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MODERN BREEDING APPROACHES FOR DURABLE RESISTANCE AGAINST THE PARASITIC PLANT *STRIGA*

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Crop losses caused by parasitic plants of the genus *Striga* pose a great danger to the livelihoods of millions of smallholder farmers in Africa. The parasite attaches to host crops and siphons nutrients leading to severe retardation and crop death. Controlling *Striga* is difficult because of the parasite's ability to produce large amounts of seeds that can remain dormant in the soil for decades – only germinating in response to chemical cues (strigolactones) from the host. In recent years, breeding crops for host-based resistance has been prioritized. However, such programs have not taken into account *Striga's* ability to overcome host resistance. As a result, introduced resistance fails because of increased *Striga* virulence (infection severity). This article reviews technologies for a new paradigm in *Striga* resistance breeding that incorporates host resistance breeding with well-informed knowledge of parasite resistance in order to ensure durability of resistance.

KEY WORDS: STRIGA, HOST BASED RESISTANCE, GENOME WIDE ASSOCIATION MAPPING, RNA SEQUENCING

Introduction

One of the greatest challenges to staple cereal production in Africa is the parasitic plant *Striga* that kills crops by attaching to their roots and siphoning nutrients. The *Striga* genus has over thirty species distributed over 50 countries in Sub-Saharan Africa (SSA), causing an estimated 7 billion dollars´ worth of crop losses every year (Ejeta, 2007). Of the many *Striga* species, S. *hermonthica*, S. *asiatica* – both parasitic to cereal crops – and S. *gesneriodes* – specific to cowpea – are the deadliest. It is difficult to control *Striga* because of its ability to produce extremely large numbers of seeds – as each *Striga* flower spike can produce over 50,000 seeds (Yoder and Scholes, 2010). These seeds can remain viable in the soil for up to 14 years, and germinate only in reaction to chemical cues given by the host plant's root exudates (Bouwmeester et al., 2007).

Desperate to suppress *Striga* infestations, smallholder farmers in Africa have battled the plant using cultural and agronomic practices such as hand-weeding, crop rotation (Kanampiu et al., 2018), use of resistant crops that do not allow *Striga* to attach (Cissoko et al., 2011) use of tolerant crops that withstand *Striga* attachment with no yield reduction, and use of 'trap crops' to encourage parasite germination on incompatible hosts (Midega et al., 2010). Although these methods have been extensively encouraged over the years, crop losses and the host range of these parasites have continued to increase.

Low success rates in achieving significant *Striga* management has led to extensive searches for cultivars and wild relatives of sorghum that are resistant to the parasite (Mbuvi et al., 2017; Mutinda et al., 2018; Rich et al., 2004). These resistant varieties have been introduced in some breeding programs in SSA (Ngugi et al., 2016). Still, the resistance is often weak and sometimes breaks down due to the appearance of new *Striga* ecotypes (Botanga and Timko, 2007).

Resistance breakdown in most host-pathogen interactions occurs when a pathogen manages to avoid the host defense surveillance mechanism. This arms race (host resistance versus pathogen virulence) is described well in the 'zigzag' model first proposed by Florr, (1971). In this two-level defense model, Patterns Triggered Immunity (PTI) is first induced by the perception of different Pathogen Activated Molecular Patterns (PAMPs) followed by Effector Triggered Immunity (ETI), that is induced in response to pathogen factors – effectors (Alfano & Collmer, 2004).

To avoid the activation of the host immune response – and consequently resistance breakdown, the parasite rapidly changes its PAMPs as well as effectors. This phenomenon has been well documented in plant-microbe interactions. For example, (Van de Wouw et al., 2010) describe the breakdown of the Rlm1 gene that confers resistance against blackleg fungus, *Leptosphaeria maculans* in *Brassica napus* (canola) due to evolution of the avirulence gene, AvrLm1, in fungal populations.

