Chapter 6

Molecular Breeding for *Striga* Resistance in Sorghum

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Abstract

Among the biotic stresses affecting dryland cereals, especially sorghum, *Striga hermonthica* is the most damaging obligate parasite, and is an important bottleneck to yield increases by smallholder farmers, yet it has been neglected by research in recent years. Integrated *Striga* management packages have been designed, but these will continue to require new cultural and chemical treatments, resistant varieties, and integrated approaches to manage both *Striga* and soil fertility. This review attempts to assess recent advances in bioassay development that are specific to resistance mechanisms, genomics such as New Generation Sequencing tools, RNA interference (RNAi) technologies in advancing knowledge of resistance and susceptibility to *Striga* including diversity in striga populations, and molecular marker technology in accelerating the development of *Striga*-resistant cultivars of sorghum. Recent advances in developing effective bioassays involving several modifications of rhizotrons and sand-packed titer plate assay will help dissect resistance mechanisms into component traits and increased understanding of the specific resistance mechanisms, which will directly help in efficient introgression and selection of several striga resistance mechanisms in breeding population. The current studies for identification of parasite genes specifically involved in haustorigenesis through transcriptomic and/or proteomic studies and more recently RNAseq studies will help understand susceptibility or resistance genes in striga. Release of improved version of cultivars resistant to striga developed by marker-assisted backcrossing of several striga resistance QTLs in Sudan had shown the power of integrating genomics and molecular breeding tools/techniques into routine breeding for tackling the complex constraint such as striga. Application and utilization of advance techniques in genomics and molecular breeding appropriately can further enhance the efficiency of integrated striga management practices, and thus crop productivity.

Introduction

Witchweeds (*Striga* spp.) are important pests of agricultural crops in much of Africa, especially East Africa, and had been a problem in parts of Asia and in the United States. These parasitic weeds have become the greatest biological constraint to cereal food production in resource-limited agricultural areas (Ejeta and Butler 1993; Gressel et al. 2004) as nearly 50 million hectares

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of field crops are infested with *Striga* annually, especially *S. hermonthica* and *S. asiatica*. These species infest maize, sorghum, pearl millet, finger millet, and upland rice, causing severe stunting. Yield loss attributable to *Striga* is acute, perhaps even exacerbated, ranging from 30% to 90% (Kroschel et al. 1999; van Ast et al. 2005; Ejeta 2007; Joel et al. 2007). Impacts are greatest on infertile soils, and the poorest subsistence farmers are the most severely affected. According to The Food and Agricultural Organization of the United Nations (FAO) (http://www.fao.org/), *Striga* infests over 40% of the cereal producing areas of sub-Saharan Africa, is continuing to spread, and accounts for US$7.4 billion in lost crops annually (Sharma 2006), with significant negative impact on the food supply of several million people. Effective control of *Striga* has been difficult to achieve through conventional agronomic practices, since the parasite exerts its greatest damage before its emergence above ground. Estimates on the extent of crop damage in a country or region in the African continent vary depending on the crop cultivar and degree of infestation (Parker and Riches 1993). However, *Striga* infestation is most severe in eastern Africa, especially in Ethiopia, where over 50% of sorghum production field are infested by several *Striga* species, and the invasion by the parasite is expanding at an alarming rate, often resulting in total crop losses annually on many farms. An expansion of *Striga* infestation is also occurring in West Africa. The impact of *Striga* in these regions is compounded by its predilection for attacking crops already under moisture and nutrient stress, conditions very acute in these regions and getting to be prevalent in much of the semiarid tropics.

Despite its agricultural importance, the molecular mechanisms controlling the establishment of parasitism are not well understood. The major species affecting sorghum in East Africa is *S. hermonthica*, and its life cycle is unique and well adapted to its parasitic lifestyle. The seeds need to be exposed to germination stimulants exudated from the host roots, such as strigolactones and ethylene; otherwise they can remain dormant in the soil for several decades (Bouwmeester et al. 2007). The seeds are tiny and possess limited amounts of nutrients, and this restricts their growth without a host connection. When a potential host is recognized through the sensing of strigolactones or other germination stimulants, the seeds that are close to the host roots (within 5 mm) can germinate. The germinated seedlings form haustoria—round-shaped organs specialized in host attachment and penetration (Yoder 2001). The formation of haustoria also requires host-derived signal compounds. The haustoria penetrate the host roots and finally connect with the vasculature to rob the host plant of water and nutrients. This dramatic developmental transition from an autotrophic to a heterotrophic lifestyle occurs within several days.

Intensive efforts in the scientific community, mainly in the United States during the 1960s, lead to the identification of some germination stimulants. This was followed by the development of a "suicidal germination" strategy to eradicate *Striga* weeds (Rispail et al. 2007). By this strategy, a germination stimulant (in this case ethylene) was mixed in the soil to trigger germination in the absence of the hosts. This approach was used successfully to eradicate *Striga asiatica* in North Carolina. The suicidal germination approach was not applicable for African farmers due to the high cost of the strategy and the much larger scale of infestation.

Integrated *Striga* management packages have been designed that include: *Striga* resistant varieties (Rodenburg et al. 2006); judicious and appropriate timing and application of fertilizers in combination with organic fertilizers, suitable crop rotations, and trap cropping (van Ast et al. 2005; Oswald 2005; Joel et al. 2007); intercropping with forage legume *Desmodium uncinatum* (Khan et al. 2007) and seed coating with amino acids, fusarium spps, and herbicides (Kanampiu et al. 2003); and water conservation measures. *Striga* management will continue to require cultural and chemical treatments,

resistant varieties, and an integrated approach to both *Striga* and soil fertility. Of these approaches, development of resistant crop cultivars has been recognized as the most effective and feasible method. To date, *Striga*-resistant sorghum cultivars – such as N13, SRN39, Framida, 555, ICSVs, SRN39 derivatives (P401 to P409), Soumalemba (IS15401), and Seguetana CZ and CMDT45 – have been identified and, as has been observed by Tabo et al. (2006) and ICRISAT (2009), these can be integrated with available crop management options to enhance productivity.

