# Unveiling the Ecotype driven Virulence Heterogeneity among Major Ecotypes of the Striga hermonthica Population from Ethiopia

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## Abstract

*Striga hermonthica*, a root hemiparasitic weed, severely limits sorghum production in Sub Saharan Africa. The use of resistant varieties is a widely adopted strategy for controlling *Striga*. To develop durable *Striga* resistant sorghum varieties, this study investigates the interaction effects between host and parasite, as well as the virulence levels of five Ethiopian *Striga* ecotypes. A pot trial was conducted using seven resistant sorghum varieties, two susceptible checks, and five Ethiopian *Striga* ecotypes. Valuable data were generated on the interaction effects between sorghum varieties and *Striga* ecotypes based on three resistance traits: *Striga* count, *Striga* length, and dry *Striga* biomass. The findings revealed variability in sorghum responses to *Striga* infection, with significant variety by ecotype interaction effects. Notably, the virulence levels of *Striga* ecotypes varied considerably across sorghum varieties; an ecotype highly virulent to one variety exhibited reduced virulence to another. Similarly, a sorghum variety highly resistant to one ecotype showed moderate or lower resistance to others. The variety Framida generally exhibited high levels of infection, whereas N13 demonstrated stronger resistance. Importantly, sorghum varieties N13 and SRN-39 consistently showed resistance across all tested ecotypes, making them prime candidates for strategic gene pyramiding. This study highlights the presence of interaction effects, which are critical for designing effective breeding strategies in future *Striga* resistance improvement programs. Furthermore, comprehensive studies on the genetic variability of Ethiopian *Striga* ecotypes will facilitate the development of durable resistant varieties.


## Introduction

Sorghum (*Sorghum bicolor*) is one of the most important cereal crops for people in Sub Saharan Africa (SSA), serving as a source of food, feed, nutrients, and various other resources. In this region, its production is severely limited by the most significant biotic constraint: *Striga* (AATF 2011; Gethi et al. 2005). *Striga* species are obligate root parasites that infest staple crops across the Middle East and parts of Asia (Parker 2012). A large proportion of *Striga* species, over 80%, are found in Africa (Joel et al. 2018).

*Striga hermonthica* is one of the most economically damaging *Striga* species in several regions of the world, especially in SSA (AATF 2011). The geographical distribution and area coverage of *Striga* are gradually expanding due to various factors, including declining soil fertility, shorter fallow periods, cultivation shifting to marginal lands, and continuous monoculture practices (Ejeta 2007b; Emechebe et al. 2004). *Striga* significantly limits sorghum production and may result in total crop loss (Ejeta 2007a; Muchira et al. 2021). The loss has severe economic consequences for farmers, which adversely affect their livelihoods (Emechebe et al. 2004). *Striga* infestation is a major economic concern in Ethiopian sorghum growing regions. This problem is particularly prevalent in Tigray, South and North Wollo, Gonder, Gojam, North Shewa, Gambela, Benishangul Gumuz, and Eastern Hararge (Temesgen 2019; Earecho et al. 2024). Therefore, addressing *Striga* infestations through comprehensive efforts is essential to ensure sustainable farming practices and economic stability.

Integrated *Striga* Management (ISM) is now an effective method for reducing the effects of *Striga* on sorghum production. This method combines resistance genotypes with a variety of management strategies (Mrema et al. 2020; Yonli et al. 2012). Resistant varieties are now a central component of all ISM approaches in sorghum (Tesso and Ejeta 2011). The advantages of this approach include cost effectiveness, sustainability, environmental friendliness, and suitability for small scale farmers (Mrema et al. 2020; Tesso and Ejeta 2011; Yonli et al. 2012). This confirms that breeding sorghum for *Striga* resistance has long term effects on agricultural productivity, particularly in areas where *Striga* infestation is a major issue (Dossa et al. 2023).

In Ethiopia, research on *Striga* resistance or tolerance has achieved some success (AATF 2011). Since the beginning of the sorghum breeding program for *Striga* resistance, several *Striga* resistant varieties have been developed, recommended, and incorporated into breeding programs targeting *Striga* infested regions of Ethiopia. These varieties includes "Berhan," "Gobiye," "Abshir," "Framida," "N13," "SRN-39," "BTx623," and "Tetron." (Abate et al. 2017; Ahmed et al. 2024; Belay et al. 2021; Teka 2014; Gobena et al. 2017; Kawa et al. 2021; Mbuvi et al. 2017; Muchira et al. 2021; Rodenburg et al. 2005; Temesgen 2019). However, different infested regions have different levels of success with resistant varieties in reducing the effects of *Striga*.

