Weed Science

cambridge.org/wsc

KSTP 94, an Open-pollinated Maize Variety Has Postattachment Resistance to Purple Witchweed (*Striga hermonthica*)

Sylvia Mbula Mutinda[1], Joel Masanga[2], J. Musembi Mutuku[3], Steven Runo[4] and Amos Alakonya[5]

[1]Master's of Science Student, Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, [2]Doctoral Student, Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya, [3]Postdoctoral Research Fellow, Biosciences Eastern and Central Africa–International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya, [4]Senior Lecturer, Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya and [5]Senior Research Fellow, Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, and Postdoctoral Research Fellow, International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria

Weed Management

Cite this article: Mutinda SM, Masanga J, Mutuku JM, Runo S, Alakonya A (2018) KSTP 94, an Open-pollinated Maize Variety Has Postattachment Resistance to Purple Witchweed (*Striga hermonthica*). Weed Sci 66:525–529. doi: 10.1017/wsc.2018.24

Received: 8 February 2018
Revised: 18 April 2018
Accepted: 18 April 2018

Associate Editor:
Franck E. Dayan, Colorado State University

Key words:
KSTP 94; postattachment *Striga* resistance; *Striga*

Author for correspondence:
Steven Runo, Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844, 00100 GPO, Nairobi, Kenya.
(Email: runo.steve@ku.ac.ke)

Abstract

Striga spp. are obligate root hemiparasites that constrain cereal production in sub-Saharan Africa. Although purple witchweed [*Striga hermonthica* (Delile) Benth.] and Asiatic witchweed [*Striga asiatica* (L.) Kuntze] infect all cereal crops, maize (*Zea mays* L.) is particularly vulnerable to their infestations. A sustainable control strategy for *Striga* would be to breed crops with host-based resistance as part of an integrated management plan. In maize, the open-pollinated variety Kakamega *Striga*-tolerant population of the year 1994 ('KSTP 94') has been popularized as a *Striga*-tolerant/resistant variety. This resistance was earlier reported to result from production of low amounts of sorgomol, a less potent strigolactone. To determine whether KSTP 94 harbors postattachment resistance, we used a soil-free assay based on observation chambers called rhizotrons. We found that the size of *Striga* seedlings attached to 'CML 144' (a susceptible maize inbred line) were 2.5-fold longer than those on KSTP 94. In addition, KSTP 94 had significantly fewer *Striga* attachments, which corresponded to significantly lower biomass (2.6-fold) compared with CML 144. Histological analysis revealed that the low *Striga* growth and development while infecting KSTP 94 was due the parasite's inability to penetrate the host's endodermis and make effective xylem–xylem connections. We therefore conclude that in addition to preattachment resistance, KSTP 94 exhibits postattachment resistance to *S. hermonthica* and could therefore be a good genetic source for postattachment resistance breeding.

Introduction

Purple witchweed [*Striga hermonthica* (Delile) Benth.], a root hemiparasitic weed, is one of the most devastating constraints to corn (*Zea mays* L.) production in Africa. *Striga* causes yield losses of up to 100%, translating to more than US$7 billion yearly, and this affects livelihoods of more than 300 million people worldwide (Ejeta 2007). Among the five major *Striga* species (*S. hermonthica*, Asiatic witchweed [*Striga asiatica* (L.) Kuntze], cowpea witchweed [*Striga gesnerioides* (Willd.) Vatke], *Striga aspera* (Willd.) Benth., and *Striga forbesii* Benth.), *S. hermonthica* and *S. asiatica* are the most economically devastating.

Striga has a highly coordinated life cycle. Its association with the host starts with perception of strigolactones—hormones exuded by host roots that serve as germination stimulants. After germination, *Striga* immediately develops a specialized feeding structure—the haustorium—in response to haustorium-inducing factors from the host. The haustorium penetrates the host root tissues until it connects to the host xylem and siphons nutrients, leading to host growth retardation, stunting, and chlorosis. *Striga* then emerges from the soil and flowers to produce up to 20,000 viable seeds (Teka 2014).

Striga management approaches include: intercropping hosts with trap crops that induce suicidal germination of *Striga* seeds, application of soil amendments such as fertilizer and manure, hand pulling of emerged *Striga*, and application of herbicides (Atera et al. 2013; Ejeta 2007; Teka 2014). These strategies are only moderately effective, because *Striga* continues to expand its natural range and cause more crop losses.

