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Breeding maize (*Zea mays* L.) for tolerance or resistance of Striga hermontheca.

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Abstract: *Striga hermonthica is a major limiting factor in maize cultivation in sub-Saharan Africa. Based on environmental factors such as soil fertility, climatic conditions and genotype, this can result in a yield loss of up to 100%. The aim of this article is to review maize breeding methods for resistance/tolerance to Striga hermontica and to highlight the efforts made to overcome yield reduction due to Striga hermonthica. The International Institute of Tropical Agriculture (IITA) has made significant progress in developing several early inbred maize lines, open-pollinated varieties and hybrids containing Striga resistance genes. However, the levels of striga resistance of the available precocious inbreds and hybrids are not as high as desired. This is because the Striga seed bank is added to the soil after each growing season. There is therefore a need for increased Striga resistance in the available genotypes. Since resistance to S. hermonthica in maize is regulated by many genes, breeding for Striga-resistant cultivars using traditional approaches has been less effective and time-consuming. The identification of QTLs associated with Striga resistance/tolerance would facilitate the rapid development of Striga resistant/tolerant maize genotypes.*

Keywords: *Maize, Striga hermonthica, Striga resistance, Striga tolerance*

1. INTRODUCTION

Maize (Zea mays L.) the most important staple food in Sub-Saharan Africa (SSA) (FAO, 2017; IITA, 2011). It is the most important food crop on the African continent in terms of acreage and total grain production (Reynolds et al., 2015). Corn is the main food source for over 300 million people (IITA, 2011). It is a major source of carbohydrates, proteins, lipids and vitamins for millions of people in the region (Badu-Apraku et al, 2017). A high annual consumption of 79 kg per capita in Africa (De Groote, 2002). .Corn can be exploited in a variety of ways, with all parts of the plant such as kernel, cob, stalk, leaves and tassel having economic value. The grain is used as food or fermented to make a variety of beverages and beers (Anami *et al*., 2009).

However, corn production is still below the crop's potential yields due to biotic and abiotic constraints, leading to food insecurity (FAO, 2017). Among parasitic weeds, the root hemiparasite S. hermonthica is the most devastating and dominant constraint on maize production (Khan *et al*., 2014). The extent of damage depends on the timing and extent of infection. Can cause up to 100% in high infestation (Amusan et al. 2008). Losses due to Striga are estimated at $7 billion annually. Today, the weed affects over 100 million farmers (Spallek *et al*., 2013). Striga hermonthica (giant witch's herb), a parasitic plant native to Ethiopia and Sudan (Amy. B *et al*., 2011). A separate survey conducted in 1997 found that Striga hermonthica is the most widespread parasitic weed species in Ethiopia and the overall incidence rate of Striga of the 310 maize fields surveyed was 41%.

Using cultivars that are tolerant and resistant to Striga species has been recommended as the most practical approach for resource-poor smallholders (Menkir, 2006). Since 1982, scientists at IITA have been breeding maize for tolerance and resistance to S. hermonthica. Genetic resistance in the host plant is central to the success of integrated control measures to minimize the Striga threat. Decades of Striga research efforts at the International Institute of Tropical Agriculture (IITA) Ibadan have recently resulted in the successful release and registration of Striga-resistant maize inbred lines from diverse genetic backgrounds. Planting Striga-resistant maize varieties is currently considered the best control strategy and is easy to apply or deploy, especially in combination with other management practices (Gasura, E., *et al*., 2019). Resistance to S. hermonthica parasitism is mainly attributed to the low production of Striga germination stimulants by the host plant (Rodenburg, J. et *al*., 2006). When breeding for S. hermonthica resistance in maize, a combination of these resistance mechanisms is desirable to achieve effective and durable resistance (Gasura, E., *et al*., 2019). The slow evolution and spread rate of Striga-resistant genotypes is largely due to the complex genetics of resistance as well as limited knowledge of the specific mechanisms associated with resistance to Striga (Amusan, *et al*., 2008).