Due to the high genetic diversity of *Striga* species, the weak level of host resistance that tends to break down with the infestation of new *Striga* races (Rispail et al. 2007), and the rapid evolution of parasite virulence (Qiu et al. 2022), the *Striga* breeding program and the use of resistant varieties have been complex and ineffective. *Striga* populations in Ethiopia exhibit a high degree of genetic diversity, with geographical distribution playing a significant role in shaping this diversity (Welsh and Mohamed 2011). The extensive genetic diversity of *Striga* determines its virulence level and the success of host resistance (Haussmann et al. 2001).

The development of long lasting sorghum varieties resistant to various *Striga* populations across different ecotypes requires a comprehensive understanding of the polygenic nature of resistance and the associated resistance mechanisms. This includes examining resistance both within and among *Striga* race structures, as well as understanding the nature of interaction effects between *Striga* ecotypes and sorghum genotypes and different hosts (Qiu et al. 2022; Rispail et al. 2007; Scholes and Press 2008). In Ethiopia, released resistant varieties demonstrate considerable adaptability, particularly in *Striga* infested areas. However, there is limited concrete information regarding the genetic variation among sorghum varieties in *Striga* resistance, the effects of host parasite interactions, and the virulence levels of major *Striga* ecotypes on different sorghum varieties, especially given the diverse *Striga* populations across Ethiopian ecotypes (Welsh and Mohamed 2011). Moreover, utilizing known improved *Striga* resistant sorghum varieties enhances our understanding of how sorghum interacts with different *Striga* ecotypes and the virulence levels of the parasite. This information is critical for advancing future *Striga* breeding strategies and selecting appropriate varieties for wide *Striga* ecotypes. Therefore, to assess the genetic variation of *Striga* resistant sorghum varieties in response to five distinct Ethiopian *Striga* ecotypes, to determine the virulence levels of *Striga* ecotypes against seven resistant sorghum varieties, and to investigate the nature of their interaction effects, three *Striga* resistance traits were collected from a controlled pot experiment.

## Materials and Methods

# Experimental location and materials

The pot experiment was conducted in 2024 at the Debre Berhan Agricultural Research Center in Debre Berhan. Seeds of seven *Striga* resistant sorghum varieties and two susceptible checks were obtained from the National Sorghum Research Program at the Melkasa Agricultural Research Center of the Ethiopian Institute of Agricultural Research (Table 1).

Table 1
List of *Striga* resistant and susceptible sorghum varieties used in the study

| Variety | *Striga* resistance reaction | References |
|---|---|---|
| Abshir | Resistant | (Belay et al. 2021; Teka 2014) |
| Berhan | Resistant | (Abate et al. 2017; Belay et al. 2021; Teka 2014) |
| BTx623 | Resistant | |
| Framida | Resistant | (Ahmed et al. 2024; Muchira et al. 2021) |
| Gobiye | Resistant | (Abate et al. 2017; Belay et al. 2021; Teka 2014) |
| N13 | Resistant | (Mbuvi et al. 2017; Muchira et al. 2021) |
| SRN-39 | Resistant | (Kawa et al. 2021; Temesgen 2019) |
| Teshale | Susceptible | (Mulatu 2020; Temesgen 2019) |
| Shanqo Red (SQR) | Susceptible | (Gobena et al. 2017; Kawa et al. 2021) |

### Striga hermonthica seed collection

The *Striga hermonthica* seeds used in this study were collected from severely infested sorghum fields across five distinct geographical locations in Ethiopia during the 2022 cropping season. Table 2 describes the geographical locations of the *Striga* collection sites. Healthy, intact *Striga* plants with no visible flowers or only the topmost flowers remaining were carefully collected from extensively infested areas using the methods described by Berner et al. (1997). The harvested *Striga* plants were spread out to dry on a large polyethylene sheet in a well ventilated, covered space. After 20 days of drying, during which shattered *Striga* seeds fell onto the sheet, the dried plants were carefully transferred to another polyethylene sheet, with the heads gently tapped to encourage seed shedding. The threshed *Striga* seeds from both sheets were then collected into separate containers and sieved through 250 and 150 micron open sieves. Finally, pure *Striga* seeds were placed in labeled plastic containers and stored in a refrigerator at the Holeta Agricultural Biotechnology Research Center.