The most effective and sustainable control strategy is an integrated approach that uses innate host-derived resistance. Therefore, identification of new sources of *Striga* resistance has been prioritized in *Striga*-resistance breeding programs. Sources of resistance to *Striga*

have been identified in maize (Amusan et al. 2008), rice (*Oryza sativa* L.) (Gurney et al. 2006), sorghum [*Sorghum bicolor* (L.) Moench.] (Haussmann et al. 2004; Mbuvi et al. 2017; Mohamed et al. 2003), and cowpea [*Vigna unguiculata* (L.) Walp.] (Menkir 2006). Such host-based *Striga*-resistance mechanisms act either before (preattachment resistance) or after physical contact with the host (postattachment resistance). Preattachment resistance occurs when a host produces low amounts of strigolactones or when *Striga* receptors that perceive germination stimulants are insensitive to the strigolactone produced by the host. This is because strigolactone must bind to hypersensitive to light receptors in *Striga* for germination to occur. Binding causes degradation of an F-box protein, which in turn activates gene regulatory processes that lead to *Striga* germination (Lumba et al. 2017). There is a great diversity of strigolactones produced by different hosts, each with different receptor-binding efficiencies based on their chemical structures (Yoneyama et al. 2010). For example, the *Striga*-resistant sorghum variety 'SRN39' was found to produce low amounts of orobanchol, resulting in low *Striga* germination (Gobena et al. 2017). Preattachment resistance can also be due to less production of haustorial initiation factors, therefore causing failure in effective development of haustorium (Rich et al. 2004).

In contrast, postattachment *Striga*-resistance mechanisms act after *Striga* has attached and attempted to penetrate the host. These mechanisms result in physiological or biochemical barriers that prevent the *Striga* haustorium from connecting to the host's xylem. Host plants can also produce secondary metabolites that block parasite ingression or induce a hypersensitive immune response at the host–parasite interface (van Dam and Bouwmeester 2016). In some instances, *Striga* produces enzymes that degrade host tissues and barriers before making a connection to the host's xylem (Maiti et al. 1984; Rogers and Nelson 1962).

Sorghum coevolved with *Striga* in the African savanna and therefore harbor some resistance to the weed. In contrast, maize is alien to Africa and generally more susceptible to the weed. Until now, *Striga* resistance in maize has mainly come from its wild grass relatives like diploperennial teosinte [*Zea diploperennis* (Itis, Doebley & Guzman) (Amusan et al. 2008; Lane et al. 1997) and eastern gamagrass [*Tripsacum dactyloides* (L.) L.] (Gutierrez-Marcos et al. 2003). Introgression from such sources has led to development of a *Striga*-resistant maize inbred line 'ZD05' suitable for integration in breeding programs in western Africa (Amusan et al. 2008).

In eastern Africa, the open-pollinated KSTP 94 has been used as a *Striga*-resistant maize variety since 1995, especially in western Kenya, a *Striga*-prone region. KSTP 94 exhibits remarkable resistance to *Striga* under field conditions; a characteristic that has made it a subject of intense research in the region. Such research has found the resistance of KSTP 94 to be due to production of low amounts of the strigolactone sorgomol (Yoneyama et al. 2015). Sorgomol is a strigolactone that does not efficiently induce *Striga* germination, and the resistance of KSTP 94 was therefore concluded to be due to preattachment resistance.

To further characterize host-based resistance in KSTP 94, we sought to determine whether it also exhibits postattachment *Striga* resistance. To achieve this, we used a soil-free laboratory assay based on rhizotrons (Mbuvi et al. 2017) to compare the resistance response of KSTP 94 with a susceptible inbred maize line (CML 144).

Materials and Methods

Postattachment Resistance Assays for Striga

Preconditioning of Striga hermonthica Seeds

Striga hermonthica seeds (obtained from maize-growing fields in Kibos, western Kenya in 2015) were used for postattachment resistance assays. Seeds were preconditioned as described in Gurney et al. (2003) before germination. First, *Striga* seeds (25 mg) were surface sterilized using 10% (v/v) NaOCl for 10 min with gentle agitation, rinsed three times with sterilized distilled water, and then spread on a glass fiber filter paper (Whatman GFA) placed on sterile petri dishes. Approximately 5000 *Striga hermonthica* seeds were then incubated for 11 d at 29 C. Finally, seeds were germinated by treatment with 3 ml of 0.1 ppm GR24 (Chirax, Amsterdam) and incubated overnight at 29 C. Germination efficiency of the *Striga* seedlings was determined using a Leica MZ7F microscope (Leica, Germany), and only plates showing >70% germination were used to infect maize roots.