Although many efforts have been made by plant breeders (IITA) resulting in the development and release of several Striga-resistant/torerant maize inbred lines, limited success has been achieved in controlling Striga in smallholder fields in Africa. This is mainly because the Striga hermonthica has a highly specialized life cycle synchronized with host growth, a breeding behavior that maintains tremendous genetic variability, the ability to parasitize a wide range of hosts, seed longevity in soil, and the ability to to inflict the most damage to the host before emerging above ground (Ejeta, 2007; Hearne, 2009). Therefore, it is very important to develop strategies integrating different breeding techniques for the development of a S. hermonthica resistant maize variety that would help reduce losses due to this parasite infestation suffered by maize farmers in sub-Saharan Africa, including Ethiopia. Therefore, this review examines the different approaches of maize breeding for resistance (tolerance) to parasitic weeds called Striga hermontica and the aim of this paper was to review on methods of maize breeding for resistance/tolerance to Striga hermontica and highlight efforts made to overcome yield reduction due to Striga *hermonthica.*

2. LIFE CYCLE OF STRIGA HERMONTHICA

The lifecycle of *Striga* is synchronized to that of its host and involves mechanisms that coordinate the lifecycle of the parasite and that of the host (Bouwmeester *et al*., 2003). The *Striga* life cycle generally involves: germination, host attachment, formation of haustoria, penetration, and establishment of vascular connections, nutrients accumulation, flowering and production of seeds. *Striga* seeds only germinate in presence of certain hormones known as strigolactones, produced by the host and in other cases non-host species (Keyes *et al*., 2007).

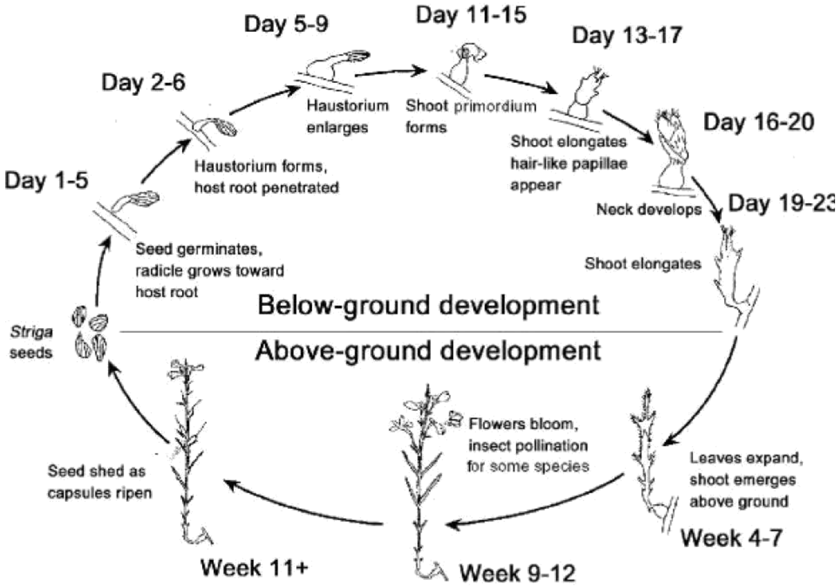

Figure1. *Major stages in the life cycle of Striga of development (Bouwmeester et al., 2003)*

The economic losses caused by Striga spp. are enormous. This is a parasite found mainly on tropical grain crops such as corn, sorghum, pearl millet and upland rice (Press et al., 2001). It causes reduced growth for the host because it disrupts its photosynthesis and uses its nutrients, causing a deficit (Joel, 2007). The weeds consume a larger portion of the host plant's solutes, resulting in wilting and early death of the plant (Ruyter-Spira et al., 2011). It is estimated that in sub-Saharan Africa alone there is an annual yield loss of more than US$7 billion due to infestation with Striga spp. (Spallek *et al*., 2013). The amount of yield loss depends on factors such as striga density, host species, land use system, amount of soil nutrients and rainfall pattern (Atera *et al*., 2012).

