differ significantly from each other (Tukey multiple comparison test, *P* > 0.05). Means for the 2010 data do not differ significantly for either artificially or naturally infested sub-plots.

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Figure 3.5 The maximum number of emerged *Striga asiatica* plants per cultivar under (a) natural and (b) artificial *Striga* infestation at Kyela, Tanzania. Data for 2010 are shown as dashed grey bars and for 2011 as light grey bars. The cultivars were ranked from the most susceptible to the most resistant based on data from 2011. Asterisks indicate missing data in 2010 due to poor rice establishment. Data are presented as means ± SE of 6 replicates. For the 2011 data, means with the same letter do not differ significantly from each other (Tukey multiple comparison test, *P* > 0.05). Means for the 2010 data do not differ significantly for either artificially or naturally infested sub-plots.

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Figure 3.6 Relationship between the biomass and number of *Striga* *hermonthica* plants per cultivar at harvest in 2010 and 2011 in the Mbita Point field trials under natural (a and b) and artificial (c and d) *S. hermonthica* infestation levels. Data are presented as means ± SE of 4 replicates. Dashed lines represent lines of best fit (Regression analysis).

and the local cultivar Supa India from Tanzania also showed relatively good levels of resistance with between 15 and 28 emerged *Striga* plants per sub-plot (Fig 3.4b). Although the overall level of infection was lower in the sub-plots with natural levels of *Striga* infestation, the ranking of the cultivars with respect to resistance was similar (Fig 3.4a). As indicated previously the ranking of cultivars for resistance in the 2010 trial was quiet similar to that observed in 2011 (with the exception of NERICAs 6 and 8), although there was no significant cultivar effect on *Striga* infection levels in 2010 (*P* > 0.05; Fig 3.4a and 3.4b).

There was a good linear relationship between the number of emerged *Striga* plants and the *Striga* biomass dry weight at harvest, irrespective of the season and infestation level (Fig 3.6). The most susceptible cultivars exhibited a greater number of emerged *Striga* plants and showed high *Striga* biomass dry weights while the most resistant cultivars supported less emerged *Striga* plants and smaller parasite biomass dry weights. In general at Mbita Point the number of emerged *Striga* plants and the *Striga* biomass dry weight at harvest (of susceptible cultivars) were much higher during the long rainy season (2011) and under artificial infestation (Figures 3.6a-d).

The difference in numbers of emerged *S. hermonthica* plants and biomass per cultivar in each sub-plot, in artificially infested plots, is illustrated by Figures 3.7a-b. The difference in the number of *Striga* plants parasitizing susceptible cultivars WAB56-50 and NERICAs 8 and 6 contrast with the good resistance of NERICAs 1, 3 and 5 and the cultivar IR49255-B-B-5-2 in Figure 3.7a. Similarly in Figure 3.7b NERICAs 1 and 17 have little emerged *Striga* whereas NERICAs 14 and 9 are highly infected (Fig 3.7b). Most of the NERICA 9 rice plants have died because of the high infection levels (Fig 3.7b).

At the Kyela field trial in 2011 the most susceptible cultivar to *S. asiatica*, with an average of 85 ± 29 emerged parasites, was Supa India, the local cultivar grown in the region (Fig 3.5a and 3.5b). While very low numbers *S. asiatica* plants emerged on the NERICA cultivars in 2010, Supa India supported significant numbers of emerged parasites under both natural and artificial infestation (Fig 3.5a and 3,5b). In the 2011 trial there was a significant cultivar effect on *S. asiatica* infection levels in both artificially (*F* = 3.1, *df* = 24, *P* = 0.001) and naturally (*F* = 2.15, *df* = 24, *P* = 0.004) infested plots (Fig 3.5). With respect to resistance to *S. asiatica*, the NERICA cultivars showed a range of *NSmax* from 12 ± 6 for the most susceptible cultivar (NERICA 6) to

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Figure 3.7 Contrasting *Striga hermonthica* infection levels between different rice cultivars in artificially infested plot 3 (a) and plot 6 (b) at the Mbita Point field trial in the long rainy season of 2011. Cultivars are delimited by yellow lines.

1 ± 0.4 for the most resistant cultivar (NERICA 2) under natural infestation, and 45 ± 13 for the most susceptible (NERICA 14) to 5 ± 1.8 for the most resistant (NERICA 5) cultivar under artificial infestation. Clearly, the overall numbers of emerged *S. asiatica* plants on the different cultivars at Kyela was less than that of emerged *S. hermonthica* observed at the Mbita Point field trial.

Supa India and NERICAs 6, 7, 8, 11, 12, 13, 14, 16 and 18 showed a *NSmax* of between 10 and 25 *S. asiatica* plants per sub-plot under natural infestation and between 25 to 85 *S. asiatica* plantsunder artificial infestation and are categorized as relatively susceptible (Fig 3.5a and 3.5b). By contrast NERICAs 1, 2, 5, 10, 17, WAB181-18 and WAB56-50 were among the most resistant cultivars under both infestation levels with an *NSmax* varying between 2 and 14 *S. asiatica* plants per sub-plot. Some cultivars, e.g. NERICAs 6, 11, 12, 15 and 16, WAB56-104 and CG14 showed different ranking positions under natural and artificial infestation levels (Fig 3.5a and 3.5b). Again there was a good correlation between the average maximum numbers of emerged *S. asiatica* plants and their biomass dry weight at harvest for the different rice cultivars under both infestation levels and across seasons (Fig 3.8).

The most resistant cultivars across sites/*Striga* species and seasons were NERICAs 1, 2, 3, 5, 10 and 17 and the most susceptible were NERICAs 7, 8, 11, 14 and 18. Some cultivars showed different responses to Sh-Mbita and Sa-Kyela. For example, Supa India, CG14, and NERICAs 12 and 13 showed good resistance to Sh-Mbita but were among the more susceptible cultivars to Sa-Kyela. In contrast, NERICA 15, WAB56-50 and IAC165 were susceptible to the *S. hermonthica* ecotype from Mbita Point but showed good resistance to the *S. asiatica* ecotype from Kyela.

1. **How resistant are selected NERICA and other rice cultivars to the two field ecotypes of *Striga hermonthica* (Sh-Mbita) and *S. asiatica* (Sa-Kyela) under controlled conditions?**

In order to determine whether the resistance of some of the NERICA cultivars, their parents, the local cultivar (Supa India) and the susceptible cultivar (IAC165) were altered by environmental conditions in the field, an experiment was carried out under controlled environmental conditions in the laboratory to determine their susceptibility to the ecotypes of *S. hermonthica* (Sh-Mbita) and S*. asiatica* (Sh-Kyela).

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Figure 3.8 Relationship between the biomass and number of *Striga* *asiatica* plants per cultivar at harvest in 2010 and 2011 in the Kyela field trials under natural (a and b) and artificial (c and d) *S. asiatica* infestation levels. Data are presented as means ± SE of 4 replicates. Dashed lines represent lines of best fit (Regression analysis).

In addition, the effect of the parasites on the host biomass (compared to uninfected plants) was also determined.

There was a significant cultivar effect on *Striga* *hermonthica* (Sh-Mbita) infection levels (*F* = 57.09, *df* = 10, *P* = 0.001) with WAB181-18, WAB56-50, CG14, Supa India and NERICA 1, 10 and 17 exhibiting good resistance (Fig 3.9a). The most resistant cultivars had few successful attachments resulting in low parasite biomass on the roots (Fig 3.10a). IAC165, WAB56-104 and NERICA 7 and 9 were highly susceptible with a large number of attachments and high parasite biomass (Fig 3.9a and 3.10a). The resistance response of these cultivars in the lab largely matched their response in the field. The exception was WAB56-50 that was characterised as susceptible in the field but showed good post-attachment resistance in laboratory studies.

Again there was a significant cultivar effect on infection levels of Sa-Kyela (*F* = 10.98, *df* = 10, *P* = 0.001). The most resistant cultivars were CG14, WAB181-18 and NERICAs 10 and 17 supporting few attachments and low *Striga* biomass (Fig 3.9b and 3.10b) whilst the most susceptible were NERICA 7, WAB56-104, WAB56-50 and IAC165, supporting the largest number and biomass of parasites on their roots (Fig 3.10b). Again, there was good correlation between the field and laboratory studies with respect to the resistance ranking of the cultivars to Sa-Kyela although there were also some differences. For example, IAC165 was not infected by Sa-Kyela to any great extent in the field but was one of the most susceptible cultivars in laboratory studies. In contrast, Supa India was very susceptible in the field, but did not support many attachments in the laboratory study. As observed in the field study, Sa-Kyela showed a different pattern of virulence on some of the NERICA cultivars compared to Sh-Mbita. For example, NERICA 9 was less susceptible to Sa-Kyela than to Sh-Mbita whereas NERICA 1 was more susceptible to Sa-Kyela than to Sh-Mbita (Fig 3.9, 3.10a and 3.10 b).

With both Sh-Mbita and Sa-Kyela, there was a negative linear relationship between the percentage biomass of infected host-plants compared to uninfected control plants and the amount of parasite biomass on the roots (Fig 3.11a and 3.11b). The most resistant cultivars (NERICAs 17, 10 and 1) showed only small (10 to 25%) reductions in biomass compared with the uninfected controls whereas the most susceptible cultivars (NERICAs 9, 7, WAB56-104 and IAC165) all lost 50 to 65% of their biomass compared to their respective control plants when infected with either Sh-Mbita or Sa-Kyela (Fig 3.11a and 3.11b). There was also a difference in growth performance

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Figure 3.9 *Striga* biomass dry weights of selected NERICA rice cultivars and their parents to (a) *Striga* *hermonthica* (Sh-Mbita) and (b) *S. asiatica* (Sa-Kyela) ecotypes collected from the two field sites at Mbita Point, Kenya and Kyela, Tanzania respectively. Rice plants were grown in rhizotrons under controlled environmental conditions. *Striga* biomass dry weight was assessed after harvesting at 21 days after infection. Data are presented as means ± SE of 4 replicates. Means with the same letter do not differ significantly from each other (Tukey multiple comparison test, *P* > 0.05).

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Figure 3.10 Relationship between the number and biomass dry weight of (a) *Striga* *hermonthica* (Sh-Mbita) and (b) *S. asiatica* (Sa-Kyela) individuals on the NERICA rice cultivars grown in rhizotrons under controlled environmental conditions.The number of *Striga* seedlings and *Striga* biomass dry weight were assessed 21 days after infection at harvest. Data are presented as means ± SE of 4 replicates.

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Figure 3.11 Relationship between the biomass dry weight of *Striga*-infected plants compared to uninfected control plants and the biomass dry weight of (a) *Striga* *hermonthica* (Sh-Mbita) and (b) *S. asiatica* (Sa-Kyela) attached to the roots of the NERICA rice cultivars 21 days after infection.Rice plants were grown in rizhotrons under controlled environmental conditions. Data are presented as means ± SE of 4 replicates.

between cultivars subjected to the same amount of *Striga* infection. For example, NERICA 17 showed 10% reduction in biomass when infected by Sh-Mbita or Sa-Kyela, while Supa India showed 40 and 50% (for Sh-Mbita and Sa-Kyela, respectively) when infected with a similar number/biomass of parasites (Fig 3.11a and 3.11b).

1. **Discussion**

Decades of research and breeding efforts towards developing *Striga*-resistant crop varieties, mainly in sorghum and maize, have resulted in an increased understanding in host resistance mechanisms but relatively few adapted cultivars with valuable levels of resistance (Rodenburg *et al.*, 2010; Yoder and Scholes, 2010). Also in gene discovery, relatively little progress has been made when one considers the enormous efforts undertaken so far. Only for one crop, cowpea, a resistance gene to *Striga* (*S. gesnerioides*) has been found (Li and Timko, 2009) and this could very well result in the development of adapted cowpea cultivars with durable resistance, in particular because the genetic variability of *S. gesnerioides* appears to be relatively low (Dube and Belzile, 2010). The situation with respect to *S. hermonthica* and to a lesser extent *S. asiatica*, on cereal crops proved however more complicated. The little tangible progress made so far on sorghum and maize is partly due to the high genetic variation in the *Striga* species involved, in particular for out-crossing *S. hermonthica* (Bharathalakshmi and Musselman, 1990). This genetic variability implies a high likelihood for any resistance to be overcome by the parasite in a few cropping seasons grown with the same cultivars (e.g. Rodenburg and Bastiaans, 2011). The current study builds on recent previous achievements where pre- and post-attachment resistance mechanisms have been found in rice cultivars (Jamil *et al.*, 2011; Cissoko *et al.*, 2011) by putting these cultivars to trial in a realistic screening environment in order to test expression and effectiveness of these mechanisms in the field under conditions typical for African upland-ecosystems. This work is particularly interesting as these previous studies have identified some cultivars of NERICA that seem to combine at least two mechanisms of resistance (pre- and post-attachment) and are therefore likely to show an effective and durable field resistance that would be more difficult to overcome by the parasite (Pickett and Hooper, 2011). The current study focused on two research questions: 1) how resistant are the NERICA rice cultivars to *Striga* spp. in the field, and 2) is the *Striga* resistance ranking of NERICA rice cultivars in field similar to that of the laboratory. These questions are discussed below.

1. **How resistant are the NERICA rice cultivars to *S. hermonthica* and *S. asiatica* in the field?**

Among the set of 25 rice cultivars screened under field conditions in Tanzania and Kenya, significant differences were found in levels of resistance against *S. asiatica* and *S. hermonthica.* Based on our results, summarized in Table 3.2, rice cultivars can be classified into three distinct types with respect to their responses to *S. hermonthica* and *S. asiatica* parasitism:

1. Type 1 cultivars are those that exhibit consistent and superior levels of resistance to both *Striga* species in the field, expressed as very low numbers and biomass of above-ground *Striga* plants. Cultivars of this type are NERICAs 1, 2, 5, 10 and 17.
2. Type 2 cultivars are consistently resistant to one *Striga* species, as evidenced by low above-ground *Striga* numbers and biomass, and consistently (moderately to highly) susceptible to the other species, based on observed high parasite numbers and biomass. Type 2 cultivars are NERICAs 3, 4, 12 and 13, CG14 and Super India resistant to *S. hermonthica* and NERICA 15, WAB56-50, WAB181-18 and IAC165 resistant to *S. asiatica*.
3. Type 3 cultivars are consistently susceptible to both *Striga* species in the field, as they supported large numbers and biomass of above-ground *Striga* plants in infested fields at both Kyela (*S. asiatica*) and Mbita Point (*S. hermonthica*). The Type 3 cultivars are NERICAs 7, 8, 11, 14, 16 and 18.

Cultivars of type 1 are as resistant as CG14. This *O. glaberrima* parent of the NERICA cultivars is known for its resistance against *S. hermonthica* as reported by Johnson *et al.* (1997) in pot and field experiments and by Kaewchumnong and Price (2008) in a pot experiment where CG14 was identified as cultivar that showed strong post-attachment resistance to *S. hermonthica*. Rice cultivars with consistently high resistance, such as found among cultivars of type 1, are suitable for rice production in sub-Saharan Africa where *S. hermonthica* and *S. asiatica* are prevalent. Currently 2 of these cultivars, NERICAs 1 and 10 have been released in Uganda (e.g. Kijima *et al.*, 2007) and NERICA 1 has proved good adaptation (more than 4 t ha-1) to low input and harsh climatic conditions in Western Kenya (Atera *et al.*, 2011).

There are a number of cultivars that exhibit different levels of susceptibility to *S. hermonthica* compared to their susceptibility to *S. asiatica* (e.g. cultivars of type 2: NERICAs 3, 4, 12, 13 and 15, CG14, Supa India and IAC165). Among them, Supa India was very susceptible to *S. asiatica* at Kyela and relatively resistant to *S. hermonthica* at Mbita Point. This cultivar has been grown for many years by the farmers at Kyela and it is likely that the virulence levels of the local parasite population against this cultivar have increased in time. *Striga asiatica* is an inbreeding parasite (Gethi *et al.*, 2005) so virulence genes are likely to have built up in the population over the years.