Infection of Maize Roots with Striga Seedlings

Maize inbred line CML 144, obtained from the International Maize and Wheat Improvement Center (Nairobi, Kenya), and open-pollinated variety KSTP 94 from the Kenya Agricultural Livestock Research Organization (Kakamega, Kenya) were screened for postattachment *Striga* resistance. Seeds were first germinated in 10 cm by 10 cm by 7 cm pots filled with vermiculite. At 5 d postplanting, maize seedlings were transferred to rhizotrons (25 cm by 25 cm by 5 cm Perspex® chambers) (Mbuvi et al. 2017) prepared as follows: chambers were lined with 25 cm by 5 cm by 5 cm foam strips at the bottom to absorb excess water and packed with vermiculite, then a 50-micron-thick mesh was placed on top. A germinated maize seedling was placed on the mesh, the chamber closed, and wrapped with aluminum foil. Plants were then maintained in the glasshouse under a 12-h light/12-h dark photoperiod with 60% humidity and day and night temperatures of 28 and 24 C, respectively. During growth on rhizotrons, plants were drip irrigated with 25 ml of 40% Long Ashton nutrient solution (Hudson 1967). Maize seedlings with well-developed roots (10 d on rhizotrons) were then infected with 25 mg pregerminated *S. hermonthica* seeds (per plant) by aligning the *Striga* seeds along the maize roots with a soft paintbrush. Five plants per genotype were screened in a randomized complete block design in three replicates (single experimental run).

Analysis of Postattachment Striga Resistance in Maize

Measures of Striga Resistance

Infected maize roots were screened for *Striga* resistance at 9 and 21 d after infection (DAI). At 9 DAI, *Striga* seedlings attached on maize roots were observed and documented using a stereomicroscope (Leica MZ4 fitted with a DFC320FX camera (Leica, Germany). At 21 DAI, all *Striga* attached to maize roots were harvested, placed on 90-mm petri plates, and photographed. Image analysis using ImageJ v. 1.45 (http://rsb.info.nih.gov/ij) was then carried out to determine the length and the number of *Striga* parasitizing each host plant. To determine the total *Striga* biomass attached on maize roots, harvested *Striga* seedlings were oven-dried for 7 d at 45 C and weighed. ANOVA was carried out to compare the means for biomass, length, and number of infecting *Striga* using statistical analysis software (SAS v. 9.1, SAS Institute, Cary, NC, USA) and presented as box plots prepared in R software.

https://doi.org/10.1017/wsc.2018.24 Published online by Cambridge University Press

Significant differences between the means were according to Tukey's honest significant difference test at a 95% confidence interval.

Histological Analysis of Striga Resistance in Maize

To determine the extent of parasite development within the host root, microscopic screening of the connection point between *Striga* and maize roots was carried out according to Gurney et al. (2003). Tissues at the point of host–parasite infection were collected from rhizotrons at 9 DAI and fixed using Carnoy's fixative (4:1 ethanol:acetic acid). For each variety (CML 144 or KSTP 94), 3 attachments from 5 rhizotrons were collected, making a total of 15 samples per variety. Samples were dehydrated with 100% absolute ethanol for 30 min, followed by pre-infiltration in ethanol-Technovit® (Haraeus Kulzer GmbH) solution for 2 h and a further pre-infiltration step in 100% Technovit® solution for 1 h. These tissues were then left in fresh 100% Technovit® for 3 d. For embedding, samples were placed in Eppendorf lid molds containing 1 part Technovit® and 15 parts hardener and left to set. Embedded tissues were then mounted on wooden blocks using the Technovit® 3040 kit following the manufacturer's instructions (Haraeus Kulzer GmbH). Small (5-micron-thick) sections were cut using a Leica RM 2145 microtome (Leica, Germany) and transferred to glass slides. The sections were stained using 0.1% toluidine blue O dye in 100 Mm phosphate buffer for 2 min, washed in distilled water, and dried at 65 C for 30 min. The microscope slides were then covered with slips using DePex (BDH, Poole, UK), observed, and photographed using a Leica DM500 microscope mounted with a Leica ICC50 camera (Leica, Germany).