Figure 2**:** A maize field infested with Striga hermonthica

3. GENETIC RESOURCE FOR STRIGA HERMONTHICA RESISTANCE AND TOLERANCE IN MAIZE

The genetic enhancement of a trait depends on the availability of genetic variability. Sources of resistance to Striga have been identified in maize (Amusan, et al., 2008), rice (Gurney et al., 2006), sorghum (Mohamed et al., 2003; Haussmann et al., 2004; Mbuvi et al., 2017) and Cowpea (Menkir, 2006). Such host-based Striga resistance mechanisms act either before (pre-attachment resistance) or after infection (post-attachment resistance). Potential sources of resistance to Striga have been found in a number of heterotic groups of maize (Table 1). Crop wild relatives offer a wide genetic pool for breeding purposes. Although there is little explicit resistance to Striga among maize landraces in Africa, some Striga-resistant landraces have recently been reported in Kenya (Charles et al. 2016).

Table1. Summary of genetic sources for Striga resistance.

Germplasm	Current source	References
Wild maize relatives	lama T. dactyloides- source of Lhf genes for haustorial developmental barriers Z. diploperensis- major source of resistance in maize	(Gurney *et al*., 2003) (Amusan *et al*., 2008)
Landraces	Sources of horizontal resistance	(Charles *et al.* (2016)
Inbred lines	IITA and CIMMYT lines	(Menkir, 2006), (Karaya et al., 2012)
Hybrids	Hybrids Resistant commercial genotypes e.g. Pioneer Hybrids and CGIAR varieties	Chitagu *et al*., 2014), (Akinwale *et al*, 2014)
OPV	IITA populations – e.g. TZL comp1 synw-1 and Acr94TZE Comp s-w	(Menkir and Kling , 2007)

Source: (Admire.T.S *et al*, 2017)

4. SCREENING AND EVALUATION OF GENETIC RESOURCES FOR STRIGA RESISTANCE

The development of Striga-resistant cultivars has been limited by the lack of reliable screening techniques (Yagoub *et al*., 2014). Some of the screening techniques that have been used in the past include laboratory analysis methods, pot screening, and field trials (Rodenburg *et al*., 2015). Although the practice of field screening helps in generating statistics about Striga infestations under natural conditions, the method is limited by the existing environmental impacts. To circumvent this and initiate reliable screening after attachment, the rhizotron screening system is ideal (Rodenburg *et al*., 2015). Rhizotrons are transparent root observation chambers that allow striga attached to the host plant to be counted, the phenotype of resistance mechanisms to be assessed, and the effect of striga on host biomass to be determined over time and with minimal disruption (Cissoko *et al*., 2011 ; Gurney et al., 2006; Runo et al., 2012; Rodenburg et al., 2015).

Laboratory Screening Methods

Traditional breeding methods by screening resistant genotypes under natural or artificial striga infestation are very expensive, time consuming and labor intensive. Using simple laboratory techniques when screening host plants for responses to Striga infestation can reveal resistance mechanisms in host plants, increase the efficiency of breeding programs and make it possible to pyramid different resistance mechanisms in a single cultivar for stable resistance responses (Ejeta, 2007). . Laboratory experiments were designed to identify resistance components that together provide the host with general resistance expression during parasite establishment (Ejeta and Gressel 2007). In vitro growth systems allow studying the architecture of host roots and their biochemical resistance mechanisms. Some laboratory assays allow the study of the release of germ inhibitors and haustorial initiation factors as well as hypersensitivity reactions (Ejeta et al. 2000). The paper roll assay was developed to analyze the early stages of Striga infection (Ejeta et al. 2000). In this case, preconditioned striga seeds are exposed to light and then spread evenly on germination paper moistened with distilled water. The striga seeds are then rolled between the germ papers along with lined host seeds. Observations are then made after three weeks when the papers are unrolled to reveal the extent of parasitic attachments to host roots and early resistance mechanisms.