Table 2
The description of the geographical locations of the *Striga* collection site

| Location | Zone | Altitude (m.a.s.l) | Location |
|---|---|---|---|
| Assosa | Assosa | 1300–1570 | 10º 04" N, 34º 31" E |
| Babile | East Harerge | 1646.88 | 09º 13' 19" N, 42º 19' 47" E |
| Fedis | East Harerge | 1055.1 | 08º 49' 43" N, 42º 00' 45" E |
| Kobe | North Wolo | 1476.49 | 12° 7' 11.52" N, 39° 37' 26.36" E |
| Shewa Robit | North Shoa | 1235 | 09º 59' 53" N, 39º 53' 53" E |

**Surface sterilization, preconditioning, and infestation of Striga seeds**

The procedure described by Taylor et al. (2024) was used for *Striga* seed surface sterilization and conditioning. A measured amount of viable *Striga* seeds from each ecotype was soaked in a separate flask containing 20% commercial bleach for ten minutes, followed by three rinses with autoclaved distilled water. The sterilized *Striga* seeds were then incubated at 30°C in the dark for 14 days. Conical pots with a volume of 5 liters, a height and top diameter of 20 cm, and a bottom diameter of 8 cm were selected for the experiment. Three kilograms of a 1:3 sand to soil mixture were placed into each pot. Before planting, approximately 7,500 (0.077 g) preconditioned *Striga* seeds were added to each pot containing the 3 kg sand/soil mixture and thoroughly mixed.

## Sorghum planting and growth monitoring under greenhouse conditions

Three or four healthy sorghum seeds were sown in a 2–3 cm hole made in the center of each pot. After twelve days, one healthy and vigorous seedling per pot was retained, and the others were carefully thinned out. For this study, the fertigation method described by Berner et al. (1997) for *Striga* screening under controlled conditions was employed. Each pot received 0.113 g of NPS fertilizer at the time of thinning, followed by 0.052 g of nitrogen in the form of urea seven days later. During the first five weeks, each pot was watered with approximately 30 ml daily. For the remainder of the experiment, each pot continued to receive the same volume of water at daily intervals.

## Data collection

Three *Striga* resistance related traits were measured ten weeks after sowing sorghum seeds: attached and emerging *Striga* count (SC), *Striga* length (SL), and dry biomass (SBM) (Joel et al. 2018; Kaubi 2016; Kavuluko et al. 2021; Mbuvi et al. 2017). *Striga* seedling attachments collected from each pot were counted and photographed on 90 mm Petri dishes. Dry *Striga* biomass was determined after the seedlings were oven dried for 24 hours at 60°C. Finally, the length of the attached *Striga* seedlings was measured using ImageJ v.1.45 software (https://imagej.net/ij/download.html), and the average length per pot was calculated (Joel et al. 2018).

# Statistical analysis

A combined three way analysis of variance (ANOVA) for three *Striga* related quantitative traits was conducted using data from a randomized complete block design with factorial arrangements, comprising four replications, and carried out over two distinct seasons under controlled conditions. This analysis was performed using the R function fat3.rbd() from the ExpDes package (Ferreira et al. 2014). The significance of the mean squares for the main and interaction effects was assessed using least significant difference (LSD) tests at a $P \leq 0.05$ probability level. The virulence levels of the five Ethiopian *Striga* ecotypes on resistant sorghum varieties alone, and including the two checks, were graphically summarized using SRplot, A free online platform for data visualization and graphing (Tang et al. 2023).

# Results

Using five Ethiopian *Striga* ecotypes, this study evaluated the genetic variability of seven improved *Striga* resistant sorghum varieties, focusing on three *Striga* related traits. The response of different sorghum varieties to *Striga* infestation was assessed by measuring the total count, mean length, and dry biomass of *Striga* attached to the root zone of each sorghum variety (Kaubi 2016; Mbuvi et al. 2017). Significant genotypic variation was observed among the *Striga* resistant varieties across all three traits. Accordingly, there was significant genotypic variation between *Striga* resistant varieties in all three traits. SC ($p < 0.001$), SL ($p < 0.05$), and SBM ($p < 0.01$) were the three *Striga* related parameters to which the resistance of varieties varied significantly on the five Ethiopian *Striga* ecotypes (Table 3). Furthermore, when including the two standard checks, "SQR" and “Teshale,” in the analysis alongside the seven improved *Striga* resistant sorghum varieties, the significant genotypic variations in all three traits remained consistent (Supplementary Table S1). Conversely, in both analyses, the tested *Striga* ecotypes did not exhibit a significant effect on any of the measured traits (Table 3 and Supplementary Tables S1).