Success has been limited because *Striga* impacts host development shortly after attaching to the root, long before the parasite emerges above ground, and yield losses are not linearly related to parasite biomass (Gurney et al. 1999). However, a lack of knowledge of the genetic factors controlling host-parasite signalling at different stages of the life cycle, the paucity of resistant host germplasm, the polygenic nature of resistance coupled with complex genotype × environment (G × E) interactions, and insufficient knowledge of parasite race structure impede progress. Genomic approaches offer new opportunities to dissect polygenic resistance traits into their underlying genetic components (quantitative trait loci [QTL]), allowing breeders to utilize marker-assisted selection (MAS) for the transfer and pyramiding of useful alleles into locally adapted cultivars. Further, sequence information and genomic tools for forward and reverse genetics open the way for identification of key genes controlling parasitism (in both host and parasite) and their genetic manipulation. Here, we critically assess the current and future roles of genomic platforms such as New Generation Sequencing tools, RNA interference (RNAi) technologies, in advancing knowledge of resistance and susceptibility to *Striga* and molecular marker technology in accelerating the development of *Striga*-resistant cultivars of sorghum. Recent status of development of *Striga*-resistant cultivars is also discussed.

Development of Bioassays and Dissecting *Striga* Resistance Mechanisms

Additionally, such combinations would offer more buffering capacity to host plant population against building virulence in *Striga* populations, as it could only result from multiple mutations to overcome these obstacles resulting in durable host-plant resistance. To date, five specific *Striga* resistance mechanisms had been described, which include resistance associated with low germination stimulant (*LGS*) production, low production of the haustorial initiation factor (*LHF*), Germination inhibitors (*GI*), hypersensitive response (*HR*), and the incompatible response (*IR*) to parasitic invasion of host genotypes (Ejeta et al. 2000). Assessing these resistance mechanisms across several lines individually is very difficult, as most of times their effects are confounding and are difficult to separate. In this scenario, breeding for *Striga* resistance would be greatly assisted by in vitro methods that allow inspection of pre-attachment and early post-attachment phases of *Striga* interaction with host root systems. Such observations could reveal underlying resistance mechanisms in source germplasm and allow selection for specific resistance mechanism, alone or in combination, in breeding populations for future exploitation. Recent approaches such as RNA interference and microRNA assays to further characterize host-parasite interactions at nucleotide level offer new avenues for improving *Striga* resistance, but require more specific assays.

In case of *Striga*, it is particularly important that control is expressed early in the parasitic life cycle since severe consequences on host health are manifested within the first few days after parasitic attachment (Gurney et al. 1999). Traditionally, breeding for most of complex traits such as *Striga* resistance had been based on field selection/phenotypic performance of germplasm, and selection efficiency is based on a well-defined trait phenotype such as emerged parasite number or severity scores in artificially infested

plots over multiple years and locations (Omanya et al. 2004). However, these filed evaluation measures of *Striga* resistance fail to distinguish between resistance/tolerant traits and the associated interactive events between host and parasite. A potential host plant may have any of several defense responses to *Striga* infection (Ejeta 2007; Joel et al. 2007; Rich and Ejeta 2007). Tolerance to the ill effects of *Striga* is also known, whereby the crop is able to produce acceptable yields despite Striga infestation (Rodenburg et al. 2006).

Adequate genetic variation for a target trait and availability of effective selection tools are essential requisites for successful plant-breeding efforts, and unfortunately selection methods that work well for improving other desirable crop traits have not operated at the same level of efficiency for *Striga* resistance (Ejeta and Butler 1993). Similar to other major complex trait, field selection for *Striga* resistance had not been successful because of the difficulty in clearly identifying resistant variants, lack of understanding on the genetic control of field resistance, and the difficulty of establishing uniform infestation of the parasite population under varying environmental conditions. Lack of rapid and efficient screening techniques had been a major constraint in the progress of *Striga* resistance breeding, and hence development of bioassays received primary focus and consideration across all *Striga* resistance breeding programs.

Rhizotrons and other in vitro growth systems of various designs have been described for co-culture of weedy root parasites and their hosts (Rubiales et al. 2006). An agar-based system and its modifications had been quite useful in identifying sorghum seedlings with resistance based on low *Striga* germination stimulant production (Omanya et al. 2004), and sorghum accessions had been useful for their ability to trigger formation of the haustorium or appressorium in *S. asiatica* (Rich et al. 2004) and to observe early post-attachment reactions on sorghum expressed as hypersensitive response to *Striga* (Mohamed et al. 2003). These methods had inherent limitation of short time for observations and inconsistency. Co-culture of *Striga* on sorghum in Petri dishes stood on end and containing moistened paper topped with glass fiber with a hole to accommodate host shoot growth (Arnaud et al. 1999) allowed observations of parasitic associations over several weeks, but attachment frequency in these rhizotrons is also low if the growth medium is too wet. Larger rhizotrons using sand (Gurney et al. 2003) or rockwool (Gurney et al. 2006; Yoshida and Shirasu 2009) sandwiched between plastic plates have been well suited to co-culture of *S. hermonthica* on cereals. A fundamental problem with all the aforementioned methods, however, is that newly attached *Striga* are so small (millimeters or less) that a microscope capable of at least 10× magnifications is required to view newly attached parasites. This means that this entire activity becomes very cumbersome, low throughput, resource intensive, and time consuming. To be useful in a breeding program, in vitro methods should mimic natural conditions, occupy little space (particularly for containment facilities), and be low cost and relatively easy to set up and maintain. They should allow nondestructive and progressive observations, preferably at multiple times during co-culture, consistent, repeatable, and with high heritability.