Table 3.2 Summary of the resistance of different rice cultivars to *S. hermonthica* and *S. asiatica* ecotypes in field and laboratory studies. Classification of cultivars as either resistant (R), susceptible (S) or intermediate (I) was based on the average maximum number of emerged *Striga* plants for the field experiments and on the average number of *Striga* plants for the laboratory studies. Information on pre-attachment resistance was based on strigolactone production from root exudates of the NERICA cultivars (from Jamil *et al.,* 2011).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |
|  | **Field resistance** | | | | **Post-attachment** | | | | **Pre-attachment** |
| Rice cultivars | **Sh-Mbita** | | **Sa-Kyela** | | **Sh-Mbita** | **Sa-Kyela** | **Sh-Kibos** | **Sa-USA** | **Sh-Medani** |
|  | **NI** | **AI** | **NI** | **AI** |  | | | |  |
| NERICA 1 | R | R | R | R | R | R | R | R | R |
| NERICA 2 | R | R | R | R | - | - | R | R | I |
| NERICA 3 | R | R | R | I | - | - | R | R | R |
| NERICA 4 | R | R | I | I | - | - | R | R | R |
| NERICA 5 | R | R | R | R | - | - | R | R | I |
| NERICA 6 | R | S | S | I | - | - | S | S | I |
| NERICA 7 | S | S | S | S | S | S | S | S | S |
| NERICA 8 | I | R | S | S | - | - | S | S | S |
| NERICA 9 | S | S | R | S | S | I | S | I | I |
| NERICA 10 | R | R | R | R | R | R | R | R | I |
| NERICA 11 | S | S | S | I | - | - | S | S | S |
| NERICA 12 | R | R | S | I | - | - | R | R | R |
| NERICA 13 | R | R | S | S | - | - | I | R | I |
| NERICA 14 | S | S | S | S | - | - | S | S | S |
| NERICA 15 | S | S | I | R | - | - | S | S | I |
| NERICA 16 | S | S | S | R | - | - | S | S | R |
| NERICA 17 | R | R | R | R | R | R | R | R | R |
| NERICA 18 | S | S | S | S | - | - | S | S | I |
| CG14 | R | R | I | S | R | R | R | R | R |
| WAB56-104 | R | R | R | S | S | S | I | I | R |
| WAB56-50 | S | S | R | R | R | I | I | R | S |
| WAB181-18 | I | I | R | R | R | R | I | R | R |
| IAC165 | S | S | R | R | S | S | S | S | - |
| IR49255-B-B-2 | R | R | - | - | - | - | - | - | - |
| Supa India | R | R | S | S | R | I | - | - | - |
| Mwangulu | - | - | R | S | - | - | - | - | - |

Sh-Mbita = *Striga hermonthica* from Mbita Point, Kenya; Sh-Kibos = *Striga hermonthica* from Kibos, Kenya; Sh-Medani = *Striga hermonthica* from Waz Medani, Sudan; Sa-Kyela = *Striga asiatica* from Kyela, Tanzania; Sa-USA = *Striga asiatica* from North Carolina, USA); NI = plots naturally infested; AI = plots artificially infested with *Striga* seeds.

Supa India has not been grown at Mbita Point prior to our screening efforts and showed good resistance to *S. hermonthica.* Based on insights presented by Huang *et al.* (2012), this wouldsuggest that the *S. hermonthica* population in this field did not have the virulence genes to overcome resistance genes in the host. At present, we do not know whether Supa India is resistant to all ecotypes of *S. hermonthica*. Further experiments are required to assess the resistance of this cultivar against different populations of both *S. hermonthica* and *S. asiatica*. IAC165 shows differential susceptibility responses; it is susceptible to *S. hermonthica* at Mbita Point but supports much less *S. asiatica* at Kyela. However in laboratory studies using isolate of *S. hermonthica* (from Mbita Point) and *S. asiatica* (from Kyela), IAC165 is again very susceptible to bothspecies. For some reason, probably linked to soil type and fertility, the screening environment of Kyela was not conducive enough (infection rates were generally low) to show consistent and significant differences between the cultivars, except for the locally adapted Super India.

More than half of the 18 upland NERICA cultivars, as well as their *O. glaberrima* parent CG14 and Supa India and IR49255-B-B-5-2, exhibited a good level of resistance to *S. hermonthica*. Observed superior resistance of IR49255-B-B-5-2, used as resistant check in the *S. hermonthica* infested field at Mbita Point (Kenya), confirms previous field and pot studies where this cultivar was highly resistant to *S. hermonthica* (Harahap et *al.*, 1993; Johnson *et al.*, 1997). While not field screened against *S. asiatica*, IR49255-B-B-5-2 showed a similar high resistance against this speciesin the laboratory and rhizotrons experiments (data not shown). Resistance to *S. asiatica* is strongly expressed in some NERICA cultivars (e.g. all NERICA cultivars of type 1) and moderately in Mwangulu, the local resistance check at Kyela (Tanzania). Both Mwangulu and *O. glaberrima* CG14 were susceptible under high level of *S. asiatica* infestation.

The lack of field resistance of the NERICA cultivars of type 3 to *S. hermonthica* and *S. asiatica* infestations are consistent with the pre- and post-attachment resistance ranking observed in laboratory studies (Jamil *et al.*, 2011; Cissoko *et al.*, 2011, Chapter 2). As these cultivars exhibited poor pre- and post-attachment resistance against both *Striga* species they are unsuitable for use in *Striga*-infested environments in these regions of Africa. It is perhaps surprising that NERICA cultivars of type 1 showed good resistance whereas some NERICA cultivars of type 3, some of which are derived from the same parental crosses, are very susceptible to *S. hermonthica* and *S. asiatica*. NERICAs 16 and 18, for instance, are susceptible while NERICA 17 showed broad resistance to both *Striga* species, and they are all offspring from resistant CG14 and moderately resistant WAB181-18. The lack of *Striga* resistance in the type 3 NERICA cultivars could be due to (a) the loss of genes involved in *Striga* resistance during crossing and back-crossing activities of the breeding of NERICA cultivars and/or to (b) the genetic introgressions of unknown non parental alleles which can happen during the breeding process, as Semagn *et al.* (2007) pointed out in a molecular profiling study on NERICA cultivars. Johnson *et al.* (2000) found similar discrepancies between reaction types of parents and offspring material. They examined F7 generation of rice progenies derived from crosses between *O. glaberrima* CG14 (as donor parent) and *O. sativa* WAB56-104 (as female parent) and found no *Striga* resistance expression in the lines when screened against *S. hermonthica*.

1. **Is the *Striga* resistance ranking of NERICA rice cultivars in field similar to that of the laboratory?**

In order to compare the resistance expressed by some NERICA rice cultivars in the field with that observed under laboratory conditions, selected NERICA rice cultivars have been screened in the laboratory against the same *Striga* ecotypes (Sh-Mbita and Sa-Kyela) collected from the respective field sites. The results showed similar general patterns for resistance to *Striga* species and ecotypes in both field and laboratory, particularly for *S. hermonthica*. Our laboratory and field studies identified cultivars that exhibited superior resistance responses to Sh-Mbita (CG14, NERICAs 1, 10 and 17) and Sa-Kyela (NERICAs 10 and 17) and those that were moderately to highly susceptible to Sh-Mbita (IAC165, WAB56-104, WAB56-50, NERICAs 7 and 9) and Sa-Kyela (WAB56-104, NERICAs 7 and 9). Similar reaction types were also observed under field and laboratory conditions for WAB181-18 (moderately resistant) to Sh-Mbita and Sa-Kyela as well as for the locally adapted cultivar Supa India which exhibited a strong level of resistance to Sh-Mbita and susceptibility interactions with Sa-Kyela. *Striga* biomass at harvest in the field, and the number of *Striga* seedlings attached to the host root at harvest in the laboratory, are both linearly and strongly correlated to the dry biomass of harvested *Striga* plants and these parameters can therefore all be used to quantify resistance against *Striga*, confirming previous studies (e.g. Haussmann *et al.*, 2000; Rodenburg *et al.,* 2005). Moreover, resistance observations obtained in the laboratory are to a large extent similar to those in the field. However, these observations cannot be used to reliably predict their resistance levels in the field as some rice cultivars exhibited different responses to parasitism in the field as compared to their response in the laboratory study when infected with Sa-Kyela or Sh-Mbita. WAB56-50 and IAC165 with their low to moderate level of post-attachment resistance against *S. asiatica* found in the laboratory, displayed good resistance responses in the field. The same was observed with WAB56-104 against *S. hermonthica*. Conversely, good levels of laboratory post-attachment resistance found with WAB56-50 and WAB181-18 against *S. hermonthica* and with CG14 against *S. asiatica* were not expressed under field conditions. This confirms earlier findings that show that resistance in the laboratory or other controlled environments do not always express the same way in the field (e.g. Haussmann *et al.*, 2000; Omanya *et al.*, 2004). There are a number of reasons that could explain differences between laboratory and field observations. The non-homogenous distribution of *Striga* seeds, variability of soil fertility and soil moisture, flora and fauna of infested fields creates a different screening environment compared to the fully controlled situations in the laboratory where the infestation or even the infection level (i.e. with pre-germinated seeds) can be completely controlled (e.g. Haussmann *et al.*, 2000; Ejeta, 2007). Besides *Striga* seeds distribution in the soil, the characteristics of the host root system could play a role in the responses of cultivars to *Striga* infection. Again in the laboratory study, equal amounts of pre-conditioned *Striga* seeds were aligned along the totality of host roots even though apparent differences are observed in root morphology or architecture of rice cultivars tested. These apparent differences between cultivars with respect to their root system could help to avoid or escape *Striga* parasitism in the field. Some cultivars had less extensive root systems which allow them to escape the soil layer in which parasite seeds are more likely to be present resulting in fewer attachments (Ejeta *et al.*, 1992; Berner *et al.*, 1995). Root systems that form a smaller proportion of root length in the topsoil (e.g.Van Delft *et al.*, 1996; Arnaud *et al.*, 1999) or are much simpler, less branched (Cherif-Ari *et al.*, 1990) could possibly facilitate fewer attachments. This phenomenon known as avoidance (or escape) was also reported by Amusan *et al.* (2008), in the study of resistance to *S. hermonthica* in maize inbred lines derived from *Zea diploperennis* and by Oswald and Ransom (2004) where some short growth-cycle maize cultivars with their rapid root development give less time to the parasite to attach.

Soil fertility could also have played an important role. The two field sites (particularly Kyela) are characterized by poor soil fertility, caused by continuous crop production without nutrient replenishment by appropriate fertilizer applications. Soil fertility depletion is a general and persistent problem in sub-Saharan Africa as observed by Smaling (1993), Stoorvogel and Smaling (1998) and more recently by Vanlauwe and Giller (2006). Mghase *et al.* (2010) carried out an analysis of soils in 4 locations in Tanzania including Kyela (9°34’60’’S-33°55’0’’E) near the field used for our *Striga* resistance trials. They showed that Kyela soils were clay loam with poor nitrogen (N = 0.16%) and available phosphorus (P = 0.5 mg kg-1) and with medium carbon contents (1.65%). Soils at Mbita Point (0°25’0’’ S - 34°13’0’’E) according to Weisskopf *et al.* (2009), consisted of a clay loam with also poor N (0.09-0.12%), medium available P (6.3-13.3 mg kg-1) and poor organic carbon contents (1.13-1.25%). This poor soil fertility, in particular in Kyela, has certainly negatively affected crop performance and root growth and this in turn might have lowered the expression of resistance/susceptibility of cultivars that are less adapted to local conditions. This might have influenced the extent of expression of reaction types of the newly introduced NERICA cultivars compared to the locally adapted Super India and Mwangulu.

Soil fertility levels could also affect the expression of pre-attachment resistance in rice cultivars. Root exudates from plants grown under N and P limited conditions markedly induced germination of parasite seeds via increased levels of strigolactone production as demonstrated by Yoneyama *et al.* (2007a) with sorghum. Others studies have reported the enhancement of strigolactone exudation to P deficiency in tomato (Lopez-Raez *et al.*, 2008), in red clover (Yoneyama *et al.*, 2007b) and in rice (Umehara *et al.*, 2010; Jamil *et al.*, 2011). Some cultivars with pre-attachment resistance (i.e. those with lower strigolactone production levels) may exhibit a weaker expression of resistance under field conditions characterized by extreme deficiencies (and/or micro-variability) in nutrient elements such as nitrogen (N) and phosphorus (P). Conversely, under conditions where N and P are not limited, the susceptible cultivars (i.e. those with higher strigolactone production levels) may show relatively low levels of *Striga* infection due to overall lower strigolactone production. Both conditions could reduce the contrast between resistant and susceptible cultivars in the field.

NERICAs 2, 5 and 10 were among the most *S. hermonthica*-resistant cultivars identified in the laboratory and field studies. These cultivars showed intermediate production rates of strigolactones (Jamil *et al.*, 2011) indicating their moderate to low pre-attachment resistance. This highlights the risk of screening for a single resistance mechanism as it could lead to some cultivars with useful and effective post-attachment resistance being discarded. This was previously concluded by Haussmann *et al.* (2000) and Rodenburg *et al.* (2005).

The field resistance observed in the current study largely reflects the pre- and post-attachment resistance against *S. hermonthica* (Sh-Kibos) and *S. asiatica* (Sa-USA) as previously observed in the laboratory and rhizotrons studies of Jamil *et al.* (2011) and Cissoko *et al.* (2011). The reaction of the NERICA cultivars to both *S. asiatica* ecotypes Sa-Kyela and Sa-USA as well as to both *S. hermonthica* ecotypes Sh-Mbita and Sh-Kibos was identical in laboratory experiments (Cissoko *et al.*, 2011 Chapter 2; Table 3.3). NERICA 1 and 17 showed both pre- (Jamil *et al.,* 2011), post- (Cissoko *et al.*, 2011) and field resistance against both *Striga* species. NERICAs 3, 4, 12 and NERICA parents CG14 and WAB56-104, shown by Jamil *et al.* (2011) to possess pre-attachment resistance against *S. hermonthica*, also showed good field resistance in the *S. hermonthica* infested fields in the current study. Likewise NERICAs 2, 3, 4, 5, 10 and 12, shown by Cissoko *et al.* (2011) to have post-attachment resistance against *S. hermonthica*, also showed good field resistance in the *S. hermonthica* infested fields in the current study. While against *S. asiatica*, the post-attachment resistance of NERICAs 2, 5 and 10 (Cissoko *et al.*, 2011) was also expressed in the field. Conversely, NERICAs 4, 12, 13 and *O. glaberrima* cultivar CG14 showed good post-attachment resistance against *S. asiatica* in rhizotrons studies (Cissoko *et al.*, 2011, Chapter 2) but proved moderately susceptible under field conditions.

The optimization of resistance of the NERICA rice cultivars will require a good management and application of fertilizers. Improving soil fertility will allow rice cultivars to reduce *Striga* infestation and increase their yield as was shown by Adagba *et al.* (2002). Application of 90 to 120 kg N ha-1 in upland rice helped to reduce the number of emerged *Striga* plants and boost yield of host plants grown in *Striga* infested fields. In fact NERICA cultivars are known to have higher yield potential and strong response to the use of inputs such as fertilisers (Somado *et al.,* 2008). In addition, as a soil fertility replenishment and *Striga* control option, the use of a legume, as intercrop or rotation crop, could be an alternative strategic approach in this region as many resource-poor farmers cannot afford mineral fertilizers (see references in Rodenburg et al. (2010)).

**Conclusions**

This study showed that some NERICA cultivars display good level of resistance and tolerance to *Striga* species. Nevertheless, notable and sufficient genetic variability exists. A number of NERICA cultivars, notably NERICAs 1, 2, 5, 10 and 17 for *S. asiatica* and NERICA 1, 2, 3, 4, 5, 10 and 17 for *S. hermonthica*, possess superior resistance in the field. Superior field resistance against those two *Striga* species observed in NERICA 1, 5, and 17 was based on at least one pre- and one post-attachment resistance mechanism, as confirmed in recent previous studies. NERICAs 1 and 17 have also been identified as two of the more tolerant cultivars among the group of cultivars included in this study. They suffered less *Striga*-inflicted total plant biomass reduction compared to some other cultivars when subjected to similar *Striga* infection (biomass) levels. This study showed that the use of *in-vitro* methods to identify resistance based on single mechanisms are useful for the identification of superior breeding material in particular when such methods are used in succession to identify material with resistance based on multiple mechanisms.

The resistant cultivars identified in this study, in particular the ones showing resistance at the pre- and post-attachment stages, and those that are resistant against the two *Striga* species studied here, could be used in breeding programs aiming at the development and improvement of *Striga* resistance in adapted and high yielding rice cultivars. Such cultivars are also suitable for inclusion in an integrated *Striga* control program for resource-poor rice farmers typically working in the poorly fertile, drought-prone and *Striga*-infested upland ecosystems commonly found in sub-Saharan Africa.

# Chapter 4

# Identification of *Striga* resistance QTL in a Chromosome Segment Substitution Line (CSSL) mapping population

## 

## Introduction

Rice is one of the most important cereal crops in sub-Saharan Africa but its growth and production suffers from many biotic and abiotic stresses. Among the biotic constraints, the parasitic weed *Striga* has been identified as one of the main limiting factors of rice production in many areas. Infection by *Striga* species causes losses in grain yield (Johnson *et al.*, 1997; Ejeta, 2007) that range from 40% to complete crop failure in highly *Striga*-infested fields (Dugje *et al.*, 2006). Various biological, chemical, cultural and genetic control strategies have been developed and employed to control *Striga* parasitism in rice (Rodenburg and Johnson, 2009) but the use of resistant and tolerant cultivars is considered to be one of the most cost-effective and sustainable control methods for African subsistence farmers (Rodenburg *et al.*, 2006a; Scholes and Press, 2008).