Glasshouse Screening

Screening in pots was also an integral part of Striga resistance assessments (Ahonsi *et al*., 2002). Pots have been used extensively for screening for cultivar resistance, host-parasite nutritional relationships, growth stimulant analysis, and herbicide efficacy. Various pot-strainer techniques such as the polybag and the seed pan have been described in detail by (Rao, 1985). The methods are recommended for their effectiveness in screening for sorghum resistance to S. hermonthica. Of note is the development of the Eplee bag pot screening technique developed by (Eplee, 1992). Striga seeds are placed in small micromesh bags, tied to string and buried near the plant roots. At a certain moment, the threads are pulled to observe the germination of the striga. The method can also be used under field conditions to observe Striga germination under natural conditions. Several studies demonstrate the validity of the Eplee-Bag technique as a screening method (Gurney *et* al. 1995; Ahonsi *et al*. 2002). The most important consideration in greenhouse evaluations is its compatibility with experiments on the effectiveness of biological control agents such as Fusarium oxysporum f. sp. striae. The technique allows for a continuous, uninterrupted assessment of the plant rhizosphere, as demonstrated by (Ahonsi et al., 2002) and (Yonli et al. 2006) in their assessment of potential biological control agents in control.

Field Techniques

Confusing effects of environmental conditions on polygenic inheritance of traits associated with Striga resistance make field screening imperative despite advances made through laboratory and pot experiments. The art of increasing the accuracy and efficiency of field screening for Striga resistance has been perfected over the years. In corn improvement, an efficient grading scale must be used to estimate the breeding value of a single genotype for Striga. Striga damage rating value, striga occurrence, and agronomic traits that contribute to grain yield are commonly used in selection for resistance in corn. Notwithstanding the shift in focus from selection for tolerance to that in favor of resistance, the Striga damage score still provides a basis for maize improvement for Striga resistance (Menkir 2006). Tolerance, determined by the Striga damage score, is based on a scale of 19, where 1 means no symptoms of damage and the genotype is considered highly tolerant, while 9 means very susceptible and severe damage (Kim and Adetimirin 1997). It has been found that a low Striga damage rate is directly associated with an increase in grain yield at the same infestation level as the susceptible maize genotypes (Badu-Apraku 2007; Menkir and Kling 2007). However, selection for tolerance leads to the accumulation of a high striga seed bank. Genetic variants in crop plants have been advanced for further improvement based on their ability to suppress parasitic attachment ladders.

5. EVALUATION OF GENETIC RESOURCES FOR STRIGA RESISTANCE

The development of genetically improved strains with Striga resistance is often straightforward given the availability of reliable sources of resistance and the availability of efficient and easily controllable as well as practical screening methods to create sufficient selection pressure (Rubiales, 2003). Various controlled environment and field screening methods have been developed and applied in Striga improvement programs. Evaluation of germplasm for resistance to parasitic weeds can be performed in controlled and field environments. Controlled environments include laboratory and greenhouse conditions under artificial infestation, while field trials are conducted in either hotspot areas with additional infestation to increase selective pressure.

6. BREEDING TECHNIQUES FOR S. HERMONTICA RESISTANCE IN MAIZE

Conventional Breeding

Conventional breeding techniques have been predominantly used in conferring superior combinations of Striga resistance alleles among susceptible cultivated crops (Menkir et al. 2004).Hybrid breeding, recurrent selection, half-sib selection, full-sib and, S1 family selection schemes have been successfully utilized in developing resistance to most virulent Striga species in legumes and cereal. It is relevant to explore the applicability of most conventional breeding techniques as they have been utilized in various Striga resistance breeding programs. Striga resistance traits have been accumulated successfully through recurrent selection in cereal crops. Through recurrent selection, genetic gains in grain yield in segregating populations has been reported by (Menkir *et al.* 2004) and (Badu-Apraku et al. 2006).