A sand-based rhizotron for monitoring *Striga* parasitism with the aid of a scanner during the critical attachment and early post-attachment phases was recently developed (Amusan et al. 2011). The sand-packed titer plate assay (SPTPA) was used to examine *Striga*-susceptible and -resistant maize (Amusan et al. 2008) and sorghum, previously identified in field trials, to pinpoint the stage at which *Striga* seedlings stop growing or died on the host roots. These modifications and the ones to follow will help dissect the different *Striga* resistance mechanisms into the component traits, which in turn will allow for easier selection and introgression in breeding programs. Use of these assays had enhanced the ability more systematically to evaluate and exploit sorghum germplasm as sources

of *Striga* resistance by focusing on each stage of the parasitic process individually. The bioassays had also provided further insights to the interactive biological processes between *Striga* and the roots of host plants during the early stages of the infection process, giving an increased understanding of the specific mechanisms of resistance associated with each source of host genotype (Cai et al. 1993). Furthermore, defense responses triggered by infection could also be monitored and exploited via these assays. Hence, disrupting these interactions offers unique opportunities for controlling *Striga* through identification of genetic variants with single or multiple interventions at key critical stages throughout the life cycle, which are likely to be simply inherited and therefore easy to manipulate through conventional breeding or via other biotechnological approaches for the development of *Striga*-resistant crop cultivars.

Understanding Host-Parasite Biology: Exploring Pathway Stages as Entry Points for Breeding Resistance to *Striga*

Genetic resistance is a vital component of an effective integrated *Striga* management program (Ejeta 2007). Durable resistance for a trait like *Striga* is most securely based on multiple traits that block the establishment of weedy root parasites on their hosts (Rispail et al. 2007). This involves several metabolic pathways, both in host and parasite, that play a very dynamic role during expression of resistance or susceptibility for host and of virulence or avirulance for parasite. Successful parasitism is therefore a series of interactive processes between host and parasite conditioned by a large number of genetic and physiological events, each possibly influenced by additional array of environmental factors. Host plant resistance based on observation of emergence above ground of parasitic seedlings and level of infestation, therefore, is a complex, quantitatively inherited trait that is difficult to select for using conventional approaches of plant breeding. Characterization and dissection of *Striga* resistance into specific mechanisms based on a series of host-parasite signal exchanges should be the central focus and premise for any research approach and effort aimed at developing *Striga*-resistant cultivars (Ejeta et al. 1991).

The life cycle of *Striga* is intimately linked to that of its host and depends on a complex exchange of chemical signals; this poses a challenge and an opportunity for control, both before and after attachment of *Striga* to the host root. Understanding host-parasite biology at each life cycle stage is essential for the design of novel control strategies. The first committed step of the *Striga* life cycle is germination, which occurs only in response to specific secondary metabolites in the root exudates of host and some non-host plants. There are several classes of germination stimulants such as strigolactones (Bouwmeester et al. 2003), most commonly present in the exudates of many cereals species (Awad et al. 2006). Some low-germination-stimulant-producing sorghum cultivars have been identified and often perform well when used as part of an integrated control program (Joel et al. 2007). Manipulation of the production of germination stimulants requires further knowledge of their biosynthetic pathways. Forward and reverse genetic approaches may identify key genes involved in strigolactone synthesis such as in maize (Bouwmeester et al. 2003), and the use of maize mutants revealed that strigolactones are derived from the carotenoid pathway (Matusova et al. 2005; Bouwmeester et al. 2007) and can facilitate the identification of genes involved in the synthesis and regulation of strigolactones (Sun et al. 2007) in rice and sorghum as model crops.

Following germination, haustorial inducing factors (HIF) are required to trigger differentiation of the parasite haustorium (Keyes et al. 2000; Keyes et al. 2007). To initiate the identification of parasite genes specifically involved in haustoriogenesis, *Triphysaria versicolor*, a facultative parasite closely related to *Striga*, is being used as a model system. An EST database

(Pscroph) (http://pscroph.ucdavis.edu/) (Torres et al. 2005) has been developed representing transcripts differentially regulated in Triphysaria before and after contact with host roots, in response to host root exudates and to the HIF 2,6-dimethoxybenzoquinone (DMBQ) (Yodler et al. 2007). Approximately 40,000 ESTs from the different suppressive subtraction hybridization (SSH) libraries have been sequenced and annotated (http://www.plantgdb.org/prj/ESTCluster/progress.php). This approach is being extended to produce EST collections from *Striga* and *Orobanche* facilitating identification of potentially essential genes for germination stimulant perception, haustorial formation, and parasite development. Stable and transient transformation systems for *Triphysaria* (Tomilov et al. 2007) and transformation of *Striga* have also been successful, allowing the function of parasite genes to be tested by silencing or overexpression. These approaches may allow to design novel control strategies, for example, by utilizing gene-silencing vectors designed against essential parasite specific genes. If such vectors were transformed into cereals and the RNAi signal moved from host to parasite, silencing of the parasite gene could be lethal or inhibit parasite development. Post-transcriptional gene silencing (PTGS) has been demonstrated to work against viruses and nematodes, but its applicability for *Striga* control is unproven. Preliminary evidence suggests that this approach may work for the holoparasitic *Orobanche* where mRNA levels encoding the enzyme mannose-6-phosphate were reduced by 60–80% in the parasite when grown on transgenic tomato plants containing the appropriate silencing construct (Rady 2007). However, a key difference between *Orobanche* and *Striga* is that only the former establishes direct phloem connections with the host as the silencing signal is thought to be phloem mobile (Tournier et al. 2006).