The discovery of resistance to *Striga* species in cereal crops including rice is beginning to open the way to our understanding of the genetic basis of some resistance mechanisms. Resistance to *S. hermonthica* is thought polygenic and controlled by many genes of major and minor effect (Scholes and Press, 2008). A possible reason for this is that *S. hermonthica* is an obligate, outbreeding parasite, thus populations of *Striga* individuals are extremely genetically variable (e.g. Huang *et al.*, 2012). In addition, as *Striga* seeds can remain viable in the soil for 20 years or more (Parker and Riches, 1993) the seed bank is a reservoir of genetic diversity which suggests that cultivars may require many genes to defend themselves against attack. In contrast to *S. hermonthica*, *S. gesnerioides* (the latter attacks dicotyledonous crops such as cowpea) is an inbreeding parasite that exhibits less genetic variation within specific seed populations (Timko *et al.*, 2007). Resistance to *S. gesnerioides* is mainly monogenic in character and there is clear evidence of race specific resistance responses (Botanga and Timko 2006; Li *et al.*, 2009). These authors have clearly shown that particular cultivars of cowpea are resistant (or susceptible) to specific races of the parasite suggesting a gene-for-gene interaction. Li and Timko (2009) carried out a positional cloning study to identify the resistance gene(s) involved in resistance of cowpea cultivar B301 to race SG3 of *S. gesnerioides*. They isolated a gene called *RSG-301*, a resistance (R) gene homolog that encodes a protein which has a coiled-coil (CC) protein-protein interaction domain at the N-terminus, a nucleotide binding site (NBS) and a leucine-rich repeat domain at the C-terminus. Thus, this protein is structurally similar to other CC-NBS-LRR class R proteins identified in plants (Caplan *et al.*, 2008). Li and Timko (2009) also showed that when this gene was silenced in the resistant cultivar it became susceptible to *S. gesnerioides* race SG3. At present this is the only example of a *Striga* resistance gene that has been identified, cloned and functionally validated.

Resistance in cereals to *S. hermonthica* or *S. asiatica* has been mostly identified through quantitative trait loci (QTL) analysis of segregating populations such as advanced recombinant or backcross inbred lines (RIL or BIL), doubled haploids (DH) and self-pollinating F2 or F3 populations. For example, the low germination stimulant (*lgs*) gene for resistance to *S. hermonthica* has been identified in some sorghum F1 and F2 backcross progenies derived from the *Striga* resistant cultivar SRN39 and three susceptible cultivars, Shanqui Red (SQR), P-954063 and IS 4225 (Vogler *et al.*, 1996; Ejeta, 2007). The resistant progenies possessing this gene have the ability to produce low amounts of *Striga* germination stimulants which reduce parasite germination prior to attachment to host roots systems (Vogler *et al.*, 1996). Haussmann *et al.* (2001b, 2004) used different RIL populations of sorghum to identify many different QTL for resistance to *S. hermonthica*. They developed two RIL populations from crosses between a line IS9838 (a low germination stimulant producer) and a susceptible cultivar E-36, and cultivar N13 (which shows post-attachment resistance) and E-36. The two populations were grown in the field in Africa at 5 different locations in Mali and Kenya over two seasons. Many QTL were identified for resistance to *Striga*, some were specific to one of the populations and some were only seen in a particular environment in a particular season. However 5 QTL were common to both RIL populations and occurred independently of site and season. These QTL are very important as they are associated with broad spectrum resistance to different populations of *S. hermonthica* and were not affected by environment. These QTL are being transferred to other cultivars via marker assisted breeding but it would be valuable to know the identity of the genes involved to enable the design of novel control strategies.

Fewer studies have been carried out to investigate resistance to *Striga* in rice. However, post-attachment resistance to *S. hermonthica* was found in rice cultivar Nipponbare by Gurney *et al.* (2006). The resistance phenotype is characterised by a lack of ability of the parasite to penetrate through the endodermis thus the parasite cannot establish vascular continuity with the host. Nipponbare is a parent of two Backcross Inbred Line (BIL) populations developed by Professor Masahiro Yano at the Japanese Rice Resource Centre. Gurney *et al.* (2006) screened the BIL population developed from a cross between Nipponbare and Kasalath (an *O. sativa* spp. indica cultivar) for resistance to an ecotype of *S. hermonthica* from Kibos, Kenya. This study revealed seven QTL for post-attachment resistance to *Striga* on chromosomes 1, 4, 5, 6, 7, 8 and 12. The two largest QTL (explaining the greatest percentage phenotypic variance in the population) were on chromosome 4 (where the resistance allele came from Kasalath) and chromosome 12 where the resistance allele came from Nipponbare. In addition, two other QTLs (on chromosome 1 and 8; Nipponbare alleles) were also identified in a study by Kaewchumnong and Price (2008) using a different F6 RIL population derived from a cross between the cultivars Bala (susceptible) x Azucena (tolerant/resistant).

In chapters 2 and 3, it has been shown that some of the upland NERICA rice cultivars exhibited strong broad-spectrum resistance to *Striga* species. A greater understanding of the genetic basis underlying this resistance is essential for identification and characterisation of the genes that are involved. The African rice species (*O. glaberrima*) is known to be well adapted to biotic and abiotic stresses and clearly contains resistance genes to *Striga* species, particularly to *S. hermonthica,* as illustrated in pot and field experiments carried out by Johnson *et al.* (1997), Kaewchumnong and Price (2008) and Cissoko *et al.* (2011) (see chapters 2 and 3). In all of these studies the *O. glaberrima* cultivar CG14 showed good resistance to *S. hermonthica* ecotypes.

In order to understand the genetic basis of resistance in African rice germplasm to *Striga* species, a mapping population derived from a cross between an *O. glaberrima* and *O. sativa* cultivar was used in order to identify QTL for resistance to *Striga*. Several populations of Chromosome Segment Substitution Lines (CSSLs), have or are being developed by Dr Mathias Lorieux and colleagues at the Rice Genetics and Genomics group at the International Center of Tropical Agriculture (CIAT, Cali, Columbia) and the Institut de Recherche pour le Developpement (IRD, Montpellier, France) through a Generation Challenge Program (GCP). This GCP aimed to develop rice genetic resources relevant to African Agriculture. CSSLs are defined as inbred lines where each line carries a single or a few chromosomal segments of a donor genome within a pure genetic background of the recurrent (recipient) parent. Good CSSL populations consist of introgression lines where the whole of the donor genome is represented in the background of an adapted cultivar (Ali *et al.*, 2010).

Three CSSL populations have been produced at CIAT by crossing three wild relative accessions, *O. barthii* (cv. IRGC101937), *O. rufipogon* (cv. IRGC105491) and *O. meridionalis* (OR44) with a tropical *O. sativa* subspecies japonica cultivar, Curinga. A further two CSSL populations have been produced by crossing the *O. glaberrima* cultivar TOG5681 with the tropical *O. sativa* (spp. indica) cultivar, IR64 (Bocco *et* *al.*, 2012) and the *O. glaberrima* cultivar MG12 with the tropical *O. sativa* (spp. japonica) cultivar, Caiapo (Gutierrez *et al.*, 2010). An aim of this chapter was to screen the MG12 x Caiapo introgression lines for resistance to *S. hermonthica*.

Many different studies have shown the importance of CSSL populations for trait improvement. Because the introgression lines contain small segments of the donor genome, any differences in the trait of interest must be due to QTL and genes present in the introgressed segments. Thus both QTL/genes of minor and major effect can be separated and identified. The lines that exhibit interesting phenotypes can then be used to generate Near Isogenic Lines (NILs) for fine mapping and identification of the genes involved. Bocco *et al.* (2012) evaluated the effects of drought on agro-morphological traits of 54 CSSLs derived from the cross between the high yielding *O. sativa* cultivar IR64 and the drought tolerant cultivar TOG5681. These authors identified some introgression lines (e.g SEN-Ll3-2 and MPL-15-3) that showed a much higher yield potential under drought conditions in the field than their parental cultivars. Additionally, using 64 CSSLs which carried *O. glaberrima* MG12 chromosomal segments in the genetic background of the *O. sativa* cultivar Caiapo, Gutiérrez *et al.* (2010) identified fourteen QTL for important agronomic traits such as plant height, tiller number per plant, grain yield and a highly significant resistance QTL to the *rice stripe necrosis virus* (RSNV). Other CSSL populations derived from crosses between cultivated and wild rice species have also demonstrated their usefulness for the identification of QTL and genes for many important traits such as seed shattering (Sanchez *et al.*, 2001) and resistance to leaf hoppers (Fujita *et al.*, 2004; Deen *et al.*, 2010). The identification of ‘novel’ QTL in wild relatives is very valuable for breeding and crop improvement as wild relatives are often excellent sources of genes that have been lost from elite cultivars during breeding (Ali *et al.*, 2010).

The aims of this chapter are to:

1. Evaluate the parental cultivars of several CSSL populations for resistance to *S. hermonthica* to determine their potential for identification of QTL for resistance.
2. Determine the effect of *S. hermonthica* on the growth and biomass partitioning of the cultivars.
3. Identify QTL underlying resistance to *S. hermonthica* (Sh-Kibos 1997) in a CSSL population derived from a cross between the *O. glaberrima* cultivar MG12 and the tropical *O. sativa* spp. japonica cultivar Caiapo.
4. Determine whether introgression lines with high levels of resistance to *S. hermonthica* (Sh-Kibos) also exhibit broad spectrum resistance against other ecotypes of *S. hermonthica* and *S. asiatica.*
5. Begin a crossing programme to develop Near Isogenic Lines (NILs) for fine mapping of *Striga* resistance genes.
6. **Materials and Methods**

### Plant materials

Selected rice cultivars CG14, MG12 and TOG5681 (*Oryza glaberrima*), Caiapo and Curinga (*O. sativa* spp. japonica), IR64 (*O. sativa* spp. indica) and three wild species, *O. barthii* (cv. IRGC101937), *O. rufipogon* (cv. IRGC105491) and *O. meridionalis* (OR44) and a CSSL population of 64 BC3DH lines (derived from cross between MG12 and Caiapo) were evaluated for resistance to *Striga* in the current study. All the rice materials were supplied by Dr Mathias Lorieux at the International Center for Tropical Agriculture (CIAT), Columbia, except for CG14 which was obtained from Africa Rice Center, Cotonou, Benin. The seeds of *S. hermonthica* and *S. asiatica* used in this study were the same as those shown in Chapter 2 (Table 2.1).

### Evaluation of post-attachment resistance of Asian, African and wild rice species and cultivars to *S. hermonthica* ecotype Sh-Kibos 1997

Rice seeds were germinated, transferred to rhizotrons and plants infected with 12.5 mg of pre-germinated *S. hermonthica* seeds (16 days after sowing) as described in section 2.2.2. Five replicates of control and infected plants were established for each cultivar. Plants were grown in a controlled environment chamber with day/night temperatures of 28 °C and 24 °C, respectively, 60% relative humidity and a 12 hours photoperiod. Irradiance was provided by a mixture (50:50) of Sylvania Metal Halide E39 POM lamps and Philips HalogenA BTT46 CL E27 lamps. Irradiance increased gradually over the first hour of the period until it reached 500 mol m-2 s-1 at plant height. Irradiance then decreased gradually during the last hour of the photoperiod. Rhizotrons were supplied with 25 ml of 40% Long Ashton nutrient solution (Hewitt, 1966) containing 2 mM of ammonium nitrate, four times each day via an automatic watering system. Twenty one days after infection, individual *Striga* seedlings were removed from the roots of each host plant in order to determine their number, length and weight as described in section 2.2.3. Rice plants were harvested and separated into roots, stem and leaves and dried for one week at 48 °C. Biomass was determined 7 days later. The effect of *Striga* on host growth was quantified by expressing the above-ground biomass of infected plants as a percentage of uninfected control biomass.

Student *t*-tests or analysis of variance (ANOVA) were used to test whether differences in resistance levels of the parents of each CSSL population were statistically significant. Statistical tests were performed using Minitab version 14.

### Mapping *Striga* resistance QTL in 64 BC3DH lines derived from a cross between MG12 (*O. glaberrima* donor parent) and Caiapo (*O. sativa* background parent)

The development of the CSSL population from a cross between Caiapo (recurrent or background parent) and MG12 (donor parent) was carried out at CIAT, Columbia as described by Gutiérrez *et al.* (2010). The population consists of 312 BC3DH lines that have been genotyped with 200 polymorphic Single Sequence Repeat (SSR) markers that are well distributed across the 12 chromosomes. A computer programme, CSSL Finder version 0.9a14 (http://mapdisto.free.fr./CSSLFinder/) was used to identify a subset of 64 lines that contained donor segments (with average size of 10 cM) which spanned the entire *O. glaberrima* genome as described in Gutiérrez *et al.* (2010). Figure 4.1 shows the graphical genotypes of the 64 selected lines.

In order to evaluate the 64 lines and the two parental cultivars (MG12 and Caiapo) for resistance to *S. hermonthica* (Sh-Kibos), plants were grown, infected and maintained as described in section 4.2.2. Four replicates of each CSSL and the two parental cultivars MG12 and Caiapo were established. Post-attachment resistance to Sh-Kibos 1997 was assessed 21 DAI by determining total *Striga* biomass on each rice plant as described in section 4.2.2. The plants were grown in four batches with replicates of each CSSL randomized within and between batches. All four batches were grown in the growth chamber at the same time but they were set up at two-day intervals to allow time for infection and harvesting of plants (Fig 4.2).

Analysis of *S. hermonthica* resistance QTL was carried out using *S. hermonthica* dry biomass data as the trait variable. Molecular marker genotypes of the CSSLs were determined by Dr Mathias Lorieux’s group (IRD-CIAT). Identification of *Striga* resistance QTL was carried out using CSSL Finder v. 09a14 (http://mapdisto.free.fr./CSSLFinder/). This programme was used to generate and display the graphical genotypes of the 30 most contrasting phenotypes (the 15 most resistant lines and the 15 most susceptible). This way, chromosomal segments were

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| Figure 4.1 Graphical genotyping of a sub-set of 64 BC3DH lines (out of 312) displaying contiguous introgressed segments from *O. glaberrima* donor parent MG12 (in brown) and *O. sativa* background parent Caiapo (in pink). The heterozygous and missing segments are dark green and dark grey, respectively, while genotype 3 and 4 representing non parental segments are light grey. These lines were evaluated for resistance to *S. hermonthica* (Sh-Kibos). The genotypes are indicated horizontally whilst the 12 chromosomes and 200 SSRs markers are displayed vertically. |

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| Figure 4.2 Screening of the interspecific lines of a CSSL population (Caiapo (*O. sativa*) x MG12 (*O. glaberrima*)) for resistance to *S. hermonthica* (Sh-Kibos 1997). (a) Controlled environment growth room used for the experiment showing different batches of plants growing in rhizotrons. (b) Close up view of rice plants growing in rhizotrons. |

analysed for their putative association with the trait by graphical genotyping, in order to determine the QTL position. Additionally, the 64 lines were analysed by ANOVA1, in order to confirm the statistical association between markers identified by graphical genotyping. Here, it is worth mentioning that, due to unbalanced sizes of the two sub-populations defined by each couple of marker alleles, the statistical properties of ANOVA1 are not optimal and may lead to identification of false positives or artefacts. Nevertheless, combining graphical genotyping and ANOVA1 allows a fairly robust QTL identification.

1. Development of materials for fine mapping of resistance genes

In order to confirm and fine map QTL identified in this study, CSSLs that contained a segment of the *O. glaberrima* genome conferring resistance to *S. hermonthica* were crossed (as the male parent) with Caiapo (the recurrent parent) to produce BC4F1 seeds (Fig 4.3). Some of these seeds were grown and plants allowed to self-pollinate to produce the BC4F2 generation. As panicles emerged from the sheath they were enclosed in pollen proof seed bags (prior to anthesis) to prevent cross-pollination. These crosses have been completed. Some of the BC4F2 seeds have been grown to produce the BC4F3 generation. In order to produce seeds, plants were grown in pots using John Innes N° 3 soil and maintained in the controlled environment growth room, as described in the section 4.2.2.

In future, seeds from both the BC4F2 and BC4F3 generations will be grown and simultaneously phenotyped for resistance to *S. hermonthica* and genotyped with SNP markers to select informative recombinants in the QTL region. In the BC4F3 generation genotyping will allow selection of recombinants that are homozygous for the region of interest. These plants will be allowed to self-pollinate to produce families of BC4F4 homozygous lines. These plants will then be evaluated for *Striga* resistance allowing fine mapping of the *Striga*-resistance QTL.

1. Extraction of DNA for molecular marker analyses

DNA was extracted from a 0.5 mm disc of leaf tissue taken from all plants to be analysed using a hole punch supplied with a Phire Plant Direct PCR kit (Thermo Scientific Inc, Finland). The small leaf disk was placed in a PCR reaction tube containing 1 X Phire Plant PCR buffer (including dNTPs and MgCl2), 0.5 µM of each

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| Figure 4.3 Production of Near Isogenic Lines (NILs) from lines of a CSSL population (*O. glaberrima* (MG12) x *O. sativa* (Caiapo)) for fine mapping of *Striga*-resistant QTL. |

forward and reverse primers for the SSR marker RM101 and Phire Hot Start II DNA Polymerase (20 µl total reaction volume). PCR amplification was carried out in Flexigene TC-500 (Techne) with the following program: 98 ºC for 5 min followed by 35 cycles of 98 ºC for 5 s, 55 ºC for 5 s, 72 ºC for 20 s with a final extension of 72 ºC for 1 min. PCR products were separated on a 3 % agarose gel at 100 V for 1 hour with a DNA Hyperladder V (Bioline, London, UK). Gels were stained with ethidium bromide and visualized using an Epi Chemi II Darkroom (UVP Ltd, Laboratory Products, UK) gel documentation system. The sequences of the forward and reverse primers for marker RM101 are: Forward primer 5’-3’, GTGAATGGTCAAGTGACTTAGGTGGC; Reverse primer 5’-3’, ACACAACATGTTCCCTCCCATGC.