Results and Discussion

An effective measure of host resistance to *Striga* is achieved by determining the number and size of the parasite seedlings infecting the host and their biomass. A resistance response is characterized by fewer, smaller, and less biomass relative to a susceptible host (Figure 1A and B). We found that CML 144, the susceptible maize inbred line, had significantly more *Striga* attachments (Figure 2A), longer *Striga* seedlings (Figure 2B), and a higher *Striga* biomass (Figure 2C). CML 144 had an average of 72.93 ± 9.40 attachments per plant, while KSTP 94 had significantly fewer attachments (44.80 ± 10.22). Similarly, longer *Striga* seedlings were observed on CML 144 (2.03 ± 0.39 mm) compared with those on KSTP 94 (0.80 ± 0.12 mm). Finally, the biomass of *Striga* seedlings harvested from CML 144 was 39.93 ± 7.46 mg, which was significantly higher than that of seedlings from KSTP 94, which averaged 14.88 ± 4.07 mg per CML 144/KSTP 94 plant.

To further elucidate the underlying resistance mechanism of KSTP 94 after infection, we carried out histological analyses of *Striga*–host interactions at the attachment point at 9 DAI. *Striga* parasitism is considered to be successful when the vascular connection between host and parasite is established followed by efficient nutrient flow into the parasite. We found that in KSTP 94, 53% ($n = 15$) of *Striga* seedlings penetrated host tissue up to the cortical cells but did not go beyond the endodermis, hence failing to make xylem–xylem connections (Figure 3Ai and ii). However, this was not the case with CML 144, in which the parasite successfully attached and established xylem–xylem connections in all tissues sectioned (Figure 3Bi and ii).

Figure 1. *Striga hermonthica* seedlings growing on the roots of maize lines screened on rhizotrons 21 d after infection with a *S. hermonthica* ecotype from Kibos. (A) Susceptible maize inbred line CML 144, characterized by numerous *S. hermonthica* attachments. (B) Resistant open-pollinated maize KSTP 94, characterized by fewer and smaller attachments. White arrows indicate parasite attachment points to the host. Scale bar: 0.2 mm.

The ability of *Striga* to penetrate a host and make vascular connections is critical for the survival of this parasite. Our results suggest that *Striga* was able to successfully achieve parasitism in the susceptible variety CML 144 about 2.6-fold more frequently compared with KSTP 94. The significantly larger *Striga* size in the susceptible variety resulted in higher parasite biomass on susceptible line CML 144. A comparison of *Striga*'s ability to penetrate its host and complete its life cycle from our study and previous work on a resistant maize inbred line ZD05 developed from wild maize show striking similarities (Amusan et al. 2008). The frequency of formation of xylem–xylem connections between *S. hermonthica* and ZD05 was 12%, resulting in 88% fewer infections. This translated to significantly fewer *Striga* attachments.

Our study and earlier studies have reported host and *Striga* incompatibility. For example, Gurney et al. (2006) described this resistance mechanism between rice variety 'Nipponbare' and *S. hermonthica*. Similarly, the inbred line ZD05 described earlier showed incompatibility with *S. hermonthica* in Amusan et al. (2008). In all these cases, the parasite penetrates the host cortex but is deflected before it gets to the endodermis. The exact mechanism for this parasite's inability to penetrate the endodermis is unknown, but it seems plausible that molecules that

https://doi.org/10.1017/wsc.2018.24 Published online by Cambridge University Press