Once the haustorium has attached to the host, *Striga* penetrates the cortex and endodermis to form a direct link with host xylem vessels. Identifying host genotypes that block penetration and identification of the genes involved is a major focus of *Striga* research. There are a few cultivated and many more wild sorghums that exhibit partial post-attachment resistance; several sorghum cultivars and a wild accession P47121 exhibit a hypersensitive response phenotype when infected with an ecotype of *S. asiatica* (Mohamed et al. 2003; Rich et al. 2004), and the sorghum cultivar N13 appears to exhibit "mechanical" resistance at the root endodermal barrier (Haussmann et al. 2004). The molecular basis and signalling pathways underlying resistance or susceptibility in plant-plant interactions are obscure, and rice offers the opportunity for molecular dissection of these traits. Preliminary analyses of changes in gene expression in susceptible and resistant cultivars using rice microarrays revealed that many of the genes upregulated in Nipponbare are those classically associated with defense responses to microbial pathogens, for example, pathogenesis related (PR) genes, cytochrome P450s, and WRKY transcription factors (Scholes et al. 2007).

Understanding the mechanistic basis of different types of resistance to *Striga* will facilitate pyramiding of resistance genes, via genetic engineering strategies or MAS with aim of providing durable polygenic resistance. Relatively few transcriptomic or proteomic studies to identify susceptibility or resistance genes in *Striga* are known to be under way. Increasing availability of microarrays for sorghum and newer expression-profiling tools such as RNAseq would address this in the near future.

Striga Diversity, Racial Differentiation, and its Implications on *Striga* Resistance Breeding

The issue of genetic diversity among *Striga* species and within species populations structured by geography, host crop, and other environmental factors affecting adoption plays an important role in host-plant interaction and expression of

resistance. It is clear that a much greater knowledge of *S. hermonthica* and *S. asiatica* diversity/race structure in relation to host species and cultivar specificity is urgently needed to inform breeding programs in different regions of Africa. There is growing evidence from field studies and molecular analysis that there is host specificity and adaptation in *S. asiatica* and *S. hermonthica* populations. Different *Striga* populations show specific genetic adaptation to host and host genotypes displaying variable virulence (Riopel and Timko 1995). In addition to variation in the genetic diversity between populations of a species of *Striga*, there is likely to be a difference between the species in within population diversity. *Striga hermonthica* is a self-incompatible outbreeder, whereas *S. asiatica* is autogamous, self-pollinating prior to floral opening, and, as such, it is highly inbred. The difference in reproductive biology is likely to have a significant impact on the within-population diversity of these species, *S. hermonthica* having a higher within-population genetic diversity than *S.asiatica* (Safa et al. 2006; Scholes et al. 2007). What implication this has for plasticity of populations regarding host specificity needs to be determined. Evaluation of the genetic diversity of *Striga* populations and determination of the influence of parasite genotype on virulence in differing hosts would better enable *Striga* researchers to ensure that potential control products are fully evaluated. This knowledge is key to the generation of more durable technologies and will enable better targeting of dissemination of control technologies to specific localities. A recent study by Estep et al. (2011) investigated genetic diversity of *S. hermonthica* populations collected from four different regions in Mali using SSR markers. The *Striga* populations were characterized by broadly distributed allelic diversity with little genetic differentiation and large amount of gene flow. It was also observed that population structure did not correlate with local environment or host species (sorghum versus pearl millet). These understandings can help plant breeders identify race/population specific resistance genes/genotypes, which can further be used to identify individual resistance genes in crop (host) germplasm and pyramid multiple resistant genes into a targeted crop plant. In order to fully characterize the existence of "races" and the factors driving their formation, further collections of *S. hermonthica* populations and their hosts are needed. The recent development of a high-throughput microarray-based Diversity Arrays Technology (DArT) (Wenzl et al. 2004) for several crop species, including sorghum, and Next Generation Sequencing (NGS) based Genotyping-by-Sequencing (GbS) tools could substantially accelerate knowledge of *Striga* diversity. The development of a *Striga*-specific assays would allow screening of thousands of molecular markers in parallel and facilitate comparison of *Striga* populations from micro (within a field) to macro (between countries) scales.

QTL Analysis and Marker-Assisted Selection for Improving *Striga* Resistance

The availability of sequence information from EST and genome-sequencing projects has led to the development of dense molecular genetic maps for many cereals including rice, sorghum, and maize (Varshney et al. 2004; Mace et al. 2009). Most resistance to *Striga* appears to be polygenic. The use of mapping populations, QTL analysis, and advanced backcross QTL analysis (AB-QTL) (for transferring important traits from wild relatives into a crop variety (Tanksley et al. 1996) combined with marker-assisted selection (MAS) is a promising approach that is beginning to yield results for the development of resistant cultivars. Several QTL and AB-QTL studies have been performed under laboratory conditions to identify the genetic basis of resistance in cultivated and wild relatives of sorghum (Haussmann et al. 2004; Grenier et al. 2007). An advanced backcross mapping population derived from a cross between PQ434 (low-HIF-producing wild sorghum) and Shanqui Red (cultivated,

high-stimulant-producing sorghum) allowed the *Lhf* (low haustorial factor) locus to be mapped to 19.3 cM from the microsatellite marker *Xtxp358* on linkage group nine (= sorghum chromosome SBI-09 short arm) (Grenier et al. 2007). Similarly, a cross between the wild sorghum species *S. arundinaceum*, which exhibits a hypersensitive like resistance response, with two cultivated sorghum species revealed that the resistance trait was controlled by two nuclear genes *HR1* and *HR2*, which were mapped at 7.5 cM from *Xtxp096* on SBI02 and 12.5cM from *SbKAFGK1* on SBI05, respectively (Mohamed et al. 2003; Grenier et al. 2007).