## Results

### Evaluation of post-attachment resistance of Asian, African and wild rice species and cultivars to *S. hermonthica* ecotype Sh-Kibos 1997

In order to select a CSSL population to begin to map resistance to *S. hermonthica* the parents of different CSSL populations were evaluated for post-attachment resistance to Sh-Kibos. Figure 4.4 shows the biomass and number of *S. hermonthica* individuals (Sh-Kibos 1997) attached to each cultivar at 21 DAI. The different cultivars exhibited different susceptibilities to Sh-Kibos 1997. Some rice cultivars (e.g. Caiapo and Curinga) were very susceptible supporting large numbers of emerged *Striga* seedlings with more than 100 well-developed parasite plants per host root system (Fig 4.5 and 4.6). The other cultivars exhibited good levels of post-attachment resistance to Sh-Kibos 1997 although none were completely immune to the parasite. All the *O. glaberrima* cultivars, wild species, and the *O. sativa* cultivar IR64 showed good resistance and supported only a few small *Striga* seedlings on their root system (Fig 4.5 and 4.6). As the *O. sativa* cultivars Caiapo and Curinga were parents of one of the CSSL populations these populations represent excellent resources for mapping *Striga* resistant QTL. The only population where both parents were quite resistant to *S. hermonthica* (and therefore not suitable for mapping resistance QTL) was the IR64 (*O. sativa*)x TOG5681 (*O. glaberrima*) population. Thus, in order to begin to analyse the genetic basis of resistance in the *O. glaberrima* genome, the Caiapo (*O. sativa*) x MG12 (*O. glaberrima*) population was used in this study. Figure 4.7 illustrates the difference in susceptibility of these parental cultivars.

### The impact of *Striga hermonthica* on the biomass of resistant and susceptible rice cultivars

In order to assess the effect of *S. hermonthica* on the growth of the host plants, the biomass of the roots, stems and leaves of infected plants were compared to those of the uninfected control plants (Figures 4.8 - 4.10 and Table 4.1). Infection of all cultivars by *S. hermonthica* significantly reduced the biomass of roots, stems and leaves. The above ground biomass (stems and leaves) was more severely affected than the roots (Fig 4.8) in all cases. Figure 4.11 shows the relationship between the total biomass of *Striga*-infected plants as a percentage of the biomass of their respective control plants, and the amount of *Striga* biomass on the roots for each of the rice cultivars. The reduction in

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| Figure 4.4 Evaluation of post-attachment resistance of different rice species and cultivars to *S. hermonthica* (Sh-Kibos 1997). *Striga* dry biomass (a) and number of attached *Striga* seedlings (b). *Striga* plants were assessed at harvest 21 days after infection. Values represent mean ± SE of5 replicate plants. For each trait the significance of a genotype effect was calculated using student *t*-test (for the *O. sativa* x *O. glaberrima* parents) or one-way ANOVA (for the *O. sativa* x wild relative parents). Within each set of coloured bars, bars with the same letter are not significantly different. Level of significance. \*, *P < 0.05*; \*\*, *P < 0.01*; \*\*\*, *P < 0.001*. |

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| Figure 4.5 Phenotype of resistance in rice cultivars Caiapo, MG12, IR64 and TOG5681 infected with *S. hermonthica* (Sh-Kibos 1997) at 21 days after infection. Scale bar represents 5 cm. |

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| Figure 4.6 Phenotype of resistance in rice cultivars Curinga, IRGC105491, OR44 and IRGC101937 infected with *S. hermonthica* (Sh-Kibos 1997) at 21 days after infection. Scale bar represents 5 cm. |

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| Figure 4.7 The parents of the CSSL population derived from a cross between the *O. sativa* cultivar Caiapo and the *O. glaberrima* cultivar MG12 exhibit different levels of resistance/susceptibility to *S. hermonthica* (Sh-Kibos, 1997). Individual *S. hermonthica* plants taken from the roots of an individual plant of cultivar Caiapo plant fill 3 Petri dishes whereas those taken from one plant of cultivar MG12 are shown in one Petri dish. |

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| Figure 4.8 Effect of *S. hermonthica* (Sh-Kibos 1997) on the biomass of roots, stems, and leaves of different rice species and cultivars at 21 days after infection. The letters C and I indicate uninfected control and infected plants, respectively. Data presented are means ± SE of 5 replicate plants. Statistical analysis of differences between roots, stem and leaf biomass in control and infected plants are shown in Table 4.1. | | | | | |
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| Figure 4.9 Representative images of different rice species grown in rhizotrons with and without S. hermonthica (Sh-Kibos 1997) for 21 days. The letters C and I indicate uninfected control and infected plants, respectively.   |  | | --- | |  | | Figure 4.10 Representative images of different rice species grown in rhizotrons with and without *S.* *hermonthica* (Sh-Kibos 1997) for 21 days. The letters C and I indicate uninfected control and infected plants, respectively.  Table 4.1 Summary of the results of Student *t*-tests for the effect of *S. hermonthica* on the biomass of roots, stems and leaves in infected compared to control plants for wild and cultivated rice species. Significance levels:NS, not significantly different, *P > 0.05*; \*, *P < 0.05*; \*\*, *P < 0.01*; \*\*\*, *P< 0.001*. Data are shown in Figure 4.8. | | | | | | |
| **Genotype** |  | ***t-value*** | ***df*** | ***p-value*** | **Significance** |
| **Caiapo** | Root | 3.52 | 4 | 0.025 | \* |
|  | Stem | 13.23 | 4 | 0.001 | \*\*\* |
|  | Leaf | 10.55 | 3 | 0.002 | \*\* |
| **MG12** | Root | 2.24 | 4 | 0.088 | NS |
|  | Stem | 5.36 | 4 | 0.006 | \*\* |
|  | Leaf | 6.44 | 4 | 0.003 | \*\* |
| **IR64** | Root | 3.55 | 2 | 0.071 | NS |
|  | Stem | 6.89 | 2 | 0.020 | \* |
|  | Leaf | 9.24 | 2 | 0.012 | \* |
| **TOG5681** | Root | 1.94 | 2 | 0.192 | NS |
|  | Stem | 5.46 | 3 | 0.012 | \* |
|  | Leaf | 3.35 | 3 | 0.044 | \* |
| **Curinga** | Root | 5.83 | 3 | 0.010 | \*\* |
|  | Stem | 8.8 | 3 | 0.003 | \*\* |
|  | Leaf | 8.3 | 4 | 0.001 | \*\*\* |
| **IRGC105491** | Root | 0.58 | 2 | 0.628 | NS |
|  | Stem | 4.72 | 2 | 0.042 | \* |
|  | Leaf | 1.8 | 3 | 0.169 | NS |
| **OR44** | Root | 2.4 | 4 | 0.075 | NS |
|  | Stem | 4.69 | 2 | 0.042 | \* |
|  | Leaf | 4.59 | 3 | 0.019 | \* |
| **IRGC101937** | Root | 0.28 | 4 | 0.790 | NS |
|  | Stem | 10.78 | 4 | 0.001 | \*\*\* |
|  | Leaf | 3.99 | 3 | 0.028 | \* |
| **CG14** | Root | 2.21 | 2 | 0.158 | NS |
|  | Stem | 5.1 | 3 | 0.015 | \* |
|  | Leaf | 3.99 | 3 | 0.028 | \* |

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| Figure 4.11 Relationship between total biomass of infected plants as a percentage of control plants and the amount of Sh-Kibos 1997 biomass on the root system of host plant. Rice cultivars are indicated in different colours with parents of cross in the same colour (dotted for the recurrent parent). Data presented are means ± SE of 5 replicate plants. |

host biomass of infected compared to control plants at harvest was at least 40% for all rice cultivars when compared with controls despite the fact that the *O. glaberrima* cultivars CG14, MG12 and TOG5681 and the wild relatives all showed good levels of resistance to the parasite. The most severely affected cultivars were the very susceptible cultivars Caiapo and Curinga, which lost 72% and 78% of their biomass respectively. As observed in the experiment to determine the effect of *S. hermonthica* on the biomass of the NERICA cultivars (Chapter 2), not all cultivars were affected to the same extent when infected by a similar parasite biomass, i.e. they exhibited different levels of tolerance to the parasite. In this experiment IR64, CG14, MG12, IRGC105491, TOG5681 and *O. meridionalis* (OR44) supported similar amounts of parasite biomass on their roots but displayed reductions in host biomass of 50%, 51%, 57% 44%, 51% and 75% respectively when compared to their control plants.

### Screening of 64 CSSLs and their parents, Caiapo and MG12, for resistance to *S. hermonthica* (Sh-Kibos 1997) and identification of QTL underlying resistance

Figure 4.12 shows the biomass of *S. hermonthica* (Sh-Kibos 1997) attached to the roots of Caiapo, MG12 and the 64 interspecific CSSLs at 21 DAI. The introgression lines varied widely in their post-attachment resistance to Sh-Kibos 1997 and were ranked from the most susceptible to the most resistant. Some CSSLs were very susceptible to Sh-Kibos 1997 witnessed by the high biomass of *Striga* seedlings on the roots (Fig 4.12). For example, some CSSLs (e.g. 86, 181 and 186) were more susceptible than the recurrent *O. sativa* parent Caiapo (transgressive segregation). Other CSSLs showed levels of susceptibility that were intermediate between Caiapo and MG12. However, only two CSSLs (149 and 194) showed the same very strong resistance phenotype as the donor parent MG12 as shown by CSSL 194 in Fig 4.13. These two CSSLs supported very few attached *Striga* seedlings and low amounts of parasite biomass (Fig 4.12 and 4.13).

Figure 4.14 shows the graphical genotypes of 30 lines that displayed extreme phenotypes for resistance to Sh-Kibos 1997 based on *Striga* dry biomass (15 smallest and 15 highest values) using CSSL Finder program. The graphical genotyping together with a QTL analysis identified a major QTL located on chromosome 12 with an *F*-test value of more than 10. The two resistant CSSLs 149 and 194, shared only one chromosomal segment of the donor parent MG12 in common on chromosome 12. This

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| Figure 4.12 Screening of 64 CSSLs and their parents Caiapo and MG12 for resistance to *S. hermonthica* (Sh-Kibos 1997). *Striga* dry biomass was assessed at harvest 21 days after infection. Asterix indicate the most resistant CSSLs 149 and 194. Data shown are means ± SE. Genotype effect was highly significant (one-way ANOVA, *P* < 0.001) for *Striga* dry biomass. |

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| Figure 4.13 Phenotype of resistance to *S. hermonthica* (Sh-Kibos 1997) in cultivars Caiapo (susceptible) and MG12 (resistant) and CSSLs 194 at 21 days after infection (in rhizotrons). |

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| Figure 4.14 Identification of the major resistance QTL to *S. hermonthica* (Sh-Kibos 1997) on chromosome 12 using CSSL Finder. *Oryza glaberrima* donor parent MG12 segments are indicating in black in *O. sativa* background parent Caiapo in light grey. The heterozygous and missing segments are dark grey and dark blue, respectively, while genotype 3 and 4 representing non parental segments are white. On the right, red bars indicate the 15 smaller and 15 higher values of *Striga* dry biomass with corresponding CSSLs named on the left. The 2 most resistant lines 149 and 194 shared the donor *O. glaberrima* segment in common located within the red rectangle. The *F*-test (ANOVA1) is considered significant when its value is superior to 10. |

QTL has been designated as STR12.1 and explains at least 80% of the variation for resistance to *S. hermonthica* (Sk-Kibos 1997) in this CSSL population and suggests that resistance is likely to be due to a single gene or a few genes of major effect.

### How broad spectrum is the resistance identified in the CSSLs and their parental lines?

In order to determine whether the resistance QTL on chromosome 12 was broad spectrum or confined to the ecotype of *S. hermonthica* collected in Kibos in 1997, the two most resistant CSSLs 149 and 194 and their two parent cultivars MG12 and Caiapo were evaluated for resistance to two additional ecotypes of *S. hermonthica* (Sh-Kouto, collected from rice in 2009 and Sh-Kibos also collected in 2009) and to two ecotypes of *S. asiatica* (Sa-USA and Sa-Kyela). See Table 2.1 (Chapter 2) for details.

Figure 4.15 presents the dry biomass (a), number (b) and average length (c) of attached *Striga* seedlings of each *Striga* ecotype on the two CSSLs and their parents. A similar pattern of resistance/susceptibility to all of the *S. hermonthica* and *S. asiatica* ecotypes was observed in the parental cultivars. MG12 showed good resistance to all *Striga* ecotypes whilst Caiapo was susceptible to all ecotypes. For example there were approximately 100 well-developed parasite seedlings per root system for both Sh-Kibos (1997) and Sh-Kibos (2009) and over 200 parasite seedlings per root system for Sa-USA (Fig 4.15b and 4.15c). The two CSSLs 194 and 149 displayed the same strong post-attachment resistance to both ecotypes of Sh-Kibos(1997 and 2009) and to Sa-USA as MG12. The two CSSLs also showed some resistance to Sh-Kouto (2009) although it was not as strong as the resistance exhibited against the Kibos ecotypes of *S. hermonthica*. The total biomass and size of *Striga* seedlings attached to CSSL194 and 149 were less than on Caiapo (Fig 4.15a and 4.15c) but the number of attached seedlings was similar (Fig 4.15b).

Interestingly, a different pattern of resistance / susceptibility of the CSSLs to the *S. asiatica* ecotype from Kyela, Tanzania was observed. MG12 was strongly resistant and Caiapo was susceptible to Sa-Kyela (as seen with the other ecotypes) but both CSSLs 194 and 149 were as susceptible to this ecotype of *Striga* as Caiapo (Fig 4.15). In this study the biomass and number of *S. asiatica* attachments on Caiapo was quite low. In order to determine whether this was due to a difference in virulence of the ecotype or to the lower germination of seeds (approximately 50%; data not shown), an additional

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| Figure 4.15 Resistance response of Caiapo and MG12, and the most resistant CSSL149 and 194 to a range of *S. hermonthica* and *S. asiatica* ecotypes 21 days after infection. *Striga* dry biomass (a), number (b) and average length (c) of *Striga* seedlings. *Striga* plants were assessed 21 days after infection. Data presented are means ± SE. For the three traits, genotype effect is shown as: \*, *P < 0.05*; \*\*, *P < 0.01*; \*\*\*, *P < 0.001* (One-way ANOVA for each *Striga* ecotype). Mean with different letter differ significantly (Tukey multiple comparisons test, *P < 0.05*). |

experiment was carried out but 25 mg of germinated seeds were used to infect each plant (two times the normal amount used). The results of this screen are shown in Figure 4.16. The number and biomass of *Striga* individuals attached to the susceptible cultivar Caiapo was almost double that of the previous experiment. However the pattern of resistance on Caiapo, MG12 and the two CSSLs was the same as seen in the first study. There was no significant difference in the biomass of *Striga* on Caiapo and CSSLs 194 and 149; all were very susceptible. MG12 remained very resistant to Sa-Kyela. This suggests that the resistance observed in MG12 to this ecotype is likely to be controlled by different loci to those controlling resistance to the other ecotypes of *S. hermonthica* and the ecotype of *S. asiatica* from USA.

### Development of Near Isogenic Lines (NILs) for fine mapping studies

In order to decrease the size of introgressed MG12 target segment involved in the *Striga* resistance QTL on chromosome 12, the two resistant CSSLs (194 and 149) were crossed with the *O. sativa* cultivar Caiapo (as the female or male in different crosses) to produce the BC4F1 generation as shown in Table 4.2. In total 600 seeds were produced from many independent crosses of CSSL149 (female) with Caiapo (male) and 300 from crosses between CSSL194 (female) and Caiapo (male). Sixty four and 4 seeds were produced from crosses between CSSLs 149 and 194 (as the male parent) and Caiapo (as the female parent), respectively (in these crosses the lower number of seeds produced was due to fewer crosses not to problems of sterility).

In order to check whether crosses had been successful, seeds of BC4F1 generation were grown and analysed with molecular marker RM101. The SSR marker RM 101 is associated with the *Striga* resistance QTL on chromosome 12 and is polymorphic between Caiapo and MG12 (Fig 4.17). As seen by the amplification products, MG12 and Caiapo have different alleles at this marker and CSSLs 194 and 149 have the MG12 allele (Fig 4.17). All seeds of the BC4F1 generation should be heterozygous and have both parental alleles if the cross has been successful. Figure 4.17 shows that some plants were heterozygous indicating a successful cross whilst others just have the Caiapo allele. Heterozygous individuals were selected and self-pollinated to produce the BC4F2 generation (Table 4.2).