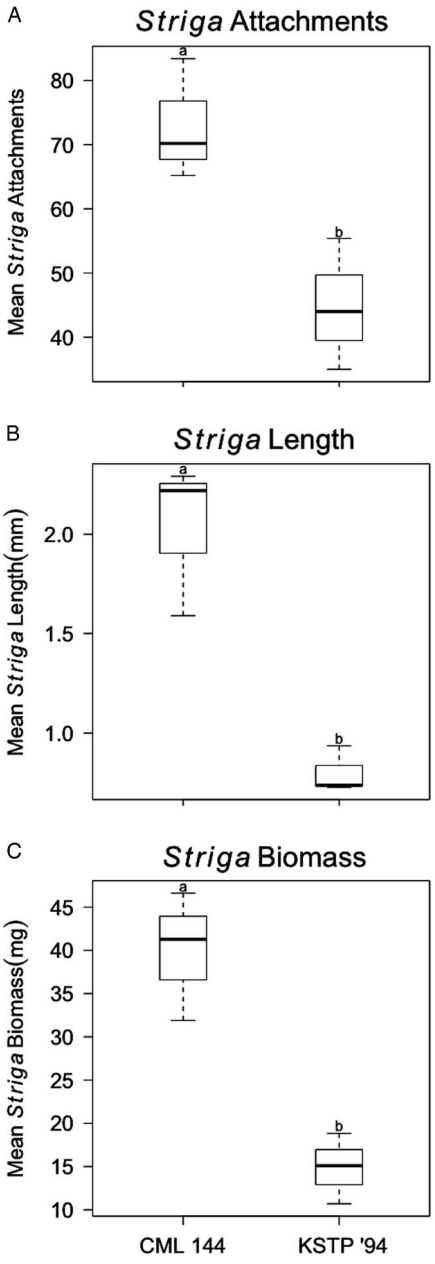

Figure 2. Postattachment resistance evaluation of maize inbred line CML 144 and KSTP 94 following infection with *S. hermonthica* seeds. (A) Mean number of *Striga* seedlings attached to host roots; (B) mean length of *Striga* seedlings; and (C) mean *Striga* dry biomass of parasite seedlings attached to the roots of each host. Data were collected 21 d after infection and are a mean of three replicates. Letters above each bar indicate significant differences ($P \leq 0.05$).

mediate interactions between *Striga* and hosts play an important role in resistance. Particularly, the resistance can be attributed to biochemical or physiological barriers from the host (Yoshida and Shirasu 2009) such as a tough sclerenchyma (Amusan et al. 2008).

Our findings emphasize the need to continuously screen germplasm for pre- and postattachment *Striga* resistance. We have identified an additional mechanism of resistance that protects against *Striga* in maize. Postgermination *Striga* resistance had been previously demonstrated in maize. However, this is the first time it has been shown in an open-pollinated maize variety that is not introgressed with wild germplasm. These results have significant *Striga* management implications in eastern Africa and demonstrate

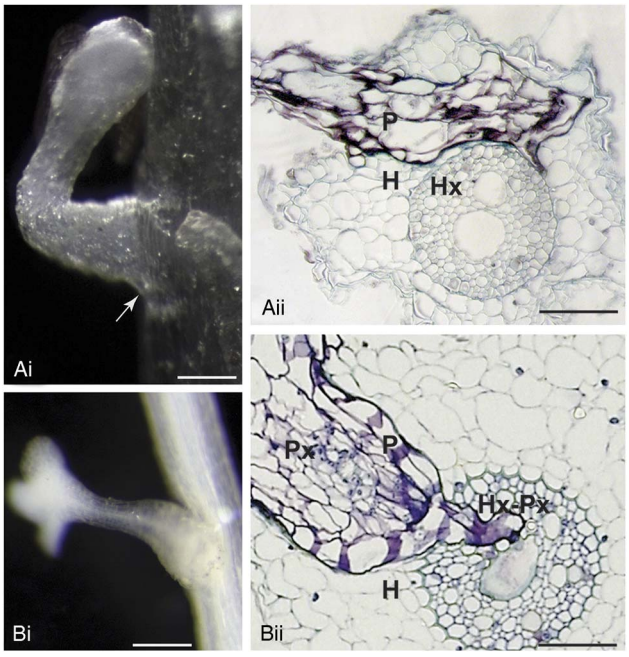

Figure 3. Histological analysis of postattachment resistance mechanisms to *S. hermonthica*. (Ai) Resistance interaction in KSTP 94, in which the parasite penetrates the host but exits (white arrow). Scale bar: 1 mm. (Aii) A transverse section through the haustorium of the resistant maize line KSTP 94. The parasite penetrates the cortex but is unable to breach the endodermal barrier and grows around the host vascular cylinder. Scale bar: 0.1 mm. (Bi) Susceptible interaction in CML 144 showing host penetration by the parasite for xylem–xylem connection. Scale bar: 1 mm. (Bii) Transverse section of a stained root tissue of CML 144 maize line 9 d after infection showing penetration of the host root cortex and endodermis as well as connections between the host and parasite xylem (Hx-Px). Scale bar: 0.1 mm. H, host, P, parasite, Px, parasite's xylem. In the susceptible interaction, the parasite penetrates the cortex and endodermis and connects to the xylem vessels of the host, allowing the haustorium to differentiate.

the greater importance of KSTP 94 than previously thought. This research underscores the need for further integration of KSTP 94 in breeding programs as well as determination of genetic mechanisms underlying this resistance.