Although never described for *Striga*, increasing evidence points to a possible role of pathogen effectors in race specific interactions of S. *gesneriodes*. In the aforementioned report, the authors cloned a gene-for-gene *Striga* resistance gene (RSG3-301) which has a coiled-coil nucleotide binding site leucine-rich repeat domain (Li and Timko, 2009). Resistance as a result of the gene is only activated by a specific hypervirulent S. *gesneriodes* race SG3 from Niger (Timko et al., 2012), pointing to the possibility of avirulence gene action.

Evidence for possible involvement of effectors in *Striga*-host interactions underscore the importance of developing new paradigms in breeding against *Striga* that take into account virulence and host-pathogen specificity.

Exploiting host resistance as a control strategy against Striga

Host based resistance provides an attractive breeding strategy because it is cost effective and sustainable. Previous research has shown that sorghum is an attractive source for resistance against S. *hermonthica* and S. *asiatica* compared to limited sources of resistance from maize (Mutinda et al., 2018), rice (Cissoko et al., 2011; Gurney et al., 2006) and their wild relatives (Amusan et al., 2008). Such resistance is evident in *Striga* infested fields where if maize and sorghum are grown side by side, maize is more adversely affected by the parasite (Figure 1). Sorghum's high capacity for *Striga* resistance can be explained by the fact that it co-evolved with *Striga* in northeastern Africa. This region which harbors the greatest diversity of both wild and cultivated sorghum (Paterson, 2008), is also the natural range of the *Striga* parasite (Musselman and Hepper, 1986).

Striga resistance can either act before (pre-attachment resistance) or after infection (post-germination resistance). Some host crops do not induce *Striga* to germinate because the

hosts do not produce sufficient amounts of *Striga* germination stimulants – called Strigolactones, or because *Striga* receptors that perceive germination stimulants are insensitive to the strigolactone produced by the host. These hosts demonstrate what is known as pre-germination resistance and are referred to as low germination stimulant (*LGS*) varieties (Jamil et al., 2011).

For example, some sorghum varieties have the *LOW GERMINATION STIMULANT* (*LGS*) trait and these have been exploited as a control strategy against *Striga* (Hess and Ejeta, 1992). The genetic cause of this resistance has recently been found to be due to a natural mutation in the *LGS* loci of sorghum (Gobena et al., 2017). Plausibly, many more varieties of sorghum habouring a mutation on the *LGS* loci exist but have not been identified. The vast genetic diversity of sorghum globally as well as the explosion of sequencing technology now allows screening and identification of such mutations.

An effective strategy to perform such screening and identification is genome wide association (GWAS) mapping. In this strategy, a large number of diverse individuals, or a mapping population is genotyped with high resolution genetic markers. This is followed by an analysis of the desired trait (phenotype). Subsequently, genotype and phenotype are correlated to determine association of the trait with particular genetic loci – identifiable by a genetic marker. With regard to pre-germination resistance against *Striga*, it is possible to measure trait data by determining the frequency of sorghum's resistance to stimulating Striga seed germination. Further pre-germination resistance data can be obtained by analysis of the amounts and types of strigolatones present in root exudate.

In addition to the low stimulation of *Striga* germination, some *Striga* hosts exhibit post-attachment *Striga* resistance mechanisms that act after *Striga* attachment and attempted penetration into host. These mechanisms result in physiological or biochemical barriers, which prevent the *Striga* haustorium from connecting to the host xylem (Maiti et al., 1984). Host plants can also produce secondary metabolites that block parasite ingression (Mbuvi et al., 2017) or induce a hypersensitive immune response at the host-parasite interphase (Mohamed et al., 2003). In yet other instances, *Striga* produces enzymes that degrade host tissues (Rogers and Nelson, 1962).

This resistance has been described as quantitatively inherited (Haussmann et al., 2004). In line with this assertion, *Striga* resistance quantitative trail loci (QTL) has been identified in sorghum (Haussmann et al., 2004) and rice (Swarbrick et al., 2009) in a Recombinant Inbred Line (RIL) population that was derived from a cross between the resistant cultivar N13 and a susceptible cultivar E36-1 (Haussmann et al., 2004). Such QTLs have been further utilised in breeding programs in Africa (Masiga et al., 2014; Yohannes et al., 2015).