In the BC4F2 generation most panicles were not fertile and did not produce seeds. From the 13 plants (5 from Caiapo x CSSL149 and 8 from Caiapo x CSSL194) that

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| Figure 4.16 Dry biomass (a) and number (b) of *S. asiatica* (Sa-Kyela 2009) seedlings collected from parental lines Caiapo and MG12, CSSLs 194 and 149 at 21 days after infection. Data presented are means ± SE of 6 replicate plants. One-way ANOVA indicated that the genotype effect is significant (*P < 0.05*) for each trait. The letters above each bar indicate the different significance groups after Tukey’s pairwise comparisons. |

Table 4.2 Generation of Near Isogenic lines (NILs) for fine mapping of *Striga* resistance QTL.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Number of seeds produced** | | |
| **Cross and self-pollination** | | **BC4F1** | **BC4F2** | **BC4F3** |
| **Female ♀** | **Male ♂** |  |  |  |
| BC3F1DHCSSL149 | Caiapo | 600 **(5)** | - | - |
| BC3F1DHCSSL194 | Caiapo | 300 **(8)** | - | - |
| **Self-Pollination**  BC4F1DHCSSL149/Caiapo |  | - | 45 **(45)** | - |
| BC4F1DHCSSL194/Caiapo |  | - | 06 **(6)** | - |
| BC4F2DHCSSL149/Caiapo |  | - | - | >2000 |
| BC4F2DHCSSL194/Caiapo |  | - | - | >5000 |
| **Female ♀** | **Male ♂** |  |  |  |
| Caiapo | BC3F1DHCSSL149 | 64 **(4)** | - | - |
| Caiapo | BC3F1DHCSSL194 | 04 **(4)** | - | - |
| **Self-Pollination**  Caiapo/BC4F1DHCSSL149 |  | - | 62 **(0)** | - |
| Caiapo/BC4F1DHCSSL149 |  | - | 22 **(0)** | - |

Numbers in brackets indicate the number of plants allowed to self-pollinate to produce the next generation.

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| Figure 4.17 Amplification products of SSR marker RM101 for the rice cultivars MG12 (*O. glaberrima*) and Caiapo (*O. sativa*) and for CSSL149 (interspecific introgression line). MG12 and Caiapo show different alleles at this marker. The BC4F1 progeny of crosses between Caiapo and CSSL 149 are also shown. Progeny with both alleles result from a successful cross. Hypperladder V (Bioline London UK; V) was used as size standard (number in bp). R = good resistance, S = susceptible. |

were self-pollinated only 51 seeds (45 from Caiapo x CSSL149 and 6 from Caiapo x CSSL194) were produced in total. All these seeds were grown and self-pollinated to produce the BC4F3 generation. In this generation there were no fertility problems and large numbers of seeds were produced (Table 4.2).

### Is the resistance found in the NERICA rice cultivars associated with the QTL on chromosome 12?

The SSR marker RM101 is located within the QTL region on chromosome 12 of the *O. glaberrima* cultivar MG12. As described above, MG12 and Caiapo have different alleles at this marker. In order to determine whether the *O. glaberrima* and *O. sativa* parents of the NERICA cultivars have the same alleles as MG12 and Caiapo respectively, an analysis of the parents and all 18 NERICA cultivars was carried using marker RM101. Figure 4.18 shows the amplification products of RM101 for these cultivars. CG14 has the same allele (size 275 bp) as MG12. However all the *O. sativa* parents have also the same allele (size 275 bp) as MG12 at RM101, i.e. the marker is monomorphic (Fig 4.18). Some of the NERICA cultivars have the same allele as the parents but some have a different allele (size 325 bp) at this marker independent of the *O. sativa* parent (Fig 4.18a, 4.18b and 4.18c). Interestingly there is an exact correlation between the presence of the ‘MG12 allele’ and resistance to *S. hermonthica*. All NERICA cultivars that have this allele show good resistance to the parasite whereas those that have the non-parental allele are susceptible (Fig 4.18 and Fig 2.1).

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|  |
| Figure 4.18 Amplification products of SSR marker RM101 for the 18 upland NERICA rice cultivars and their parents; (a) shows the NERICA cultivars 1 – 11 which derived from cross between CG14 (*O. glaberrima*) and WAB56-104 (*O. sativa*); (b) shows the NERICA cultivars 12, 13 and 14 from cross between CG14 (*O. glaberrima*) and WAB56-50 (*O. sativa*) and (c) shows the NERICA cultivars 15-18 from cross between CG14 (*O. glaberrima*) and WAB181-18 (*O. sativa*). Hypperladder V (Bioline London UK; V) was used as size standard (number in bp). R = good resistance, I = intermediate susceptibility, S = susceptible. |

## Discussion

### How resistant and/or tolerant are the parent cultivars of different inter-specific rice CSSL populations to *Striga hermonthica*?

In order to understand the molecular genetic basis of resistance to *Striga* species, the parent cultivars of several different rice CSSL populations were screened to determine their susceptibility/resistance to *S. hermonthica* (Sh-Kibos) and thus their suitability for mapping *Striga*-resistance QTL. Ideally parents of mapping populations should show clear differences in the trait of interest. In this study, the three wild relative accessions, *O. rufipogon* (IRGC105491), *O. meridionali*s (OR44) and *O. barthii* (all donor parents in CSSL populations developed or under development at IRD-CIAT) showed very good levels of post-attachment resistance to *S. hermonthica* (Sh-Kibos) whilst the recurrent *O. sativa* parent (Curinga) was very susceptible. The CSSL populations derived from these parental crosses therefore represent excellent resources for the identification of *Striga* resistance QTL in future studies. Wild species of rice contain more genetic variability for important traits such as biotic and abiotic stress resistance and yield characteristics when compared to cultivated rice which have lost alleles during domestication (Sun *et al.*, 2001; Jena, 2010). For example, several CSSL populations and BC2F5 introgression lines using *O. rufipogon* IRGC105491 as donor parent and *O. sativa* ssp. indica Zhenshen 97B as recurrent parent have been developed with the objective of improving yield-related traits, drought tolerance and grain quality (McCouch *et al.*, 2007). Similarly Cheema *et al.* (2008), and Xie et *al.* (2006) identified and introgressed useful alleles associated with the yield-enhancing genes from *O. rufipogon* (IRGC105491) into adapted cultivated rice species. Chen *et al.* (2010) identified QTL for white-backed plant hopper resistance from an interspecific cross (*O. sativa* x *O. rufipogon*), and Prasad and Eizenga (2008) identified rice sheath blight resistance in accessions of *O. nivara*, *O. barthii* and *O. meridionalis*. There are also many examples of the discovery and use of QTL/genes from wild rice species for resistance to abiotic stresses (drought, iron toxicity and salinity) (reviewed in Ali *et al.* (2010)).

In this study, the *O. glaberimma* cultivars CG14, MG12 and TOG5681 exhibited much stronger levels of resistance to *S. hermonthica* (Sh-Kibos) than some Asian cultivated rice species (e.g. Caiapo and Curinga). This result is consistent with the previous findings of Johnson *et al.* (1997) and Kaewchumnong and Price (2008), where *O. glaberrima* cultivars were generally much more resistant to *Striga* under field and laboratory conditions respectively, than *O. sativa* cultivars. The only *O. sativa* cultivar that showed good resistance to *S. hermonthica* (Sh-Kibos) in the current study was IR64. However, this cultivar showed high susceptibility to an ecotype of *S. hermonthica* from Samanko in Mali-West Africa in the study of Kaewchumnong and Price (2008) illustrating differences in host-parasite specificity. A key aim of this current PhD study was to begin to identify the molecular basis of resistance to *S. hermonthica* in the inter-specific NERICA cultivars. Clearly the CSSL population derived from the cross between *O. glaberrima* (TOG5681) and *O. sativa* (IR64) was not suitable for this purpose as both parents exhibited good levels of resistance to *S. hermonthica*. However the CSSL population derived from the cross between *O. glaberrima* (MG12) and *O. sativa* (Caiapo) was ideal for this purpose as the *O. glaberrima* parent was much more resistant than the *O. sativa* parent.

### Identification of QTL underlying resistance in the CSSL population derived from a cross MG12 x Caiapo

In this current study, a major QTL underlying post-attachment resistance to parasitic weed *S. hermonthica* (Sh-Kibos 1997) was identified in the mapping population of CSSLs generated from the resistant cultivar MG12 (*O. glaberrima*) and the susceptible cultivar Caiapo(*O. sativa* ssp. japonica). This significant QTL (designated STR12.1) was located on chromosome 12 and explained a large proportion (at least 80%) of the resistance in MG12 to Sh-Kibos 1997. This strongly suggests that one (or a very small number) of genes on chromosome 12 conferred the majority of the resistance to this ecotype of *Striga*. The fact that some of the other CSSLs were slightly more resistant to *S. hermonthica* than Caiapo also suggests that there are genes of minor effect in other regions of the *O. glaberrima* genome. However, the finding that one or a few genes are likely to be responsible for the majority of the resistance is very important from a breeding perspective. Interestingly, the location of QTL STR12.1 overlapped with a *Striga*-resistance QTL on chromosome 12 of Nipponbare discovered in mapping study by Gurney *et al.* (2006). These authors conducted a QTL analysis of post-attachment resistance to the same *S. hermonthica* ecotype (Sh-Kibos 1997) in a Backcross Inbred Line (BIL) population consisting of 98 BC1F14 lines derived from a cross between Nipponbare (*O. sativa* spp. japonica) and Kasalath (*O. sativa* spp. indica). At present, both QTL are too large to determine whether the gene(s) responsible for resistance are the same but fine mapping studies are underway to aid identification of candidate resistance genes. It is interesting to note that the region of chromosome 12 where QTL STR12.1 is located also contains QTL and resistance genes against other biotic stresses. For example Boisnard *et al.* (2007) identified a QTL located in a very similar position on chromosome 12 for partial resistance to *rice yellow mottle virus* (RYMV). They showed by using *in silico* studies and the evaluation of Near Isogenic Lines (NILs) that genes encoding eukaryotic translation initiation factors (eIF) were good candidates for a role in the resistance to this virus.

In our current study, development of resources for fine mapping studies was carried out by crossing the 2 resistant CSSLs 149 and 194 (both BC3F1 lines) as the donor parent with Caiapo as the female parent to produce advanced backcrossed lines (BC4F1) followed by several generations of self-pollinations (3 in total) to successively generate BC4F2, BC4F3 and BC4F4 lines as indicated in Figure 4.3. During this process, reduced viability and fertility of BC4F1 hybrids was noticed at BC4F2 level (as shown by the low number of seeds obtained - Table 4.2). This is the consequence of interspecific sterility barriers that occur during crossing between cultivars of *O. sativa* and *O. glaberrima* (Sano, 1990; Li *et al.*, 2008; Deng *et al.*, 2010; Garavito *et al.*, 2010). These authors have shown that the sterility barrier is due to the interaction of several rice sterility loci, but most importantly the complex locus S1 on chromosome 6 of *O. glaberrima*. This barrier affects both male and female fertility. However when the seeds of the BC4F2 generation that were obtained, were self-pollinated the following generations were fertile and produced a lot of seeds as reported in studies by Ghesquiere *et al.* (1997) and Gutierrez *et al.* (2010). In the future, the BC4F3 or BC4F4 lines (developed after our backcross and self-pollination process) will be genotyped with SNP markers for the presence of target segments (*Striga* resistance) from the donor parent MG12 and for their Caiapo genetic background. Lines carrying STR12.1 target regions will be phenotyped to test their level of resistance to *Striga* and will serve as valuable source for gene detection and development of improved cultivars carrying *Striga* resistance for breeding programmes.

The characterisation of the genes underlying the *Striga* resistance QTL STR12.1 using genetic and genomic approaches (such as fine mapping, identification of candidate genes and positional cloning) will be important for our understanding of the molecular genetic basis of host defence mechanisms to *Striga* species in general and *S.* *hermonthica* in particular and for breeding strategies that aim to pyramid different resistance genes that are effective against different *Striga* ecotypes into adapted germplasm. The way in which QTL STR12.1 is used in breeding strategies depends to some extent on whether the underlying gene(s) confer resistance to many ecotypes of *Striga* or just to the ecotype Sh-Kibos. In order to determine this, the resistance QTL found in our study was tested to determine whether it confers resistance to other *Striga* ecotypes and species collected from different locations in Africa in different years.

### How broad spectrum is QTL STR12.1 against different *Striga* ecotypes

In this current study, post-attachment resistance was quantified in MG12, Caiapo and the two resistant CSSLs 149 and 194 against three ecotypes of *S. hermonthica* and two of *S. asiatica* in order to determine whether the resistance QTL STR12.1 provided broad-spectrum resistance or was specific to one ecotype of the parasite. The result has shown that the susceptibility of the two parental cultivars MG12 and Caiapo was confirmed for all ecotypes of *Striga* species tested; cultivar MG12 (*Oryza glaberrima*) exhibited very good resistance whilst the *O. sativa* cultivar Caiapo was highly susceptible. Interestingly, the two CSSLs 149 and 194 showed good resistance to both of the ecotypes of *S. hermonthica* collected from the Kibos region of Kenya in 1997 and 2009, despite the long time difference between collections. The CSSLs also showed good resistance to the ecotype of *S. asiatica* from the USA indicating that the resistance is effective against a different *Striga* species and ecotypes and suggesting that a similar genetic basis may be involved in the resistance. However, although the CSSLs also showed some resistance to another ecotype of *S. hermonthica* collected in Kouto, Côte d’Ivoire (Sh-Kouto) the resistance was not as strong as that observed against the other *S. hermonthica* ecotypes. It is interesting to note that this is the ecotype that was collected from NERICA 1 where the farmers had observed an increase in virulence of this ecotype against NERICA 1 over a period of several years of growing this normally quite resistant NERICA cultivar (Cissoko *et al.*, 2011, chapter 2). The fact that the two CSSLs are also more susceptible to this ecotype of *S. hermonthica* may suggest that the resistance in NERICA 1 may be due in part to genes located on chromosome 12. However, a much larger screen of different *Striga* ecotypes is required to identify different patterns of resistance to ecotypes of *S. hermonthica*.

The two CSSLs displayed a completely susceptible interaction with the ecotype of *S. asiatica* from Kyela-Tanzania, thus STR12.1 is not effective against this ecotype of *Striga*. However, the parental cultivar MG12 was still very resistant to this *Striga* ecotype suggesting that the resistance is governed by other genetic loci within the donor genome of *O. glaberrima*. The *S. asiatica* ecotype from Kyela is likely to be genetically very different from the other ecotypes tested in this study as it came from a region where only 2-3 cultivars of rice have been grown for many years (Rodenburg pers. comm.). It is possible therefore that the virulence genes within this population differ markedly from those of the other populations tested. This emphasizes the need to understand the population genetics of virulence in *Striga* in relation to host-parasite specificity as suggested by Huang *et al.* (2012).

These results confirm that the identification and transfer of multiple QTL/genes underlying post-attachment resistance mechanisms against different species and ecotypes of *Striga* in the elite cultivated rice, is likely to be necessary to insure durable host resistance over the time. They also illustrate that additional resistance QTL/genes could be identified in other defined donor segments or CSSLs of the same mapping population (MG12 x Caiapo) and confirms the powerfulness, usefulness and efficiency of these genetic resources.

### Is the resistance found in the NERICA rice cultivars due, at least in part to QTL STR12.1?

The NERICA rice cultivars are *O. sativa* x *O. glaberrima* inter-specific cultivars. One of the reasons for using the CSSL population derived from a cross between MG12 and Caiapo was to maximise the chance of identifying *Striga* resistance QTL that may also be relevant to the resistance seen in some of the NERICA cultivars to *S. hermonthica* and *S. asiatica* ecotypes. The SSR marker RM101 is within the peak area of QTL STR12.1 and is polymorphic for MG12 and Caiapo. It was therefore used as a marker of the region of chromosome 12 associated with the resistance QTL. I aimed to test the hypothesis that CG14 and the *O. sativa* parents of the NERICA cultivars would be polymorphic at this marker and that the more resistant NERICA cultivars would possess the resistance allele at this marker.

The results of the analysis were unexpected. This analysis revealed that NERICA parents CG14 (*O. glaberrima*) and WAB56-104, WAB56-50 and WAB181-18 (*O. sativa*) were monomorphic for this marker that ran at the same position on the gel as the MG12 allele (Fig 4.18a, b). Thus RM101 is not a suitable marker for analysing differences in this region of chromosome 12 in the NERICA cultivars. As RM101 was monomorphic between the NERICA parents, it would be expected to be monomorphic in the NERICA cultivars. However some NERICA cultivars displayed the parental allele whilst others possessed a new non-parental allele that ran at a different position on the gel. This strongly suggests that at some point during the development of the NERICA cultivars outbreeding has occurred and is consistent with the study of Semagn *et al.* (2007). These authors carried out a detailed molecular profile (using 130 SSR markers) of all the breeding lines of NERICAs 1-7 to examine the relative contribution of each of the parent to the genomes of these inter-specific cultivars and to determine the extent of any outbreeding. They discovered that 83% of the lines contained non-parental alleles that contributed an average of 2.7% of the genome. It is interesting that in this study the same non-parental allele was present in some NERICA cultivars that derived from each of the three original crosses (CG14 x WAB56-104 or WAB56-50 or WAB181-18).

A very interesting observation in this study was that allele of RM101 present in all the NERICA parent cultivars and MG12 was also present in all of the NERICA rice cultivars that were identified as being most resistant to *Striga* and absent in those that were very susceptible. This was an exact correlation; those cultivars that possessed the non-parental allele at RM101 were very susceptible. This suggests therefore that at least some of the resistance seen in the most resistant NERICA cultivars may well be due to genes present within the QTL STR12.1 region. A much more detailed molecular marker analysis profile (or sequencing) of this region of chromosome 12 in some of these cultivars is required to understand the genetic nature of the resistance in this particular chromosomal region.