Emphasizing the consequences of interactions, the two way interaction effects of variety by ecotype had a significant influence on SC and SBM in both analyses, including and excluding the standard checks traits (Table 3 and Supplementary Tables S1). However, a notable variety by ecotype interaction effect on SL was observed when evaluating the seven improved *Striga* resistant sorghum varieties without the two susceptible checks. This strong variety by ecotype interaction suggests that the responses of resistant varieties to the three parameters were significantly affected by the varying *Striga* ecotypes. A strong and significant three way interaction effect was detected for SBM in both analyses, with and without the standard checks ($p < 0.001$). Therefore, the impact of these interactions on SBM should be examined across different environmental levels. Consequently, using the seven improved *Striga* resistant sorghum varieties, this study primarily focused on the two way interaction effects of *Striga* ecotypes and varieties on SC and SL.

Table 3
Combined three way ANOVA for seven improved resistant varieties across five *Striga* ecotypes and two seasons on SC, SL, and SBM, analysis of ecotypes inside each level of varieties for SC and SL, and analysis of varieties within each level of ecotype for SC and SL.

| **Source of Variations** | **DF** | **Mean Squares** | | |
|---|---|---|---|---|
| | | **SC** | **SL** | **SBM** |
| Variety | 6 | 131.57*** | 13.45*** | 0.68* |
| Ecotype | 4 | 28.71 | 2.43 | 0.32 |
| Environment | 1 | 65.09* | 11.34 | 2.32** |
| Variety x Ecotype | 24 | 54.69*** | 5.48* | 0.61** |
| Variety x Environment | 6 | 35.02* | 3.10 | 0.34 |
| Ecotype x Environment | 4 | 2.05 | 3.31 | 0.22 |
| Variety x Ecotype x Environment | 24 | 7.6 | 4.97 | 0.73*** |
| Ecotype x variety Abshir | 4 | 58.84** | 10.17* | |
| Ecotype x variety Berhan | 4 | 29.96 | 0.97 | |
| Ecotype x variety BTx623 | 4 | 32.65 | 10.15* | |
| Ecotype x variety Framida | 4 | 40.35* | 10.09* | |
| Ecotype x variety Gobiye | 4 | 99.9*** | 0.84 | |
| Ecotype x variety N-13 | 4 | 1.23 | 0.36 | |
| Ecotype x variety SRN39 | 4 | 93.94*** | 2.74 | |
| Variety x Ecotype Assosa | 6 | 42.37* | 9.11* | |
| Variety x Ecotype Babile | 6 | 52.91** | 1.04 | |
| Variety x Ecotype Fedis | 6 | 34.37* | 7.94* | |
| Variety x Ecotype Kobo | 6 | 118.41*** | 14.05*** | |
| Variety x Ecotype S/Robit | 6 | 102.28*** | 3.21 | |

****, **, * indicate significant at 0.1%, 1%, and 5% probability level respectively, SC: Striga count, SL: Striga Length, and SBM: Striga Biomass*

The analysis of ecotypes within each variety level is presented in Table 3, which shows that the interaction effects of ecotypes within each variety level were significant. Specifically, the interaction effects of ecotypes with the varieties Abshir ($p < 0.001$), Framida ($p < 0.05$), Gobiye ($p < 0.001$), and SRN-39 ($p < 0.001$) on SC were significant. Similarly, the average SL of Abshir, BTx623, and Framida was influenced by their interactions with different *Striga* ecotypes.