Acknowledgments. The authors wish to acknowledge the International Foundation for Science for funding the project and the Plant Transformation Laboratory at Kenyatta University where this work was done. No conflicts of interest have been declared.

References

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 Phytol 178:157–166

Atera EA, Ishii T, Onyango JC, Itoh K, Azuma T (2013) *Striga* infestation in Kenya: status, distribution and management options. Sustain Agric Res 2:99–108

Ejeta G (2007) The *Striga* scourge in Africa: a growing pandemic. Pages 3–16 *in* Gressel J ed, Integrating New Technologies for *Striga* Control: Towards Ending the Witch-Hunt. 1st ed. London: World Scientific

Gobena D, Shimels M, Rich PJ, Ruyter-Spira C, Bouwmeester H, Kanuganti S, Mengiste T, Ejeta G (2017) Mutation in sorghum *LOW GERMINATION STIMULANT 1* alters strigolactones and causes *Striga* resistance. Proc Natl Acad Sci USA 114:4471–4476

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 Phytol 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 Phytol 169:199–208

https://doi.org/10.1017/wsc.2018.24 Published online by Cambridge University Press

Gutierrez-Marcos JF, Pennington PD, Costa LM, Dickinson HG (2003) Imprinting in the endosperm: a possible role in preventing wide hybridization. Biol Sci 358:1105–1111

Haussmann BI, Hess DE, Omanya GO, Folkertsma RT, Reddy BV, Kayentao M, Welz HG, Geiger HH (2004) Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. Theor Appl Genet 109:1005–1016

Hudson J (1967) Ecology and Classification of North American Freshwater Invertebrates. 3rd ed. San Diego: Academic. Pp 1022–1194

Lane JA, Child DV, Moore T, Arnold GM, Bailey JA (1997) Phenotypic characterisation of resistance in *Zea diploperennis* to *Striga hermonthica*. Maydica 42:45–51

Lumba S, Holbrook-Smith D, McCourt P (2017) The perception of strigolactones in vascular plants. Nat Chem Bio 13:599–606

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

Mbuvi DA, Masiga CW, Kuria E, Masanga J, Wamalwa M, Mohamed A, Odeny DA, Hamza N, Timko MP, Runo S (2017) Novel sources of witchweed (*Striga*) resistance from wild sorghum accessions. Front Plant Sci 8:116

Menkir A (2006) Assessment of reactions of diverse maize inbred lines to *Striga hermonthica* (Del.) Benth. Plant Breed 125:131–139

Mohamed A, Ellicott A, Housley TL, Ejeta G (2003) Hypersensitive response to *Striga* infection in sorghum. Crop Sci 43:1320–1324

Rich PJ, Grenier C, Ejeta G (2004) *Striga* resistance in the wild relatives of sorghum. Crop Sci 44:2221–2229

Rogers WE, Nelson RR (1962) Penetration and nutrition of *Striga asiatica*. Phytopathology 52:1064–1070

Teka HB (2014) Advance research on *Striga* control: a review. Afr J Plant Sci 8:492–506

van Dam NM, Bouwmeester HJ (2016) Metabolomics in the rhizosphere: tapping into belowground chemical communication. Trends Plant Sci 21:256–265

Yoneyama K, Arakawa R, Ishimoto K, Kim H, Xie X, Nomura T, Kanampiu F, Yokota T, Ezawa T, Yoneyama K (2015) Difference in *Striga*-susceptibility is reflected in strigolactone secretion profile, but not in compatibility and host preference in arbuscular mycorrhizal symbiosis in two maize cultivars. New Phytol 206:983–989

Yoneyama K, Awad AA, Xie X, Yoneyama K, Takeuchi Y (2010) Strigolactones as germination stimulants for root parasitic plants. Plant Cell Physiol 51:1095–1103

Yoshida S, Shirasu K (2009) Multiple layers of incompatibility to the parasitic witchweed, *Striga hermonthica*. New Phytol 183:180–189

https://doi.org/10.1017/wsc.2018.24 Published online by Cambridge University Press