Analyzing gene expression profiles within a segregating population such as NILs or selected backcross inbred lines (BILs) or Recombinant Inbred Lines (RILs) allows the identification of both cis and trans acting QTL, thus providing information about factors that control the expression of a gene as well as its location on a genetic map (Schadt et al. 2003). Although it is often assumed that QTL do not accurately reflect the physical location of genes on the genome underlying a polygenic trait, in many cases, the gene was located within 1–2 cM of the QTL peak (Price et al. 2006). Combining QTL analysis with transcriptomic data can help determine whether genes that are differentially regulated within the QTL regions are putative candidate resistance genes.

Haussmann et al. (2004) reported a QTL analysis of field resistance to *Striga* using two mapping populations of RILs derived from a cross between IS9830, a low germination stimulant producer, and a E36-1, a susceptible genotype (RIL-1); and N13 (mechanical resistance) and E36-1 (RIL-2). Each mapping population was divided into two sets, which were tested in sequential years at five sites in Mali and Kenya (Haussmann et al. 2001). Composite interval mapping revealed some QTL in each RIL that were stable across years and environments and some that were not. In RIL-1, the most significant QTL corresponded to the *lgs* locus but other QTL also indicated the presence of other resistance mechanisms. In RIL-2, five QTL (derived from N13) were stable across years and environments and explained between 12% and 30% of the observed genetic variation for resistance indicating that flanking molecular markers would be excellent candidates for MAS. Recently Satish et al. (2012) fine-mapped the *lgs* locus on sorghum chromosome SBI-05 toward distal end. Four tightly linked SSRs were also tagged and validated for their linkage with *lgs* locus.

Recent Development in Marker-Assisted Backcrossing for Development of *Striga* Resistance Products

The QTLs identified by Haussmann et al (2004) in RIL-2 (based on cross N13 × E36-1) were subject to two MABC projects over last few years. Following the initial QTL mapping studies, a collaborative project of ICRISAT, IER, and the University of Hohenheim, two locally adapted, farmer-preferred sorghum varieties from Mali were introgressed with up to four of the five resistance QTL by marker-assisted backcrossing (MABC) (Muth et al. 2011). In this project, 32 of the resulting backcross-two lines (BC2S3) were field-evaluated for their *Striga* resistance under natural and artificial *Striga* infestation at three sites in Mali in 2009 and 2010. Together with yield data and agronomic properties, the number of emerged *Striga* plants per experimental plot was evaluated at regular intervals over the cropping season as an integrative measure of disease severity. In parallel, the presence/absence of the targeted genomic regions from N13 neighboring the QTL was tested in all lines using flanking SSR markers mapped to the vicinity of the targeted QTLs. Preliminary analyses of the data show a resistance of the best sorghum lines equal to or exceeding the resistance of the donor parent N13. However, yield of BC2-lines was on average inferior to the recurrent parents. A strong environmental influence on resistance between trial sites was observed in the field experiments.

The same project activities in Sudan were much more advance, and recently resulted in the release of four *Striga*-resistant varieties in the genetic backgrounds of popular, but *Striga*-susceptible, improved sorghum varieties "Tabat," "Wad Ahmed," and "AG8" (personal communication with Dr. Abdalla Mohamed, Sudan). The backcross/QTL validation project advanced to the second backcross generation (BC2) in several locally adapted farmer-preferred open-pollinated varieties. The resulting early-generation backcross progenies, although *Striga* resistant, were not agronomically elite enough to be submitted to national trials and considered for release. The national programs in Sudan, Eritrea, and Kenya, led by Dr. Abdalla Mohamed and with ICRISAT providing backstopping, then obtained funding through the regional agricultural science network (ASARECA Competitive Grant System for 2006) to fine-map the *Striga* resistance QTLs and complete the task of recovering recurrent parent eliteness for materials in the genetic backgrounds of farmer-preferred improved sorghum varieties from Sudan. Three more backcrosses were executed along with foreground selection, with QTL flanking SSRs at each stage and with background selection with DArT markers in BC4F1 progenies.

Standard variety trials were conducted in *Striga* sick plots over three rainy seasons (2009 to 2011) at the Gezira Research Station (GRS), Damazine, Sinnar, and Gedaref in Sudan. Results from these trials revealed that backcross-derived lines T1BC3S4, AG6BC3S4, AG2BC3S4, and W2BC3S4 were *Striga* resistant and agronomically superior, giving 180% to 298% increases in grain yield over their recurrent parents in the infested sick plots. These four experimental varieties in Sudan were approved by the National Crop Variety Release Committee, as "ASARECA.T1" (T1BC3S4), "ASARECA.W2 Striga" (W2BC3S4), "ASARECA.AG3" (AG2 BC3S4), and "ASARECA.AG4" (AG6BC3S4).