Variety Abshir exhibited higher SC with ecotypes from Assosa (6.13) and Kobo (9.75), whereas the number of attached *Striga* on variety Gobiye was lower for ecotypes from Assosa (2.50) and Kobo (2.63). Additionally, variety SRN-39 showed a high mean SC on the ecotype from Babile (9.38), while variety Framida exhibited significantly high and low mean SC on ecotypes from Assosa (3.75) and Fedis (4.75), respectively (Fig. 1 and Supplementary Table S2). The mean SL of variety Abshir was significantly lower on ecotypes from Babile (0.87 cm) and Fedis (1.22 cm). In contrast, sorghum variety BTx623 had significantly higher mean SL on ecotypes from Fedis (3.6 cm), and variety Framida showed higher mean SL on ecotypes from Assosa (2.17 cm) and Kobo (3.76 cm) (Fig. 2 and Supplementary Table S3). Consequently, the mean SC and SL responses of ecotypes within each variety confirmed the immune response of certain varieties to the tested *Striga* ecotypes, such as Abshir for ecotypes from Babile, Fedis, and S/Robit; BTx623 for ecotypes from Kobo and S/Robit; and Gobiye for ecotypes from Assosa and Kobo. Furthermore, the strong immune response of N13 was confirmed across all tested *Striga* populations. Overall, all examined resistant varieties, except Berhan and N13, had a significant effect on the mean performance of ecotypes for SC and SL (Table 3).

The virulence levels of five Ethiopian *Striga* ecotypes were investigated by analyzing their interaction effects with seven improved *Striga* resistant sorghum varieties for SC and SL. The analysis of the seven improved resistant varieties within each ecotype level for SC and SL (Table 3) showed that, under the *Striga* ecotypes Assosa ($p < 0.05$), Babile ($p < 0.01$), Fedis ($p < 0.05$), Kobo ($p < 0.001$), and S/Robit ($p < 0.001$), the number of *Striga* attached to the root zone of the varieties varied significantly. Similarly, for the sorghum varieties, performance differences in the length of attached *Striga* were significant under the *Striga* ecotypes Assosa ($p < 0.05$), Fedis ($p < 0.05$), and Kobo ($p < 0.001$) (Table 3).

The analysis of the varieties' mean SC and SL within each ecotype (Fig. 3 and Fig. 4) revealed that the Assosa *Striga* ecotype exhibited a significantly higher number of *Striga* attachments on the varieties Abshir (6.13) and BTx623 (6.88), as well as the greatest SL on Abshir (3.65 cm) and Framida (2.17 cm). Similar to the varieties Berhan and Framida, the *Striga* ecotype from Babile showed substantially higher attachments to variety SRN-39 (9.38). *Striga* ecotype Fedis on variety BTx623 (3.50 and 3.60 cm) and Gobiye (7.38 and 1.98 cm), and Kobo on variety Abshir (9.75 and 2.69 cm) and Framida (9.63 and 3.76 cm) were found to have significantly more attachments and longer mean lengths. The *Striga* ecotype from S/Robit severely affected variety Gobiye (10.88). The analysis of the nine varieties (seven resistant and two susceptible varieties) means SC and SL within each ecotype further supports the variation in virulence levels of major Ethiopian *Striga* ecotypes on the developed sorghum varieties (Supplementary Tables S4 and S5)

# Discussion

Continuous screening of sorghum genotypes and identifying the most resistant varieties under uniform *Striga* infestation conditions is crucial to mitigating the increasingly severe impact of *Striga* on sorghum production. The mean number, mean length, and dry biomass of *Striga* growing on the roots of each of the sorghum varieties were used to classify their reaction to the parasite. Resistant varieties exhibited the fewest attachments, the shortest *Striga* length, and the lowest dry biomass on their roots (Joel et al. 2018; Kavuluko et al. 2021). All resistant sorghum varieties studied exhibited some level of successful parasite attachment to the roots, as complete resistance to *Striga* has not been identified in cultivated sorghum varieties (Gurney et al. 2002). Our pot experiment revealed that released *Striga* resistant sorghum varieties respond differently to *Striga* infection on the three resistance traits. This variation suggests that some varieties may possess more effective resistance mechanisms against the parasite, while others may lack such defenses. Similar differences in resistance responses among sorghum varieties under *Striga* infested conditions have been reported by Abate et al. (2017), Ayana (2019), Fisseha et al. (2023), Mamo et al. (2020), and Mbuvi et al. (2017).