# Chapter 5

# Synopsis and General Discussion

**5.1 General Discussion**

Problems with parasitic weeds in rice are becoming more critical and more widespread in Africa. Parasitic weed species, particularly *Striga,* affect low income subsistence upland farmers in SSA and cause severe losses in yield of up to 80% in highly infested areas (Gethi *et al.*, 2005). The most economically important and widespread species are *Striga hermonthica* and *S. asiatica*. Many of the control strategies that are used to fight against both *Striga* species have had limited success in terms of efficiency and affordability and do not meet the expectations of subsistence farmers who would like a sustainable control strategy which improves their crop production. Therefore, the use of resistant and tolerant cultivars as part of an integrated control strategy seems to be a promising, low cost strategy, provided that the resistance is durable and broad-spectrum. Some resistant rice cultivars have been identified previously (for a review see Scholes and Press, 2008) but were not well adapted to African upland rice growing environments and there was little understanding of the underlying resistance mechanisms at molecular genetic level.

The introduction and adoption of the high-yielding, interspecific NERICA rice cultivars developed by Africa Rice Center has boosted rice expansion and production in SSA (Diagne *et al.*, 2006; Somado *et al.*, 2008; Wopereis *et al.*, 2008). Thus, farmers working in upland production environments are increasingly replacing maize, and even sorghum, with NERICA rice (Lamo *et al.*, 2010). However, as many of these cereal producing areas are infested by the parasitic weeds *S. asiatica* or *S. hermonthica* rice production is increasingly limited by these parasites ([Rodenburg *et al.*, 2010](#_ENREF_24)) and there is little information available about the resistance or tolerance of the different NERICA cultivars to different ecotypes of the two major *Striga* species. Thus, it is not possible to advise farmers about which cultivars may be the most resistant and suitable for areas infested with these parasitic weeds.

Thus the main aims of this thesis were firstly to determine whether the NERICA cultivars showed different levels of resistance to different *Striga* species and ecotypes under controlled environment and field conditions and secondly to begin to identify QTL underlying post-attachment resistance to *Striga* using a CSSL population derived from a cross between a biotic stress-resistant African (*O. glaberrima*) and a high-yielding Asian (*O. sativa*) rice species in order to be able to select stable, durable QTL for maker-assisted breeding programmes and development of resistant cultivars.

**5.2 How resistant are the NERICA cultivars to *Striga* ecotypes and species; implications for African rice farmers**

The work presented in chapters 2 and 3 reports the first systematic study of the susceptibility of all 18 upland NERICA cultivars and their parents to different ecotypes of *Striga hermonthica* and *S. asiatica* when screened under controlled environment conditions at University of Sheffield and at two field locations in Africa (Kyela, Tanzania and Mbita Point, Kenya). This has allowed us to determine both the genetic potential for resistance to different *Striga* ecotypes and also the impact of variable environmental conditions on the expression of resistance.

Different NERICA cultivars exhibited different levels of post-attachment resistance and tolerance against ecotypes of *Striga hermonthica* and *S. asiatica* when screened under controlled environment conditions at University of Sheffield. Some of the cultivars showed very good levels of resistance to many ecotypes of *S. hermonthica* and *S. asiatica* (characterised by a few small parasite attachments) (e.g. NERICAs 1, 2, 5, 10) and in some cases were even more resistant than their African parent CG14 (None of the NERICA cultivars or their parents showed complete immunity to the *Striga* ecotypes used in the study). Other NERICA cultivars were very susceptible to all the ecotypes of *Striga* tested and some cultivars showed good resistance to one or two ecotypes of *Striga* but not to all. The latter case is similar to a study by Harahap *et al.* (1993) where two *O. sativa* ssp. indica cultivars IR38547-B-B-2-2 and IR47697-4-3-1 showed no emergence of parasites at two sites in Kenya but were heavily infested at a site in Côte d’Ivoire. This range of responses of the NERICA cultivars was expected because of the enormous genetic variation that exists within an ecotype of *S. hermonthica* (Huang *et al.*, 2012) and between ecotypes from different geographical locations (Botanga *et al.*, 2002), resulting in different degrees of parasite population virulence. Much of the genetic variation is likely to be due to the outbreeding nature of S*. hermonthica* (Bharathalakshmi and Musselman, 1990) and to the longevity of the *Striga* seed bank (greater than 30 years).

The genetic variation present in the *Striga* seed bank also has implications for the evolution of virulence on previously resistant cultivars as illustrated by NERICA 1. Even though NERICA 1 was resistant to all ecotypes of *S. hermonthica* (from Kenya and Sudan) and *S. asiatica* ecotypes from USA and Kyela, Tanzania it was susceptible to the ecotype of *S. hermonthica* from Kouto, Côte d’Ivoire (chapter 2). This ecotype was collected from NERICA 1 growing in a farmer’s field. This farmer had been growing NERICA 1 in the field continuously for more than 5 years and strongly suggests that virulence had evolved and built up in the local *Striga* population (seed bank) over a period of time. Our findings highlight the necessity of understanding the genetic basis of host-parasite-specificity and of the need to identify multiple resistance genes and to pyramid them in cultivars to increase the durability of resistance, a key area for future studies.

It is very important for farmers that some of the NERICA cultivars showed good broad-spectrum resistance to a range of *S. hermonthica* and *S. asiatica* ecotypes. It is also interesting that such cultivars also displayed different phenotypes of resistance suggesting that multiple mechanisms may contribute to the resistance observed (Cissoko *et al.*, 2011 and chapter 2). The main phenotype of resistance observed in the incompatible interactions was similar to that of the highly resistant *O. sativa* cultivar Nipponbare where parasites died soon after attachment to the host plant and was characterised by necrosis and an inability to penetrate the endodermis of the host root (Gurney *et al.*, 2006). In many of the resistant interactions it also took the parasite longer to cross the endodermal barrier and parasites remained small. This multi-form resistance in some of the NERICA cultivars is an advantage as it could improve the stability of resistance and its durability if these mechanisms of resistance are associated with different genes.

In order to determine whether the resistant ranking of the cultivars under controlled environment conditions has any predictive value for the performance of the cultivars in the field and to explore ecotype x host genotype x environment interactions, two field trials were conducted over two crop seasons at different locations in Africa. These studies revealed that the traditionally grown ‘local’ rice cultivars were severely infested with the local *Striga* ecotypes when compared to the NERICA cultivars. However, despite high infestation levels they showed slightly better yield compared to newly introduced NERICA cultivars (data not shown). This was particularly true at Kyela in Tanzania (hot-spot for *S. asiatica*) where farmers had grown the same cultivars for many years. However, the use of cultivars that support large amounts of *Striga* is problematic because they contribute to the *Striga* seed bank. Many of the resistant NERICA cultivars identified in our laboratory screens displayed good resistance in the field under both natural and artificial *Striga* infestation whilst the susceptible cultivars showed high number of emerged parasites and some cultivars displayed different responses to *Striga* ecotypes in the field. The rhizotron-screening system thus appears to be an excellent, tool for the quantification of post-attachment resistance under controlled environment conditions and also has some relevance for assessing the likely resistance of particular cultivars in the field (especially when using the same ecotype of *Striga*).

However, perhaps the most important outcome of these studies is that we are already able to recommend/advise farmers about which NERICA cultivars are likely to be most resistant to *Striga* species in areas where *Striga* is becoming an important limitation to rice production. In addition, good levels of resistance to *Striga* will be registered in the passport data of NERICA rice cultivars as a new characteristic among any others. Based on our findings, good information will be provided for the release of NERICA cultivars in different agro-ecological areas.

**5.3 Identification of QTL underlying post-attachment resistance to *Striga* using a CSSL population derived from a cross between a biotic stress-resistant African (*O. glaberrima*) and a high-yielding Asian (*O. sativa*) rice species**

Our results have clearly demonstrated that wild relatives of rice and the cultivated African rice species *O. glaberrima* contain valuable sources of *Striga* resistance genes that could be exploited for rice breeding programmes in Africa. All the cultivars/ accessions of *O. glaberrima, O. rufipogon, O. barthii* and *O. meriodionalis* investigated in this study exhibited very good resistance to *Striga*. This is particularly interesting as these accessions are the parents of CSSL populations thus providing excellent resources for the identification of different *Striga*-resistance genes in the future. Rich *et al.* (2004), Wilson *et al.* (2000) and Gurney *et al.* (2003) also screened accessions of wild relatives of sorghum, millet and maize respectively, and reported that many exhibited excellent resistance to *Striga* species.

Some studies have identified quantitative resistance for *Striga* in cereal crops such as sorghum (Haussmann *et al.*, 2004; Grenier *et al.*, 2007) and rice (Gurney *et al.*, 2006; Swarbrick *et al.*, 2009) using RILs and advanced BILs respectively. However, to our knowledge this is the first study that identify a QTL underlying *Striga* resistance defence mechanisms using an interspecific CSSL mapping population derived from a cross between MG12 (*O. glaberrima*) and Caiapo (*O. sativa*). In our study, phenotyping 64 CSSLs from this CSSL population for post-attachment resistance against *Striga* *hermonthica* from Kibos, followed by a QTL analysis, led to the identification of a highly significant QTL for resistance to *S. hermonthica* on chromosome 12 which was responsible for a large proportion of the resistance phenotype exhibited by MG12. This finding indicates that this *S. hermonthica* resistance on chromosome 12 of the *O. glaberrima* genome involves one or a few tightly linked-genes with strong effect. The fact that our QTL coincided with that of Gurney *et al.* (2006) (where the resistance allele comes from *O. sativa* Nipponbare) indicates that the fine mapping of the overlapping QTL will enhance our understanding of the molecular genetic nature of *Striga* resistance and narrow down the interval containing candidate resistance genes. The fact that the resistant CSSLs 149 and 194 were susceptible to the isolate of *S. asiatica* from Kyela, but that MG12 was very resistant also means that this CSSL population can be used to identify genes underlying resistance to this ecotype.

The development of near isogenic lines containing smaller segments of the QTL STR12.1 region will allow us to fine map the gene(s) involved in resistance to Sh-Kibos and other *S. hermonthica* ecotypes. This process was started during this project and will be completed within the next year hopefully allowing fine mapping of the resistance QTL. Certainly fine mapping together with the availability of extensive *O. glaberrima* genome sequence information will aid the identification of candidate resistance genes. Some of the NILs may also provide excellent resources for breeding programmes to transfer the genes underlying resistance to *Striga* to other adapted cultivars even without the identification of the genes involved. An added advantage will be the fact that the NILs will not contain the *O. glaberrima* sterility loci which will facilitate breeding programmes.

In the future it would also be interesting to evaluate the different CSSL populations for *Striga* resistance (or where appropriate tolerance) in the field to identify QTL that are specific to individual host cultivar x parasite ecotype interactions, and those that are genetically stable across species and ecotypes (broad spectrum resistance to the parasite) and environment. Such QTL could provide durable and stable *Striga* resistance suitable for African poor-income farmers and plant breeding perspectives.

In conclusion, our future challenge is to explore and exploit the resistance found in germplasm of wild relatives of rice and *O. glaberrima* using available genomic technologies to understand the genetic nature of cereal resistance and tolerance to *Striga*. This will provide a better, more appropriate and durable control option for poor-subsistence African farmers in their fight against biotic constraints including parasitic witchweed *Striga*. Through the use of resistant and tolerant cultivars, with the high-yielding interspecific NERICA cultivars as the spearhead, a great contribution could be made to ensure a sustainable food security and poverty alleviation in Africa (SSA).

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# References

Ackroyd RD, Graves JD. 1997. "The regulation of the water potential gradient in the host and parasite relationship between *Sorghum bicolor* and *Striga hermonthica*." *Annals of Botany* 80: 649-656.

Adagba MA, Lagoke STO, Imolehin ED. 2002. "Nitrogen effect on the incidence of *Striga hermonthica* (Del.) Benth in upland rice." *Acta Agronomica Hungarica* 50: 145-150.

Ahonsi MO, Berner DK, Emechebe AM, Lagoke ST. 2002. "Effects of soil pasteurisation and soil N status on severity of *Striga hermonthica* (Del.) Benth. in maize." *Soil Biology & Biochemistry* 34: 1675-1681.

Ali ML, Sanchez PL, Yu S, Lorieux M, Eizenga GC. 2010. "Chromosome Segment Substitution Lines: A powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*)." *Rice* 3: 218-234.

Amusan IO, Rich PJ, Menkir A, Housley T, Ejeta G. 2008. "Resistance to *Striga hermonthica* in a maize inbred line derived from *Zea diploperennis*." *New Phytologist* 178: 157-166.

Arnaud MC, Veronèsi C, Thalouarn P. 1999. "Physiology and histology of resistance to *Striga hermonthica* in *Sorghum bicolor* var. Framida." *Australian Journal of Plant Physiology* 26: 63-70.

Ashikari M, Matsuoka M. 2006. "Identification, isolation and pyramiding of quantitative trait loci for rice breeding." *TRENDS in Plant Science* 11: 344-350.

Atera E, Onyango JC, Azuma T, Asanuma S, Itoh K. 2011. "Field evaluation of selected NERICA rice cultivars in Western Kenya." *African Journal of Agricultural Research* 6: 60-66.

Balasubramanian V, Sie M, Hijmans RJ, Otsuka K. 2007. "Increasing Rice Production in Sub-Saharan Africa: Challenges and Opportunities." *Advances in Agronomy*, 94: 55-133.

Berner DK, Kling JG, Singh BB. 1995. "*Striga* research and control: a perspective from Africa." *Plant Disease* 79: 652-660.

Bharathalakshmi CRW, Musselman LJ. 1990. "A study of genetic diversity among host-specific populations of the witchweed *Striga hermonthica* (*Scrophulariaceae*) in Africa." *Plant Systematics and Evolution* 172: 1-12.

Bocco R, Lorieux M, Seck PA, Futakuchia K, Manneh B, Baimey H, Ndjiondjop MN. 2012. "Agro-morphological characterization of a population of introgression lines derived from crosses between IR64 (*Oryza sativa* indica) and Tog5681 (*Oryza glaberrima*) for drought tolerance." *Plant Science* 183: 65-76.

Boisnard A, Albar L, Thiéméle D, Rondeau M, Ghesquière A. 2007. "Evaluation of genes from *eIF4E* and *eIF4G* multigenic families as potential candidates for partial resistance QTLs to *Rice yellow mottle virus* in rice." *Theoretical and Applied Genetics* 116: 53-62.

Botanga CJ, Kling JG, Berner DK, Timko MP. 2002. "Genetic variability of *Striga asiatica* (L.) Kuntz based on AFLP analysis and host-parasite interaction." *Euphytica* 128: 375-388.

Botanga CJ, Timko MP. 2006. Phenetic relationship among different races of *Striga gesnerioides* (Willd.) Vatke from West Africa." *Genome* 49: 1351-1365.

Bouwmeester HJ, Matusova R, Sun ZK, Beale MH. 2003. "Secondary metabolite signalling in host-parasitic plant interactions." *Current Opinion in Plant Biology* 6: 358-364.

Bouwmeester HJ, Roux C, Lopez-Raez JA, Becard G. 2007. "Rhizosphere communication of plants, parasitic plants and AM fungi." *Trends in Plant Science* 12: 224-230.

Caplan J, Padmanabhan M, Dinesh-Kumar SP. 2008. "Plant NB-LRR Immune Receptors: From Recognition to Transcriptional Reprogramming." *Cell Host & Microbe* 3: 126-135.

Carsky RJ, Singh L, Ndikawa R. 1994. "Effect of herbicide and handweeding on current and subsequent season *Striga hermonthica* density on sorghum." *International Journal of Pest Management* 40: 111-116.

Cechin I, Press MC. 1993a. "Nitrogen relations of the sorghum-*Striga hermonthica* host-parasite association: germination, attachment and early growth." *New Phytologist* 124: 681-687.

Cechin I, Press MC. 1993b. "Nitrogen relations of the sorghum-*Striga hermonthica* host-parasite association: growth and photosynthesis." *Plant Cell and Environment* 16: 237-247.

Cechin I, Press MC. 1994a. "Influence of nitrogen on growth and photosynthesis of a C-3 cereal, *Oryza sativa*, infected with the root hemiparasite *Striga hermonthica*." *Journal of Experimental Botany* 45: 925-930.

Cechin I, Press MC. 1994b. "The influence of nitrogen on growth and photosynthesis of sorghum infected with *Striga hermonthica* from different provenances." *Weed Research* 34: 289-298.

Chang M, Lynn DL. 1986. "The haustorium and the chemistry of host recognition in parasitic angiosperms." *Journal of Chemical Ecology* 12: 561-579.

Cheema KK, Bains NS, Mangat GS, Das A, Vikal Y, Brar DS, Khush GS, Singh K. 2008. "Development of high yielding IR64 x *Oryza rufipogon* (Griff.) introgression lines and identification of introgressed alien chromosome segments using SSR markers." *Euphytica* 160: 401-409.

Chen J, Huang DR, Wang L, Liu GJ, Zhuang JY. 2010. "Identification of quantitative trait loci for resistance to whitebacked planthopper, *Sogatella furcifera*, from an interspecific cross *Oryza sativa* × *O. rufipogon*." *Breeding Science* 60: 153-159.

Cherif-Ari O, Housley TL, Ejeta G. 1990. "Sorghum root length density and the potential for avoiding *Striga* parasitism." *Plant and Soil* 121: 67-72.

Cissoko M, Boisnard A, Rodenburg J, Press MC, Scholes JD. 2011. "The New Rice for Africa (NERICA) cultivars vary in their post-attachment resistance to the parasitic weed *Striga*." *New Phytologist* 192: 952-963.