There were several hurdles, which in turn had led to significant new knowledge for handling the issues related to MABC of complex traits such as *Striga* resistance. Most of the *Striga* resistance QTLs targeted for this introgression work were characterized by large confidence interval between flanking markers, scant availability of flanking SSRs (Table 6.1), and lack of polymorphism between donor and recurrent parents. This had many implications for attempting introgression of *Striga* resistance QTLs in several target genetic backgrounds. The lack of enough SSRs at initial stage of project (till BC2-generation) spread across QTL region meant high probability of losing the QTL even after flanking marker confirmation because of the possibility of recombination occurring in the putative QTL regions, linkage drag with unfavourable traits, and ultimately lower recurrent parent recovery. This was evident from the first phase of the project were we had lower recurrent parent recovery in BC2-progenies. Before we can make any further progress toward this end, we were also stuck with unavailability of tightly linked SSRs with target QTLs and hence identification of confirmed QTL heterozygote(s). Also the underlying mechanisms/traits for each target QTL were not fully understood, which was linked to lack of proper phenotyping assays for those mechanism/traits. All of the phenotyping was done with field-level screening. These issues were addressed by advancing large BC-progenies until BC4 with foreground selection to reduce the probability of losing the QTL due to crossover between the large QTL interval. We simultaneously assayed the RIL population based on cross-(N13 × E 36-1), used for QTL identification, with additional SSRs and DArT markers used for MABC. This led to identification of additional markers for target QTL interval. These additional SSRs were subsequently used for foreground selection of BC4S4-population of >150 progenies, followed by background selection with DArT markers. We identified 31 BC4S4-progenies, which were screened across several years and locations, resulting in identification and release of the four varieties.

Table 6.1. Details of sorghum SSRs used for foreground selection for marker-assisted backcrossing of *Striga* resistance QTLs

Striga resistance QTLs	Sorghum chromosome[a]	Physical distance (Mbp)[b]	Linkage distance (cM)[c]
SSRs flanking to Striga resistance QTL on SBI-01			
Xtxp213	SBI-01	6.7	NA
XmsbCIR268	SBI-01	8.7	19.2
Xcup033	SBI-01	13.6	NA
SSRs flanking to Striga resistance QTL on SBI-02.1			
Xtxp197	SBI-02	1.5	2.2
Xtxp084	SBI-02	4.9	22.3
Xtxp080	SBI-02	3.9	18.9
XmbCIR223	SBI-02	4.7	19.7
SSRs between two *Striga* resistance QTLs on SBI-02			
Xtxp050	SBI-02	5.0	22.3
Xiabtp346	SBI-02	12.7	NA
Xiabtp500	SBI-02	18.8	NA
Xtxp072	SBI-02	27.9	74.8
SSRs flanking to Striga resistance QTL on SBI-02.2			
Xiabtp444	SBI-02	55.5	NA
Xtxp013	SBI-02	56.0	82.0
Xtxp298	SBI-02	57.1	92.9
Xtxp056	SBI-02	61.6	124.0
Xtxp296	SBI-02	71.1	171.8
SSRs flanking to Striga resistance QTL on SBI-05.1			
Xtxp115	SBI-05.1	NA	27.9
Xtxp268	SBI-05.1	1.7	NA
Xtxp065	SBI-05.1	1.9	14.4
Xiabtp420	SBI-05.1	3.2	NA
Xtxp112	SBI-05.1	3.9	NA
SSRs flanking to Striga resistance QTL on SBI-05.2			
Xtxp225	SBI-05.2	NA	59.4
Xtxp014	SBI-05.2	42.3	64.1
Xtxp015	SBI-05.2	42.0	64.2
Xtxp299	SBI-05.2	NA	64.9
Xtxp262	SBI-05.2	57.9	94.1
SSRs flanking to Striga resistance QTL on SBI-06			
Xtxp317	SBI-06	50.8	90.7
Xtxp097	SBI-06	66.1	92.9
Xtxp219	SBI-06	NA	91.9
Xiabtp130	SBI-06	53.9	NA
Xcup12	SBI-06	54.5	NA
Xtxp176	SBI-06	55.9	134.1
Xtxp057	SBI-06	57.4	141.0
Xcup37	SBI-06	61.9	165.2

[a]Sorghum chromosome nomenclature as per Kim et al. (2005).
[b]Physical map distance (in MbP) as estimated by BLAST search of primer pair sequence of individual SSR with sorghum genome sequence as described in Ramu and Deshpande et al. (2010).
[c]Linkage distance (in cM) of SSRs as estimated in consensus map developed by Mace et al. (2009).

Advances in Genomics and Applications for *Striga* Resistance Research

Simple sequence repeat (SSR) markers are still the preferred choice of markers in plant-breeding programs with interest in mapping and introgression of different traits in crop species. The amenability to simple assays, multiplexing, reproducibility, and more importantly codominant nature of SSRs works to the advantage of plant-breeding science to follow the segregating population as per principles of Mendelian and Population genetics for selection of best phenotypes. SSR markers were greatly exploited for mapping of different traits in sorghum, including *Striga* resistance (Haussmann et al. 2004, Satish et al. 2012). A major limiting factor for utilization of SSR markers is the resolution power. Recent advances in sorghum genomics including availability of sorghum genome sequence (Paterson et al. 2009), access to large number of markers including DArTs (Mace et al. 2009; Ramu and Deshpande et al. 2010), and alignment of major trait genes and quantitative trait loci (QTL) to integrated linkage and physical map (Mace et al. 2011) had strengthened the foundation for better integration of molecular marker technologies to dissect complex traits such as *Striga* resistance.