The primary focus of this study was the observed two way interaction effects between *Striga* ecotypes and sorghum varieties on SC and SL, which determine the virulence levels of *Striga* ecotypes on different sorghum varieties and the resistance responses of various sorghum varieties to different *Striga* ecotypes. The presence of an interaction effect suggests that different sorghum varieties vary in their capacity to stimulate germination or withstand infections from different *Striga* ecotypes, and vice versa (Musimwa 2005; Haussmann et al. 2001). This indicates that some sorghum genotypes exhibit ecotype specific resistance, while others demonstrate strong and broad resistance across multiple ecotypes. According to Huang et al. (2012), the interaction between *Striga* populations and varieties significantly influences the success of parasites attaching to hosts. Therefore, this interaction effect has important implications for identifying resistant genotypes for breeding purposes, suggesting that breeding *Striga* resistant sorghum may be more complex than it initially appears. Similar studies have also demonstrated the interaction effects between the host and *Striga* populations, which determine host resistance and parasite virulence (Bozkurt et al. 2015; Haussmann et al. 2001; Huang et al. 2012; Rodenburg et al. 2017).

Strong interactions reflect the presence of distinct resistance responses to specific *Striga* populations and the complexity of *Striga* virulence toward particular sorghum (Bozkurt et al. 2015). An ecotype study within each variety revealed that both the length of *Striga* attachments on Abshir, BTx623, and Framida varieties, and the number of *Striga* attachments on Abshir, Framida, Gobiye, and SRN-39 varieties, were significantly influenced by changes in *Striga* ecotype. The responses of these varieties to different *Striga* ecotypes affect both the total number and length of *Striga* attachments near their root zones. Beyond differences in low *Striga* germination stimulation (Yohannes et al. 2016), the varied responses of genotypes to infection by different *Striga* populations may also result from differences in resistance mechanisms among resistant genotypes (Muchira et al. 2021). Comparatively, the mean performance of the Abshir variety confirmed its stronger resistance to *Striga* ecotypes Babile and Fedis. Framida exhibited superior resistance to *Striga* ecotypes Assosa and Fedis. Gobiye's resistance was more pronounced against ecotypes Assosa and Kobo than others. SRN-39 and BTx623 showed stronger resistance reactions across all *Striga* populations except Babile and Fedis, respectively. This inconsistency suggests that when varieties are exposed to different *Striga* populations, their genetic potential for resistance may include traits that either enhance strong resistance or confer partial resistance to *Striga*. Similarly, Haussmann et al. (2001) demonstrated an entry-by-country interaction effect, indicating that entries identified as *Striga* resistant in Kenya may not be resistant in Mali. Overall, the interaction effect highlights significant variation in *Striga* resistance, revealing that sorghum varieties from distinct geographic regions respond differently to *Striga* infestation. Sorghum variety development and recommendations targeted at specific *Striga* ecotypes may not always be effective due to the high genetic variability among *Striga* populations in Ethiopia (Welsh and Mohamed 2011). This genetic diversity enables the parasite to evolve and adapt to changing environmental conditions (Unachukwu et al. 2017), often overcoming the resistance of varieties, especially when new *Striga* races infest the area (Rispail et al. 2007).

The total number of *Striga* attachments in the root zones of sorghum varieties varied significantly when infected with *Striga* ecotypes from Assosa, Babile, Fedis, Kobo, and S/Robit. Similarly, infection by *Striga* ecotypes from Assosa, Fedis, and Kobo had a significant impact on the length of the attached *Striga* across different sorghum varieties. According to Bozkurt et al. (2015), the strong interaction effect indicates distinct variations in *Striga* virulence toward specific sorghum varieties. The variety analysis at each ecotype level further confirms the presence of variable virulence levels of *Striga* ecotypes on the tested sorghum varieties (Fig. 5). Rodenburg et al. (2017) also reported variation in *Striga* virulence across species and ecotypes. *Striga* populations in Ethiopia exhibit a high degree of genetic diversity, with geographic distribution playing a crucial role in shaping this diversity (Welsh and Mohamed 2011). The extensive genetic diversity of *Striga* is driven by the parasite's boundless variability, exceptionally high seed production and fecundity, and the geographic distance of ecotypes (Ejeta et al. 2007; Welsh and Mohamed 2011), all of which determine their virulence and the effectiveness of host resistance (Haussmann et al. 2001). Consequently, the distinct geographic origins of the five *Striga* populations likely contribute to the observed variability in *Striga* virulence against released *Striga* resistant sorghum varieties.