Cohen J. 1997. "Corn genome pops out of the pack." *Science* 276: 1960-1962.

Deen R, Ramesh K, Gautam SK, Rao YK, Lakshmi VJ, Viraktamath BC, Brar DS, Ram T. 2010. "Identification of new gene for BHP resistance introgressed from *O. rufipogon*." *Rice Genetics Newsletter* 25: 70-72.

Deng X, Zhou J, Xu P, Li J, Hu F, Tao D. 2010. "The role of S1-g allele from *Oryza glaberrima* in improving interspecific hybrid sterility between *O. sativa* and *O. glaberrima*." *Breeding Science* 60: 342-346.

Devos KM. 2005. "Updating the 'crop circle'." *Current Opinion in Plant Biology* 8: 155-162.

Diagne A, 2006. "Diffusion and adoption of NERICA rice varieties in Côte d’Ivoire." *Developing economies* 44: 208-231.

Dingkuhn M, Jones MP, Johnson DE, Sow A. 1998. "Growth and yield potential of *Oryza sativa* and *O. glaberrima* upland rice cultivars and their interspecific progenies." *Field Crops Research* 57: 57-69.

Doggett H. 1988. Sorghum. London, Longmans Green & Co. LTD.

Doi K, Yasui H, Yoshimura A. 2008. "Genetic variation in rice." *Current Opinion in Plant Biology* 11: 1-5.

Dube MP, Belzile FJ. 2010. "Low genetic variability of *Striga gesnerioides* populations parasitic on cowpea might be explained by a recent origin." *Weed Research* 50: 493-502.

Dugje IY, Kamara AY, Omoigui LO. 2006. "Infestation of crop fields by *Striga* species in the savanna zones of northeast Nigeria." *Agriculture, Ecosystems and Environment* 116: 251-254.

Ejeta G, Butler LG, Babiker AGT. 1992. "New approaches to the control of *Striga*: *Striga* research at Purdue University." Bulletin RB-991 In: Agricultural Experimental Research Station, West Lafayete, Indiana, Purdue University, USA: 27.

Ejeta G. 2007. "The *Striga* scourge in Africa: a growing pandemic." In *Integrating New*

*Technologies for Striga Control, Towards Ending the Witch-hunt*, eds. by Ejeta

G. and Gressel J. World Scientific Publishing Co. Pte Ltd, Singapore. pp.1-16.

Elliot PC, Clarisse RN, Beby R, Josue HR. 1993. "Weeds in rice in Madagascar." *International Rice Research Notes* 18: 53-54.

Elzein A, Kroschel J. 2008. "Progress on management of parasitic weeds." In: Weed management for developing countries (Addendum 1) - Fao Corporate Document Repository. <http://www.fao.org>.

Estabrook EM, Yoder JI. 1998. "Plant-plant communications: Rhizosphere signaling between parasitic angiosperms and their hosts." *Plant Physiology* 116: 1-7.

FAO. 2008. Food and Agriculture Organisation of United Nations. http:www.fao.org/.

Fernández-Aparicio M, Pérez-de-Luque A, Prats E, Rubiales D. 2008. "Variability of interactions between barrel medic (*Medicago truncatula*) genotypes and *Orobanche* species." *Annals Applied Biology* 153: 117-126.

Fischer NH, Weidenhamer JD, Bradow JM. 1989. "Dihydroparthenolide and other sesquiterpene lactones stimulate witchweed germination." *Phytochemistry*.Oxford: Pergamon Press 28: 2315-2317.

Frost DL, Gurney AL, Press MC, Scholes JD. 1997. "*Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA." *Plant Cell and Environment* 20: 483-492.

Fujita D, Doi K, Yoshimura A, Yasui H. 2004. "Introgression of a resistance gene for green leafhopper from *Oryza nivara* into cultivated rice, *Oryza sativa* L." *Rice Genetics Newsletter* 21: 64.

Gale DM, Devos KM. 1998. "Plant Comparative Genetics after 10 Years." *Science* 282: 256-259.

Garavito A, Guyot R, Lozano J, Gavory F, Samain S, Panaud O, Tohme J, Ghesquiere A, Lorieux M. 2010. "A genetic model for the female sterility barrier between Asian and African cultivated rice species." *Genetics* 185: 1425-1440.

Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch SR. 2005. "Genetic structure and diversity in *Oryza sativa* L." *Genetics* 169: 1631-1638.

Gethi GJ, Smith ME, Mitchell SE, Kresovich S. 2005. "Genetic diversity of *Striga hermonthica* and *Striga asiatica* populations in Kenya." *Weed Research* 45: 64-73.

Ghesquière A, Sequier J, Second G, Lorieux M. 1997. "First steps towards a rational use of African rice, *Oryza glaberrima*, in rice breeding through a 'contig line' concept." *Euphytica* 96: 31-39.

Gibson CC, Watkinson A. 1989. "The host range and selectivity of a parasitic plant: *Rhinanthus minor* L." *Oecologia* 78: 401-406.

Goff SA, Darrell Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Markus Lange B, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun W, Chen L, Cooper B, Park S, Todd P, Wood C, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S. 2002. "A Draft Sequence of the Rice Genome (*Oryza sativa* L. ssp. japonica)." *Science* 296: 92-100.

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Page`s V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Be´card G, Beveridge CA, Rameau C, Rochange SF. 2008. "Strigolactone inhibition of shoot branching." *Nature* 455: 189-195.

Graves JD, Press MC, Stewart GR. 1989. "A carbon balance model of the sorghum *Striga hermonthica* host-parasite association." *Plant Cell and Environment* 12: 101-108.

Graves JD, Wylde A, Press MC, Stewart GR. 1990. "Growth and carbon allocation in *Pennisetum typhoides* infected with the parasitic angiosperm *Striga hermonthica*." *Plant Cell and Environment* 13: 367-373.

Grenier C, Ibrahim Y, Haussmann BIG, Kiambi D, Ejeta G. 2007. "Marker-assisted

selection for *Striga* resistance in sorghum." In: *Integrating New Technologies for*

*Striga Control, Towards Ending the Witch-hunt*, eds. by Ejeta G. and Gressel J.

World Scientific Publishing Co. Pte Ltd, Singapore. pp.159-172.

Gurney AL, Press MC, Ransom JK. 1995. "The parasitic angiosperm *Striga hermonthica* can reduce photosynthesis of its sorghum and maize hosts in the field." *Journal of Experimental Botany* 46: 1817-1823.

Gurney AL, Press MC, Scholes JD. 1999. "Infection time and density influence the response of sorghum to the parasitic angiosperm *Striga hermonthica*." *New Phytologist* 143: 573-580.

Gurney AL, Adcock M, Scholes JD, Press MC. 2000. Physiological processes during *striga* infestation in maize and sorghum. In: *Breeding for Striga Resistance in Cereals* (eds. Haussmann BIG, Hess DE, Koyama ML, Grivet L, Rattunde HFW & Geiger HH), Margraf Verlag, Weikersheim, Germany.

Gurney AL, Taylor A, Mbwaga A, Scholes JD, Press MC. 2002. "Do maize cultivars demonstrate tolerance to the parasitic weed *Striga asiatica*?" *Weed Research* 42: 299-306.

Gurney AL, Grimanelli D, Kanampiu F, Hoisington D, Scholes JD, Press MC. 2003. "Novel sources of resistance to *Striga hermonthica* in *Tripsacum dactyloides*, a wild relative of maize." *New Phytologist* 160: 557-568.

Gurney AL, Slate J, Press MC, Scholes JD. 2006. "A novel form of resistance in rice to the angiosperm parasite *Striga hermonthica*." *New Phytologist* 169: 199-208.

Gutiérrez AG, Carabalí SJ, Giraldo OX, Martínez CP, Correa F, Prado G, Tohme J, Lorieux M. 2010. "Identification of a Rice stripe necrosis virus resistance locus and yield component QTLs using *Oryza sativa* × *O. glaberrima* introgression lines." *Plant Biology* 10: 1-11.

Harahap Z, Ampong Nyarko K, Olela JC, 1993. "*Striga hermonthica* resistance in upland rice." *Crop Protection* 12: 229-231.

Haussmann BIG, Hess DE, Welz HG, Geiger HH. 2000. "Improved methodologies for breeding *Striga*-resistant sorghums." *Field Crops Research* 66: 195-211.

Haussmann BIG, Hess DE, Reddy BVS, Mukuru SZ, Kayentao M, Welz HG, Geiger HH. 2001a. "Pattern analysis of genotype × environment interaction for *Striga* resistance and grain yield in African sorghum trials". *Euphytica* 122: 297-308.

Haussmann BIG, Hess DE, Omanya GO, Reddy BVS, Kayentao M, Welz HG, Geiger HH. 2001b. "Major and minor genes for stimulation of *Striga hermonthica* seed germination in sorghum, and interaction with different *striga* populations." *Crop Science* 41: 1507-1512.

Haussmann B, Hess DE, Omanya GO, Folkertsma RT, Reddy BVS, Kayentao M, Welz HG, Geiger HH. 2004. "Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum." *Theoretical Applied Genetics* 109: 1005-1016.

Hearne SJ. 2009. "Control - the *Striga* conundrum." *Pest Managment Science* 65: 603-614.

Hewitt EJ. 1966. "Sand and water culture methods used in the study of plant nutrition." Farnham, UK: Commonwealth Agricultural Bureaux.

Hooper MH, Tsanuo MK, Chamberlain K, Tittcomb K, Scholes JD, Hassanali A, Khan ZR, Pickett JA. 2010. "Isoschaftoside, a C-glycosylflavonoid from *Desmodium uncinatum* root exudate, is an allelochemical against the development of *Striga*." *Phytochemistry* 71: 904-908.

Huang K, Whitlock R, Press MC, Scholes JD. 2012. "Variation for host range within and among populations of the parasitic plant *Striga hermonthica*." *Heredity* 108: 96-104.

Humphrey AJ, Beale MH. 2006. "Strigol: Biogenesis and physiological activity." *Phytochemistry* 67: 636-640.

IRRI, International Rice Research Institute. http://irri.org/about-rice/rice-facts/rice-basics).

IYR, International Year of Rice. 2004. http://www.fao.org/rice2004/en/rice-us.htm.

Jamil M, Rodenburg J, Charnikhova T, Bouwmeester HJ. 2011. "Pre-attachment *Striga* resistance of NERICA cultivars based on low strigolactone production." *New Phytologist* 192: 964-975.

Jena KK. 2010. "The species of the genus *Oryza* and transfer of useful genes from wild species into cultivated rice, *O. sativa*." *Breeding Science* 60: 518-523.

Johnson DE, Riches CR, Diallo R, Jones MJ. 1997. "*Striga* on rice in West Africa; crop host range and the potential of host resistance." *Crop Protection* 16: 153-157.

Johnson DE, Riches CR, Jones MP, Kent R. 2000. The potential for host resistance to *Striga* on rice in West Africa. Breeding for *Striga* resistance in cereals: proceedings of a workshop held at IITA, Weikersheim, Germany, Margraf Verlag.

Jones MP, Dingkuhn M, Aluko GK, Semon M. 1996. New breeding approaches for upland rice improvement: the use of *Oryza sativa*/*O. glaberrima* crosses, Manila, Philippines: International Rice Research Institute (IRRI).

Jones MP, Dingkuhn M, Aluko GK, Semon M. 1997a. "Interspecific *Oryza sativa* L. x *O. glaberrima* Steud. progenies in upland rice improvement." *Euphytica* 94: 237-246.

Jones MP, Mande S, Aluko K. 1997b. "Diversity and potential of *Oryza glaberrima* Steud in upland rice breeding." *Breeding Science* 47: 395-398.

Jones MP, Mande S, Daleba A, Sehi H. 1997c. "Using anther culture to generate fertile, double-haploid interspecific progeny." *International Rice Research Notes* 22: 7-8.

Kaewchumnong K, Price AH. 2008. "A study on the susceptibility of rice cultivars to *Striga hermonthica* and mapping of *Striga* tolerance quantitative trait loci in rice." *New Phytologist* 180: 206-216.

Kamara AT, Ekeleme F, Omoigui LO, Menkir A, Chikoye D, Dugje IY. 2009. "Nitrogen fertilisation and cultivar effects on the performance of early and late maturing maize under natural infestation with *Striga*." *African Crop Science Conference Proceedings* 9: 251-257.

Kanampiu FK, Friesen D, Gressel J. 2002a. "CIMMYT collaborative efforts unveils herbicide-coated maize seed technology to curb problematic *Striga*." *Integrated Pest Management Reviews* 7: 63-64.

Kanampiu FK, Ransom JK, Friesen D, Gressel J. 2002b. "Imazapyr and pyrithiobac movement in soil and from maize seed coats to control *Striga* in legume intercropping." *Crop Protection* 21: 611-619.

Keyes WJ, Taylor JV, Apkarian RP, Lynn DG. 2001. "Dancing together. Social controls in parasitic plant development." *Plant Physiology* 127: 1508-1512.

Khan ZR, Ahmed H, Overholt W, Khamis TM, Hooper AM, Pickett JA, Wadhams LJ, Woodcock CM. 2002. "Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp., and the mechanism defined as allelopathic." *Journal of Chemical Ecology* 28: 1871-1885.

Khan ZR, Midega CAO, Hassanali A, Pickett JA, Wadhams LJ. 2007. "Assessment of different legumes for the control of *Striga hermonthica* in maize and sorghum." *Crop Science* 47: 728-734.

Kijima Y, Sserunkuuma D, Otsuka K, 2006. "How revolutionary is the "NERICA revolution"? Evidence from Uganda." *Developing economies* 44: 252-267.

Kijima Y, Otsuka K, Sserunkuuma D. 2007. "Assessing the impact of a NERICA on income and poverty in Central and Western Uganda." FASID discussion paper series on International Development Strategies No. 2007-10-001. Foundation for Advanced Studies on International Development, Tokyo: pp.46.

Kim SK. 1995. "Genetics of maize tolerance of *Striga hermonthica*." *IITA Research* 11: 1-6.

Koffi G, 1980. "Collection and conservation of existing rice species and varieties of Africa." *Agronomie Tropicale* 34: 228-237.

Kormawa PM, Keya SO, Touré AA. 2005. "Developments and future prospects for rice research and production in Africa." *Agronomie Africaine* 5: 1-16.

Kroschel J, Müller-Stöver D. 2004. Biological control of root parasitic weeds with plant pathogens. In: Inderjit, K. (Ed.), Weed biology and management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 423-438.

Kuijt J. 1969. The biology of parasitic flowering plants. Berkeley, University of California Press.

Lagoke STO, Parkinson V, Agunbiade RM. 1991. Parasitic weeds and control methods in Africa, Ibadan, Nigeria, IITA.

Lamo J, Imanywoha J, Bigirwa G, Walusimbi M, Kyetere D, Kikafunda J, Kalule T. 2010. "First NERICA rice released in Uganda tops farmers’ rankings." *International Rice Research Notes*: 1-4.

Lane JA, Bailey JA. 1992. "Resistance of cowpea and cereals to the parasitic angiosperm *Striga*." *Euphytica* 63: 85-93.

Li J, Xu P, Deng XN, Zhou JW, Hu FY, Wan JM, Tao DY. 2008. "Identification of four genes for stable hybrid sterility and an epistatic QTL from a cross between *Oryza sativa* and *Oryza glaberrima*. *Euphytica* 164: 699-708.

Li J, Timko MP. 2009. "Gene-for-gene resistance in *Striga*-cowpea associations". *Science* 325: 1094-1094.

Li J, Lis EK, Timko MP. 2009. "Molecular genetics of race-specific resistance of cowpea to *Striga gesnerioides* (Willd.)." *Pest Management Science* 65: 520-527.

Lopez-Raez JA, Charnikhova T, Gomez-Roldan V, Matusova R, Kohlen W, De Vos R, Verstappen F, Puech-Pages V, Becard G, Mulder P, Bouwmeester H. 2008. "Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation." *New Phytologist* 178: 863-874.

Lorieux M, Ndjiondjop MN, Ghesquière A. 2000. "A first interspecific *Oryza sativa* x *Oryza glaberrima* microsatellite-based genetic linkage map." *Theoretical Applied Genetics* 100: 593-601.

Lynn DG, Chang M. 1990. "Phenolic signals in cohabitation: implications for plant development." *Plant Physiol Plant Mol Biol.Palo Alto, Calif.: Annual Reviews* 41: 497-526.

Maiti RK, Ramaiah KV, Bisen SS, Chidley VL. 1984. "A comparative study of the haustorial development of *Striga asiatica* (L.) Kuntze on sorghum cultivars." *Annals of Botany* 54: 447-457.

Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ. 2005. "The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway." *Plant Physiology* 139: 920-934.

Mbwaga AM, Riches C, Massawe C, Lamboll RI. 2001. Evaluation of Sorghum lines for *striga* resistance and their performance on farmer fields in Tanzania. 7th International Parasitic Weed Symposium, Nantes, France, Université de Nantes.

Mbwaga AM, Riches CR. 2006. Reversing the yield decline of upland-rice on *Striga* infested land. International Symposium on Integrating New Technologies for *Striga* Control: Towards Ending the Witch-hunt, Addis Ababa, Ethiopia.

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD. 1988. "Molecular mapping of rice chromosomes." *Applied Genetics* 76: 815-829.