The biological and genetic mechanisms underpinning this form of resistance in *Striga* are not yet understood but it is reasonable to assume that the effect is a result of multiple genes acting to fortify the host against invasion by the parasite. It is also possible that *Striga* resistance may be acting qualitatively in a gene-for-gene resistance mechanism as was described in the case of *S. gesneriodes* (Li and Timko, 2009). Both of these forms of resistance can be identified using GWAS. To achieve this, sorghum accessions/mapping

populations can be evaluated for *Striga* resistance using a high throughput *Striga* resistance screening strategy such as the one based on rhizotrons (Mbuvi et al., 2017). In this resistance screening strategy, transparent root observation chambers are used to assay host root development and infection by *Striga*. Resistance is scored after 21 days for metrics of: number, size and biomass of *Striga* attached on host roots. Sorghum accessions can then be genotyped and associated loci can be used in *Striga* resistance breeding programs.

In addition to GWAS, resistance genes can be identified using RNA sequencing to determine genes – in resistant sorghum – that are differentially expressed upon *Striga* infection. This approach can help pinpoint resistance (R) genes activated by *Striga* infection. Ideally, RNA sequencing to obtain resistance genes should be performed on germplasm that has been screened and found to be *Striga* resistant.

The plethora of genes identified will help in development of 'an arsenal' of resistance genes for deployment into susceptible varieties using modern breeding approaches. To ensure that *Striga* does not overcome resistance, multiple genes can be combined together and introduced into crop varieties popular to farmers.

Preventing *Striga* from overcoming host resistance

Even with good resistance sources, it is possible that *Striga* can overcome resistance because of rapidly evolving pathogen virulence. It is logical to assume that *Striga* can – with time – acquire the ability to breach a host's defenses through suppression of plant immunity and promotion of pathogenesis by injecting effectors into plant cells. Such a hypothesis is supported by work on other host-pathogen interactions where it has been shown that secreted effectors can suppress both PTI immunity and ETI protein-activated immunity (Alfano and Collmer, 2004).

Plausibly, *Striga* virulence genes undergo rapid adaptive diversification in order to acquire new host specificities as has been described in other pathogens (Li et al., 2009; Meyers et al., 1998). Such evolution is accelerated by *Striga*'s high genetic diversity; high outcrossing rates; dormancy and multiple hosts. The concept of fast evolving virulence in *Striga* is supported by the striking variations in the virulence profiles of different *S. hermonthica* populations (Mbuvi et al., 2017). Although not documented, it is also likely that such variations occur among individuals of *S. hermonthica* isolated from different host sources (because different hosts have varying susceptibilities and therefore varying selection pressure), and from seeds deposited in different seasons or years (because varying environmental stressors impose different selection pressures over time).

Therefore, the *S. hermonthica* in soil is highly heterogenous with expected variations in virulence and host specificity and, to avoid resistance breakdown, future breeding programs should take into account the vast *Striga* genetic diversity. Such work should seek to genotype *Striga* from different eco-geographical regions and identify fingerprints unique to these regions. Subsequently, the specificity of *Striga* ecotypes and virulence can be linked with genetic markers. This data will in turn inform on which crop variety is appropriate for which region, thereby preventing *Striga* from overcoming host resistance.

Such knowledge can also be used as a baseline for future reference when establishing the evolution patterns and rates. Certainly, interactions between *S. hermonthica* and the environment – including the physical and biological properties of soil as well as the microbiome can lead to variations *in Striga*'s ability to infect a host and should be areas of future investigation.

Summary and perspectives

Today, the explosion of new information coming from fully sequenced genomes, as well as *Striga* sequencing initiatives, plus high throughput tools for transcriptome analysis, provide a means to complement the more traditional approaches of *Striga* management. However, to achieve meaningful success in *Striga* management, it is critical to integrate good agricultural practices with other *Striga* management strategies.

Figure 1: Sorghum is a good source of natural resistance against Striga hermonthica. Maize (left) is more adversely affected by parasite compared to the sorghum (right).

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