With the invention of Next Generation Sequencing (NGS) technologies, identification of a large number of markers, especially Single Nucleotide Polymorphism (SNPs), has become cheap as compared to the other marker systems. Utilizing Illumina NGS platform, Ed Buckler Lab at Cornell University in Ithaca, New York, developed a technically very simple and highly multiplexed (96-plex/384-plex) method for rapid sequencing and associated bioinformatics pipeline for genotyping the germplasm and is referred as Genotyping-by-Sequencing (GbS) (Elshire et al. 2011). These sequences produced are aligned to reference genome, BTx623 (Paterson et al. 2009) to identify SNPs with help of customized bioinformatics pipelines with appropriate computational power, which still remains the major challenge with handling the large datasets generated using NGS tools. This extensive coverage with a large number of SNPs across genome helps identify SNPs closest to or inside the genes associated with *Striga* resistance, and can be a part of customized SNPs assay using BeadXpress platform or CAPS markers at lower cost for further genotyping to transfer this trait to desired recurrent parent of sorghum. This will greatly improve the efficiency of introgression of component traits underlying different *Striga* resistance mechanisms of by reducing breeding cycles (for recurrent parent recovery) and further recombining these for development of improved *Striga*-tolerant varieties.

Application of NGS tools like GbS for dissecting complex traits such as *Striga* on DNA sequence level will capture most of the functional factors of genome related to trait expression. But other application of NGS tools in RNA sequencing (commonly referred as RNA-seq) will help capture the regulatory parts (Ozsolak and Milos 2011). For a complex trait such as *Striga* resistance, involving host-parasite interactions, many growth and development pathways from both host and parasite life cycles are involved in its expression. Application of RNA-seq platforms can help understand the role of regulatory and transcription factors (including small RNA, micro RNA) and their interaction with other pathways. There is big interest to utilize recent advances in RNA-seq technologies with the recombinants identified from fine-mapping exercise to move toward better understanding the *Striga* resistance in sorghum. As knowledge of QTL underlying resistance traits increases, comparative genomic approaches will aid detection of *Striga* resistance genes in syntenic regions of the rice, sorghum, maize, and pearl millet as sequence information becomes available.

Recently Yoshida et al. (2010) generated a full-length enriched cDNA library of *S. hermonthica* by sequencing over 37,000 clones and

identified over 17,000 unigenes. The comparative analysis of this unigene dataset with other plant genomes or ESTs revealed that approximately 80% of the unigenes had homologes in other dicotyledonous plants including *Arabidopsis*, poplar, and grape. Interestingly they found 589 unigenes that were conserved in the hemiparasitic *Triphysaria* species, a close relative of *Striga*, but not in other plant species. Furthermore they also identified 1,445 putative SSRs in the *S. hermonthica* unigene dataset. These recently developed extensive set of molecular resources using advanced molecular tools will help in studying *S. hermonthica* for genome annotation, gene discovery, functional analysis, molecular breeding, comparative mapping with different plant genomes, epidemiological studies, and studies of plant evolution. The recently started Parasitic Plant Genome Project (Westwood et al. 2012) aims to develop new tools for understanding the biology of *Orobanche* and *Striga*. This project had sequenced transcripts from three parasitic species and a nonparasitic relative in the *Orobanchaceae* with the goal of understanding genetic changes associated with parasitism. The species studied span the trophic spectrum from free-living nonparasite to obligate holoparasite. Parasitic species used included: *Triphysaria versicolor*, a photosynthetically competent species that opportunistically parasitizes roots of neighboring plants; *Striga hermonthica*, a hemiparasite that has an obligate need for a host such as sorghum; and *Orobanche aegyptiaca*, a holoparasite with absolute nutritional dependence on a host. *Triphysaria* is a genus of five hemiparasitic species that grow as common annuals throughout the Pacific Coast of the western United States (Hickman 1993). *Triphysaria* has a broad host range that includes maize, rice, and Arabidopsis, and is closely related to the agricultural pests *Striga* and *Orobanche*. *Triphysaria* has no agricultural significance and so can be grown without quarantine restrictions (Goldwasser et al. 2002). *Triphysaria* flowers are amenable to classical genetic manipulations and genomic resources are being developed, making *Triphysaria* a useful model species for parasite studies (Torres et al. 2005). For the genome project, tissues for transcriptome sequencing from each plant were gathered to identify expressed genes for key life stages from seed conditioning through anthesis. Importance of this project lies in that the two of the species studied, *S. hermonthica* and *O. aegyptiaca*, are economically important weeds and the data generated by this project are expected to aid in research and control of these species and their relatives. The sequences generated through this project will provide an abundant molecular resource for understanding population dynamics, as well as provide insight into the biology of parasitism and advance progress toward understanding parasite virulence and host resistance mechanisms. In addition, the sequences provide important information on target sites for herbicide action or other novel control strategies such as trans-specific gene silencing.

RNA interference (RNAi), or post-transcriptional gene silencing (PTGS), is a conserved mechanism in eukaryotes by which double-stranded RNA molecules (dsRNA), formed either by complementary base pairing of transgenic sequences or by fold-back of endogenous noncoding sequences, are processed by Dicer-like nucleases into short 21–24 nt interfering RNAs (siRNA) or micro-RNAs (miRNAs). These small RNAs are then incorporated, along with Argonaute-like proteins, into RNA-induced silencing complexes that direct the degradation of endogenous RNAs that are homologous to the siRNAs (Bartel 2004; Baulcombe 2004). When siRNAs are introduced into specific tissues of a plant by biolistics or agroinfection, siRNA moves through plasmodesmata into other tissues in a non-cell-autonomous fashion (Voinnet 2005). RNA-dependent RNA polymerase amplifies the primary siRNA, allowing further spread of the silencing signal (Himber et al. 2003).

RNAi signals can also enter the phloem and spread systemically throughout a plant, and even

across graft junctions from transgenic stocks to nontransgenic scions, although the nature of the translocated molecule is not known. *Agrobacterium*-based vectors have been developed to deliver siRNA precursors into plants in order to selectively target endogenous genes for inactivation. These vectors are designed so that the target RNA forms self-complementary, hairpin structures (hpRNA) that result in localized dsRNA regions that are cleaved into siRNA molecules by Dicer-like nucleases (McGinnis et al. 2005).