McCouch RS, Sweeney M, Li J, Jiang H, Thomson M, Septiningsih E, Edwards J, Moncada P, Xiao J, Garris A, Tai T, Martinez C, Tohme J, Sugiono M, McClung A, Yuan LP, Ahn S. 2007. "Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*." *Euphytica* 154: 317-339.

Mghase JJ, Shiwachi H, Nakasone K, Takahashi H. 2010. "Agronomic and socio-economic constraints to high yield of upland rice in Tanzania." *African Journal of Agricultural Research* 5: 150-158.

Mohamed KI, Musselman LJ, Riches CR. 2001. "The genus *Striga* (*Scrophulariaceae*) in Africa." *Annals of the Missouri Botanical Garden* 88: 60-103.

Mohamed A, Ellicott A, Housley TL, Ejeta G. 2003. "Hypersensitive response to *Striga* infection in Sorghum." *Crop Science* 43: 1320-1324.

Mohamed KI, Papes M, Williams R, Benz BW, Peterson TA. 2006. "Global invasive potential of 10 parasitic witchweeds and related *Orobanchaceae*." *Ambio* 35: 281-288.

Morishima HO. 1998. "Genetic difference between wild and cultivated rice." *Agricultural Archaeology* 49: 30-35.

Musselman LJ. 1980. "The biology of *Striga*, *Orobanche* and other root-parasitic weeds." *Annual Review of Phytopathology* 18: 463-489.

Ndjiondjiop MN, Albar L, Fargette D, Fauquet C, Ghesquiere A. 1999. "The genetic basis of high resistance to rice yellow mottle virus (RYMV) in cultivars of two cultivated rice species." *Plant Disease* 83: 931-925.

Nwanze KF, Mohapatra S, Kormawa PM, Keya SO, Bruce-Oliver S. 2006. "Rice development in sub-Saharan Africa. Perspectives." *Journal of the Science of Food and Agriculture* 86: 675-677.

Ohnuki-Tierney E. 1993. Rice as self: Japanese indentities through time.

Olmstead RG, DePamphilis CW, Wolfe AD, Young ND, Elisons WJ, Reeves PA. 2001. "Disintegration of the *Scrophulariaceae*." *American Journal of Botany* 88: 348-361.

Omanya GO, Haussmann BIG, Hess DE, Welz HG, Geiger HH. 2001. Screening methodologies for resistance of sorghum to the parasitic weed *Striga*. 7th International Parasitic Weed Symposium, Nantes, France, Université de Nantes.

Omanya GO, Haussmann BIG, Hess DE, Reddy BVS, Kayentao M, Welz HG, Geiger HH. 2004. "Utility of indirect and direct selection traits for improving *Striga* resistance in two sorghum recombinant inbred populations." *Field Crops Research* 89: 237-252.

Oswald A, Abayo G, Ransom JK, Kroschel J, Sauerborn J. 1997. Catch-cropping with Sudan grass - an option for *striga* control in subsistence agriculture, British Crop Protection Council; Farnham; UK.

Oswald A, Ransom JK, Kroschel J, Sauerborn J. 2002. "Intercropping controls *Striga* in maize based farming systems." *Crop Protection* 21: 367-374.

Oswald A, Ransom JK. 2004. "Response of maize varieties to *Striga* infestation." *Crop Protection* 23: 89-94.

Pageau K, Simier P, Le Bizec B, Robins RJ, Fer A. 2003. "Characterization of nitrogen relationships between *Sorghum bicolor* and the root-hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth. using K15NO3 as isotopic tracer." *Journal of Experimental Botany* 54: 789-799.

Paré J, Ouedraogo O, Dembele B, Salle G, Raynal Roques A, Tuquet C. 1996. Embryological studies as an efficient strategy to control production of *Striga* seeds. Advances in parasitic research.Proceedings of the Sixth International Parasitic Weed Symposium, Sevilla.

Parker C. 1991. "Protection of crops against parasitic weeds." *Crop Protection* 10: 6-22.

Parker C, Riches CR. 1993. Parasitic weeds of the world: Biology and control. Wallingford, Oxon, Cab International.

Parker C. 2009. "Observations on the current status of *Orobanche* and *Striga* problems worldwide." *Pest Management Science* 65: 453-459.

Pérez-de-Luque A, Moreno MT, Rubiales D. 2008. "Host plant resistance against broomrapes (*Orobanche* spp.): defence reactions and mechanisms of resistance." *Annals Applied Biology* 152: 131-141.

Pickett JA, Hooper AM. 2011. "Delivering resistance to a major constraint for rain-fed rice production." *New Phytologist* 192: 792-794.

Prasad B, Eizenga GC. 2008. "Rice Sheath Blight Disease Resistance Identified in *Oryza* spp. Accessions." *Plant Disease* 92: 1503-1509.

Press MC, Shah N, Tuohy JM, Stewart GR. 1987a. "Carbon isotope ratios demonstrate carbon flux from C4 host to C3 parasite." *Plant Physiology* 85: 1143-1145.

Press MC, Tuohy JM, Stewart GR. 1987b. "Gas exchange characteristics of the sorghum *Striga* host-parasite association." *Plant Physiology* 84: 814-819.

Press MC, Graves JD. 1995. Parasitic Plants. London, UK, Chapman & Hall.

Ransom JK. 2000. "Long-term approaches for the control of *Striga* in cereals: Field management options." *Crop Protection* 19: 759-763.

Rensink WA, Buell CR. 2005. "Microarray expression profiling resources for plant genomics." *TRENDS in Plant Science* 10: 603-609.

Rich P, Grenier C, Ejeta G. 2004. "*Striga* resistance in the wild relatives of sorghum." *Crop Science* 44: 2221-2229.

Riches CR, Johnson DE, Jones MP. 1996. The selection of resistance to *Striga* species in upland rice. *Advances in parasitic research.Proceedings of the Sixth International Parasitic Weed Symposium*, Sevilla.

Riches CR, Mbwaga AM, Mbapila J, Ahmed GJU. 2005. "Improved weed management delivers increased productivity and farm incomes from rice in Bangladesh and Tanzania." *Aspects of Applied Biology* 75: 127-138.

Rodenburg J, Bastiaans L, Weltzien E, Hess DE. 2005. "How can field selection for *Striga* resistance and tolerance in sorghum be improved?" *Field Crops Research* 93: 34-50.

Rodenburg J, Bastiaans L, Kropff MJ. 2006a. "Characterization of host tolerance to *Striga hermonthica*." *Euphytica* 147: 353-365.

Rodenburg J, Bastiaans L, Kropff MJ, Van Ast A. 2006b. "Effects of host plant genotype and seedbank density on *Striga* reproduction." *Weed Research* 46: 251-263.

Rodenburg J, Bastiaans L, Schapendonk AHCM, Van der Putten PEL, Van Ast A, Dingemanse NJ, Haussmann BIG. 2008. "CO2-assimilation and chlorophyll fluorescence as indirect selection criteria for host tolerance against *Striga*." *Euphytica* 160: 75-87.

Rodenburg J, Demont M. 2009. "Potential of herbicide resistant rice technologies for sub-Saharan Africa." *AgBioForum* 12: 313-325.

Rodenburg J, Johnson D. 2009. "Weed management in rice-based cropping systems in Africa." *Advances in Agronomy* 103: 149-218.

Rodenburg J, Riches CR, Kayeke JM. 2010. "Addressing current and future problems of parasitic weeds in rice." *Crop Protection* 29: 210-221.

Rodenburg J, Bastiaans L. 2011. "Host-plant defence against *Striga* spp.: reconsidering the role of tolerance." *Weed research* 51: 438-441.

Safa SB, Jones BMG, Musselman LJ. 1984. "Mechanisms favoring outbreeding in *Striga hermonthica* [*Scrophulariaceae*]." *New Phytologist* 96: 299-306.

Sakai H, Ikawa H, Tanaka T, Numa H, Minami H, Fujisawa M, Shibata M, Kurita K, Kikuta A, Hamada M, Kanamori M, Namiki N, Wu J, Itoh T, Matsumoto T, Sasaki T. 2011. "Distinct evolutionary patterns of *Oryza glaberrima* deciphered by genome sequencing and comparative analysis." *The Plant Journal* 66: 796-805.

Sallaud C, Gay C, Larmande P, Be` s M, Piffanelli P, Pie´gu B, Droc G, Regad F, Bourgeois E, Meynard D, Pe´ rin C, Sabau X, Ghesquie` re A, Glaszmann JC, Delseny M, Guiderdoni E. 2004. "High throughput T-DNA insertion mutagenesis in rice: a first step towards in silico reverse genetics." *The Plant Journal* 39: 450-464.

Sanchez PL, Sobrizal, Ikeda K, Yasui H, Yoshimura A. 2001. "RFLP mapping of genes controlling heading date found in *Oryza glumaepatula* Steud. introgression lines in rice." *Rice Genetics Newsletter* 18: pp.57.

Sano Y. 1990. "The genic nature of gamete eliminator in rice." *Genetics* 125: 183-191.

Sarla N, Swamy BPM 2005. "*Oryza glaberrima*: a source for the improvement of *Oryza sativa*." *Current Science* 89: 955-963.

Satish K, Gutema Z, Grenier C, Rich PJ, Ejeta G. 2012. "Molecular tagging and validation of microsatellite markers linked to the low germination stimulant gene (lgs) for *Striga* resistance in sorghum [*Sorghum bicolor* (L.) Moench]." *Theoretical Applied Genetics* 124: 989-1003.

Sauerborn J. 1991. The economic importance of the phytoparasites *Orobanche* and *Striga*, Nairobi, CIMMYT.

Scholes JD, Press M. 2008. "*Striga* infestation of cereal crops - an unsolved problem in resource limited agriculture." *Current Opinion in Plant Biology* 11: 1-7.

Semagn K, Ndjiondjop MN, Lorieux M, Cissoko M, Jones M, McCouch S. 2007. "Molecular profiling of an interspecific rice population derived from a cross between WAB56-104 (*Oryza sativa*) and CG14 (*Oryza glaberrima*)." *African Journal of Biotechnology* 6: 2014-2022.

Shimamoto K, Kyozuka J. 2002. "Rice as a model for comparative genomics of plants." *Annual Review in Plant Biology*  53: 399-419.

Showemimo FA, Kimbeng CA, Alabi SO. 2002. "Genotypic response of sorghum cultivars to nitrogen fertilization in the control of *Striga hermonthica*." *Crop Protection* 21: 867-870.

Sie M, Sere Y, Sanyang S, Narteh L, Dogbe S, Coulibaly MM, Sido A, Cisse F, Drammeh E, Ogunbayo SA, Zadji L, N'dri B, Toulou B. 2008. "Regional yield evaluation of the interspecific hybrids (*O glaberrima* x *O. sativa*) and intraspecific (*O. sativa* x *O. sativa*) Lowland rice." *Asian Journal of Plant Sciences* 7: 130-139.

Silue D, Notteghem J. 1991. "Resistance of 99 *Oryza glaberrima* varieties to blast." *International Rice Research Notes* 16: 13-14.

Smaling EMA. 1993. "The soil nutrient balance: an indicator of sustainable agriculture in sub." *Proceedings Fertiliser Society* 340: 1-18.

Sokal RR, Rohlf FJ. 1995. Biometry. New York, W.H. Freeman and Company.

Somado EA, Guei RG, Keya SO. 2008. NERICA: the New Rice for Africa - a Compendium.

Stewart GR, Press MC. 1990. "The physiology and biochemistry of parasitic angiosperms." *Annual Reviews of Plant Physiology and Molecular Biology* 41: 127-151.

Stoorvogel JJ, Smaling EMA. 1998. "Research on soil fertility decline in tropical environments: integration of spatial scales." *Nutrient Cycling in Agroecosystems* 50: 151-158.

Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N. 2001. "Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers." *Theoretical and Applied Genetics* 102: 157-162.

Swarbrick PJ, Huang K, Liu G, Slate J, Press MC, Scholes JD. 2008. "Global patterns of gene expression in rice cultivarsundergoing a susceptible or resistant interaction with the parasitic plant *Striga hermonthica*." *New Phytologist* 179: 515-529.

Swarbrick PJ, Scholes JD, Press MC, Slate J. 2009. "A major QTL for resistance of rice to the parasitic plant *Striga hermonthica* is not dependent on genetic background." *Pest Management Science* 65: 528-532.

Taylor A, Martin J, Seel WE. 1996. "Physiology of the parasitic association between maize and witchweed (*Striga hermonthica*): is ABA involved." *Journal of Experimental Botany* 47: 1057-1065.

Timko MP, Gowda BS, Ouedraogo J, Ousmane B. 2007. "Molecular markers for analysis of resistance to *Striga gesnerioides* in cowpea." In: *Integrating new technologies for Striga control: Towards Ending the Witch-hunt,* eds. by Ejeta G and Gressell J. World Scientific Publishing Co. Pte Ltd, Singapore. pp.115-128.

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S. 2008. "Inhibition of shoot branching by new terpenoid plant hormones." *Nature* 455: 195-201.

Umehara M, Hanada A, Magome H, Takeda-Kamiya N, Yamaguchi S. 2010. "Contribution of strigolatones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice." *Plant Cell Physiology* 51: 1118-1126.

Van Delft GJ, Graves JD, Fitter AH. 1996. Sorghum root system architecture in relation to *Striga* parasitism, Cordoba (Spain).

Vanlauwe B, Giller KE. 2006. "Popular myths around soil fertility management in sub-saharan Africa." *Agriculture, Ecosystems and Environment* 116: 34-46.

Vogler RK, Ejeta G, Butler LG. 1996. "Inheritance of low production of *Striga* germination stimulant in sorghum." *Crop Science* 36: 1185-1191.

Waddington SR, Li XY, Dixon J, Hyman G, de Vicente MC. 2010. "Getting the focus right: production constraints for six major food crops in Asian and African farming systems." *Food Security* 2: 27-48.

WARDA, West Africa Rice Development Association. 2007. Africa Rice Center (ex-WARDA) Annual report 2005-2006: Providing what is needed. Cotonou, Benin. pp.51.

WARDA, West Africa Rice Development Association. 2008. Africa Rice Center (ex-WARDA) Annual report 2006-2007: Women taking Africa forward. Cotonou, Benin. pp.60.

Webb M, Smith MC. 1996. "Biology of *Striga hermonthica* (*Scrophulariaceae*) in Sahelian Mali: effects on pearl millet yield and prospects of control." *Weed Research* 36: 203-211.

Weisskopf L, Akello P, Milleret R, Khan ZR, Schulthess F, Gobat JM, Le Bayon RC. 2009. "White lupin leads to increased maize yield through a soil fertility-independent mechanism: a new candidate for fighting *Striga hermonthica* infestation?" *Plant Soil* 319: 101-114.

Wigchert SCM, Zwanenburg B. 1999. "A critical account on the inception of *Striga* seed germination." *Journal of agricultural food chemistry* 47: 1320-1325.

Wilson JP, Hess DE, Hanna WW. 2000. "Resistance to *Striga hermonthica* in wild accessions of the primary gene pool of *Pennisetum glaucum*." *Phytopathology* 90: 1169-1172.

Wilson JP, Hess DE, Hanna WW, Kumar KA, Gupta SC. 2004. "*Pennisetum glaucum* subsp. monodii accessions with *Striga* resistance in West Africa." *Crop Protection* 23: 865-870.

Windmeijer PN, Duivenbooden N, Andriesse W. 1994. Characterization of rice-growing agro-ecosystems in West Africa: semi-detailed characterization of inland valleys in Côte d'Ivoire. Wageningen, SC-DLO-Wageningen Agricultural University.

Wopereis MCS, Diagne A, Rodenburg J, Sié M, Somado EA. 2008. "Why NERICA is a successful innovation for African farmers: a response to Orr *et al*. from the Africa Rice Center." *Outlook on Agriculture* 37: 169-176.

Xie X, Song M, Jin F, Ahn S, Suh J, Hwang H, McCouch SR. 2006. "Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between Oryza sativa and Oryza rufipogon." *Theoretical and Applied Genetics* 113: 885-894.

Yano M, Sasaki T. 1997. "Genetic and molecular dissection of quantitative traits in rice." *Plant Molecular Biology* 35: 145-153.

Yoder JI. 2001. "Host-plant recognition by parasitic *Scrophulariaceae*." *Current Opinion in Plant Biology* 4: 359-365.

Yoder JI, Scholes JD. 2010. "Host plant resistance to parasitic weeds: recent progress and bottlenecks." *Current Opinion in Plant Biology* 13: 478-484.

Yoneyama K, Xie XN, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y. 2007a. "Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for *arbuscular mycorrhizal* fungi and root parasites." *Planta* 227: 125-132.

Yoneyama K, Takeuchi Y, Sekimoto H. 2007b. "Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites." *Planta* 225: 1031-1038.

Yoneyama K, Awad AA, Xie XN, Takeuchi Y. 2010. "Strigolactones as germination stimulants for root parasitic plants." *Plant and Cell Physiology* 51: 1095-1103.

Yoshida S, Shirasu K. 2009. "Multiple layers of incompatibility to the parasitic witchweed, *Striga hermonthica*." *New Phytologist* 183: 180-189.

Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J, Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D, Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P, Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan L, Yang H. 2002. "A Draft Sequence of the Rice Genome (*Oryza sativa* L. ssp. indica)." *Science* 296: 79-92.