The IITA proposes to cluster and mate maize populations of different genetic make-up with contrasting maturity groups and grain colors to create Striga-resistant breeding populations. Quantitative trait loci (QTLs) for resistance to S. hermonthica have been identified from local populations including wild relatives and successfully transferred into adaptable maize populations by backcross breeding (Rich and Ejeta 2008). Germplasm obtained by the backcrossing method forms the basis for further development of cultivars to achieve polygenic resistance to S. hermonthica. Such inbreds of Zea diploperennis and tropical maize were essential for the development of S. hermonthica-resistant open-pollinated populations such as Zea diplo SYNW-1, TZL Comp SYNW-1. This has been a key resource for municipal corn production systems. Partial resistance to S. hermonthica has also been observed in backcross hybrids from a resistant donor T. dactyloides (Gurney *et al*. 2003). The backcrossing procedure is straightforward when a starting population or donor with a high frequency of desirable alleles for Striga resistance is available. Rapid progress in establishing resistance to S. asiatica can be achieved by identifying a donor with a high dominance of S. asiatica resistance genes. In such a scenario, ideal recurring parents would be genotypes that combine early maturity and high yield (Badu-Apraku *et al*. 2006).

Heterosis of hybrid cultivars can be useful to mitigate the effect of S. hermonthica on plant production and productivity. With the increasing use of hybrid maize seed in West and Central Africa, IITA was able to accumulate resistance to S. hermonthica in hybrid maize by crossing different inbred lines (Menkir *et al*. 2004). This gives rise to S. hermonthica-resistant hybrids that can suppress parasite emergence, with some producing high grain yields at high infestations (Karaya *et al*. 2012). The rapid progress in the development of resistant S. hermonthica hybrids in IITA programs can be attributed to the availability of stable resistant genotypes, which have been used as testers to evaluate the broad pool of inbred lines for their overall mating abilities (Menkir *et al*. 2004). . However, conventional breeding has proven to be time-consuming, largely if not entirely dependent on climatic and environmental conditions, and therefore less effective (Ejeta and Gressel, 2007; Rispail *et al*., 2007).

Marker Assisted Breeding for Striga Resistance

Although conventional breeding has made significant contributions to improving maize resistance to Striga, it has generally been slowed when targeting the complex quantitative trait of resistance to Striga. Traditional breeding methods by screening resistant genotypes under natural or artificial striga infestation are very expensive, time consuming and labor intensive. Marker-assisted selection (MAS) is an efficient approach to increase the accuracy and efficiency of selection using markers that are tightly linked to genes to complement phenotypic selection (Srivastava, R.K.; *et al*., 2020; Wang, X. ; *et al*., 2019). Applying marker-assisted selection (MAS) is a surefire way to improve the efficiency of the Striga resistance breeding program and reduce the inefficiency, lack of precision, and high costs associated with traditional field screening for resistance in host plants. MAS can be facilitated through the use of in vitro assays. These laboratory tests can aid in the rapid and effective phenotyping of separating populations for specific resistance mechanisms to Striga. Marker assisted selections are an indispensable element of most breeding programs as they reduce selection errors associated with phenotypic assessments. In addition, phenotyping large pools of germplasm for Striga resistance is expensive, making it challenging to generate sufficient data for high-resolution maker trait association and QTL detection. Currently, there are limited reports on QTL conditioning Striga resistance in maize. However, a recent study involving marker-assisted recurrent selection for grain yield under drought stress and Striga infestation elucidated the importance of this technique in accumulating favorable genes for quantitative traits (Abdulmalik *et al.* 2017).