Tomilov et al. (2008) studied the transspecific gene silencing between host and parasite plants using transgenic roots of the hemiparasitic plant *T. versicolor* expressing the GUS gene to parasitize transgenic lettuce roots expressing a hairpin RNA containing a fragment of the GUS gene (hpGUS). These experiments described movement of RNAi molecules between parasitic and host plants. Using an *Agrobacterium rhizogenes*-mediated transformation system, Tomilov et al. (2008) developed root cultures of *Triphysaria* that express the GUS reporter gene (Jefferson et al. 1987). These roots retained their ability to develop haustoria in response to host factors and to invade host roots. The results of these experiments indicated that RNAi signals are translocated across haustorial junctions in both directions and mediate gene silencing in both parasite and host plants. Once genes critical to parasite growth and development are identified, these parasite-specific sequences could be cloned into hairpin vectors and transformed into plants for silencing of parasite genes by generating siRNA in the host. This could provide a novel strategy for controlling parasitic weeds.

Similarly, recent cloning and functional characterization of a race-specific R gene from cowpea (Timko et al. 2012) opens the door for further exploration of the mechanism of host resistance and provides a focal point for studies aimed at uncovering the molecular and genetic factors underlying parasite virulence and host selection.

Managing *Striga* in Sorghum: Current Technologies and Strategies

Farmers impacted by *Striga* occupy a very heterogeneous biophysical, cultural, social, economic, and political landscape with common key abiotic constraints of water and nutrient availability (Waddington et al. 2010). Failure to recognise this fact will invariably hinder the adoption of *Striga* control approaches. To harness the impact of variable efficacy of individual control practices, many advocate integrated *Striga* control (ISC) approaches – combinations of cultural and, where available and applicable, seed-based technologies (Schulz et al. 2003; Kamara et al. 2007). Nonetheless, just as there is no magic bullet for *Striga* control, there is no magic shotgun cartridge either (Douthwite et al. 2007). Technologies need to be packaged in such a way as to suit the abiotic, biotic, and market-access constraints the farmers experience. It is perhaps common sense that technologies that fit in with the current time-tested and culturally inherited farming systems are more likely to be rapidly adopted by the majority of farmers than those that demand significant modification to farming practices.

Information dissemination is key to the adoption of *Striga* control technologies. For example, weed management is the primary bottleneck to yield increases by smallholder farmers, yet has been neglected by researchers in recent years on the premise that this is a straightforward crop husbandry practice in which farmers should invest. However, because of labor shortages, most farmers prefer to use herbicides for controlling weeds other than *Striga*. As patents for key herbicides (such as glyphosate and atrazine) and others expire, their availability and use in cereal production areas are increasing, in many areas without technical guidance or understanding potential health risks involved. This information should include a simple explanation to farmers as to how control measures work; to explain, for example, that IR maize is a

combination of variety and herbicide, that both are needed to control the *Striga*, and that farmers should wash their hands after handling imazapyr-treatedmaize seed before planting other seed. Information should also be provided on complementary control technologies to increase awareness of ISC options and value for *Striga* control and for wider yield improvement (e.g., fertilization). Research support is thus needed to guide safe and efficient use and to develop alternative options for diverse dryland cereal production environments.

Integrated *Striga* management packages have been designed that include: *Striga* resistant varieties; judicious and appropriate timing and application of phosphate, nitrogen, and composite fertilizers in combination with organic fertilizers; and water conservation measures using tied ridges (or local alternatives). When demonstrating ISC technologies to farmers, including at least one method in all packages that gives rapid *Striga* control would facilitate sustained interest in ISC, allowing the sustained adoption of longer-impact technologies such as tools to improve soil fertility to continue (Douthwite et al. 2007). Of these approaches, development of resistant crop cultivars has been recognized as the most effective and feasible method. To date, *Striga*-resistant sorghum cultivars – such as N13, SRN39, Framida, 555, ICSVs, SRN39 derivatives (P401 to P409), Soumalemba (IS15401), Seguetana CZ, and CMDT45 – have been identified and, as has been observed by Tabo et al. (2006) and ICRISAT (2009), these can be integrated with available crop management options to enhance productivity. However, understanding the molecular nature of the plant-plant interactions is a major barrier. High genetic variability of parasite populations coupled with large, long-lived *Striga* seed banks makes it unlikely that single-gene resistance will be useful in the field. The development of durable polygenic resistance requires pyramiding of appropriate resistance genes and will depend on knowledge of the relationship between host resistance and parasite race structure. QTL studies and MAS certainly have the potential to aid the development of resistant cultivars in the short-to-medium term. Looking forward, a major challenge is to exploit genomic technologies to further advance our understanding of the biology of susceptible and resistant interactions allowing the development of novel control strategies that are appropriate for the agricultural and socioeconomic environment where this parasite is such a devastating problem.

Conclusion

Integrated *Striga* control remains the most effective way to manage *Striga* infestations in sorghum as a long-term approach. Increased understanding of the host-parasite interactions and possibility of identification of newer resistance factors by employing recent technologies such as NGS tools, RNA sequencing applications, RNAi technology, and precise phenotyping platforms will pave the way for developing cultivars with improved resistance. With molecular biology tools being practiced successfully in Africa and improved access to recent technologies such as GbS spreading to remote sorghum breeding programs, development of resistant cultivars with different resistance factors stacked together will form best short- and medium-term approaches toward *Striga* control.

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