In general, corn breeding for Striga resistance has relied too heavily on field screening, the accuracy of which can be confounded by a plethora of uncontrolled variation. As reported by (Ejeta and Gressel, 2007), some Striga resistance genes are recessive, therefore some sources of resistance can be discarded without molecular mapping. Therefore, the current S. hermonthica resistant genetic resource, developed through lengthy and costly breeding cycles, could represent half of its potential. Searching for striga resistance QTL in maize can also use the same approach of testing for resistance QTL for low induction of striga germination and for genomic regions associated with field resistance. This can be achieved by genotyping the maize gene pool at different stages of parasite development. Crosses can be performed between recombinant inbred lines (RIL) derived from susceptible and resistant backgrounds as reported in (Menkir *et al*. 2004). The resulting hybrids can then be examined prior to fingerprinting in agar gel assay tests for histological response mechanisms to parasitic infections for the lsgs gene as described by (Haussmann et al., 2004) and (Mutengwa *et al*. 2005).

Role of Genetic Engineering in Improving Maize Resistance to S. Hermonthica

Genetic engineering to improve host plant resistance to Striga is a promising approach and offers new opportunities to develop improved cultivars. Over the years, scientists have increased research against parasitic weed species, including Striga, by taking advantage of technological advances that allow insertions, deletions of target genes, manipulation of specific protein sequences, and regulation of plant metabolites involved in the synthesis of cellular structural components (Yoder 2013; Kirigia *et al*. 2014). The studies aimed at the molecular characterization of host-plant-parasite interaction and host resistance through expression analysis of the genes, proteins and metabolites involved in these processes are the subject of increasing interest and offer weed researchers the opportunity to improve the use of genetic transformation tools to control them of parasitic plants (Rispail *et al*., 2007; Aly, 2012). So far, no transgenic Striga-resistant millet plants have been reported and approved for cultivation. However, understanding the biology of the early stages of Striga parasitism will help identify potential barriers to the success of this technology.Although significant strides have been made in genetic transformation, there have been very few reports of sorghum crops lagging behind other cereals such as maize in terms of genetic transformation (Visarada and Kishore, 2015). Regardless of the varying successes recorded by different workers in attempts to induce Striga resistance, all reports reflect a great potential of RNAi in the development of transgenic maize capable of suppressing the parasite's proliferation. (Yoder et al. 2009) and (Runo *et al*. 2011) have investigated the use of RNA interference (RNAi) technology as a means of enhancing host resistance to parasitic weeds. However, this approach was unsuccessful in controlling the maize striga parasite interaction (Yoder and Scholes, 2010). Interfering double-stranded ribonucleic acid (dsRNA) constructs in maize plants could silence the expression of genes responsible for susceptibility to S. hermonthica parasitism (Kirigia et al. 2014).

7. CONCLUSION AND RECOMMENDATION

Among biotic constraints, Striga hermonthica is the most devastating and dominant factor in maize production, causing up to 100% yield loss. The extent of damage depends on the timing and extent of infection. Planting Striga-resistant maize varieties is currently considered the best control strategy and is easy to apply, especially in combination with other management practices. Maize genotypes with significant resistance to S. hermonthica are being developed by the International Institute of Tropical Agriculture (IITA) and other national breeding programs. This was a significant milestone in providing resource-poor corn growers in SSA with a cost-effective and effective Striga control option. These genetic resources can serve as useful parents for breeding programs.

Conventional breeding techniques have been predominantly used to confer superior combinations of Striga resistance alleles among susceptible crops. Since resistance to S. hermonthica in maize is regulated by many genes, breeding for Striga-resistant cultivars using traditional approaches has been less effective and time-consuming. The identification of QTLs associated with Striga resistance/tolerance would facilitate the rapid development of Striga resistant/tolerant maize genotypes using MAS due to the polygenic nature of the host-parasite relationship and its interaction with environmental factors. Genetic engineering is another option for breeding Striga-resistant strains.

It is a very promising approach and offers new opportunities for the development of improved varieties. An ideal mix of inbred lines can combine both resistance and tolerance attributes in resulting hybrids. As suggested by many researchers, a successful breeding program is one that can identify and combine striga resistance and tolerance. Therefore, it is essential for any breeding program to provide the breeder with a wide pool of genetic variation. The integration of different breeding techniques to develop S. hermonthica-resistant corn varieties would help to reduce losses to corn farmers from this parasite infestation.

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