The mean number and length of attached *Striga* plants surrounding the root zone of improved *Striga* resistant sorghum varieties within each *Striga* ecotype were used to determine the virulence levels of the five *Striga* ecotypes. For the seven improved *Striga* resistant sorghum varieties, the virulence level of each evaluated Ethiopian *Striga* ecotype varied significantly. This study showed that the Assosa ecotype exhibited greater virulence toward the varieties BTx623, Framida, and Abshir, while demonstrating reduced virulence against N13 and SRN-39. The Babile ecotype was less virulent to N13 and Abshir but more virulent to SRN-39, Framida, and Berhan. The Fedis ecotype was significantly less virulent to N13 and SRN-39 compared to its effects on Gobiye, Berhan, Framida, and BTx623. The Kobo ecotype was less virulent to Berhan, BTx623, Gobiye, N13, and SRN-39, but more virulent to Abshir and Framida. The final tested *Striga* ecotype, S/Robit, showed significantly higher virulence toward Gobiye and lower virulence toward N13, BTx623, and SRN-39. According to Haussmann et al. (2001), the interaction of *Striga* ecotype and host variety influences *Striga* resistance; they also observed that *Striga* ecotypes highly virulent to one variety may not be virulent to another. Even if *Striga* ecotypes had varying effects on different varieties, this study found that every ecotype exhibited a lower virulence towards variety N13 and SRN-39 while having higher virulence towards variety Framida.

The development of resistant sorghum varieties has proven to be quite challenging for plant breeders due to the varying resistance responses of sorghum genotypes across different ecotypes and the differing infection levels of *Striga* populations on these genotypes. Consequently, cultivated sorghum varieties are not completely resistant to *Striga* (Gurney et al. 2002). In this study, varieties N13 and SRN-39 consistently exhibited higher resistance across all *Striga* ecotypes. Other studies also demonstrate that N13 (Joel et al. 2018; Mohamed et al. 2010) and SRN-39 (Ezeaku and Gupta 2004; Mohamed et al. 2010; Muchira et al. 2021) effectively defend against various *Striga* infections. The resistance mechanisms in N13 and SRN-39 are reported to involve low germination stimulation and a mechanical barrier to *Striga* penetration, attributed to their high lignin and suberin deposition, respectively. Understanding these resistance mechanisms facilitates gene pyramiding (Muchira et al. 2021). The consistent resistance and distinct mechanisms of these two sorghum varieties may aid *Striga* resistance breeding programs and enhance *Striga* management efforts. Furthermore, knowledge of how *Striga* ecotypes differ in virulence simplifies the identification of resistance genes capable of producing genotypes resistant to multiple ecotypes (Unachukwu et al. 2017). Overall, this study provides crucial information for mapping the N13 and SRN-39 genes in adapted high yielding genotypes and screening them under diverse *Striga* infected conditions. Evaluating breeding materials across multiple environments against different host specific *Striga* populations is essential for selecting stable resistance (Haussmann et al. 2001).

# Conclusions

The genetic variation of sorghum varieties, the interaction effect of varieties with *Striga* ecotypes, and the virulence differences of five major Ethiopian *Striga* ecotypes for the resistance varieties were assessed in this activity. The significant interaction effect of major Ethiopian *Striga* ecotypes with resistant sorghum varieties articulates the complexity of *Striga*'s virulence. The result of this study revealed that certain *Striga* ecotypes showed higher virulence levels to some of the sorghum varieties but lesser to the others; similarly, certain sorghum varieties showed more resistance or susceptibility to each of the ecotypes. Such strong virulence level variation highlights the importance of ecotypes in determining varietal selection for *Striga* resistance and developing targeted breeding strategies that enhance resistance to *Striga* infestations. The clear differences in resistance reaction are shown by the contrasting behaviors of Framida and N13 across all ecotypes. Varieties N13 and SRN-39 showed a consistently higher resistance reaction across multiple ecotypes, which may facilitate the strategic pyramiding of different resistance genes within identified high-yielding genotypes, and researchers can enhance breeding programs aimed at developing *Striga*-resistant sorghum varieties. One of the main factors influencing population structure (virulence) in Ethiopia is the wide geographic range of sorghum-growing locations. Therefore, exhaustive studies on the genetic variability of Ethiopian *Striga* ecotypes facilitate the development of durable resistant genotypes. Furthermore, combining controlled testing and multi-ecotype evaluation of genotypes with multiple resistance mechanisms may serve as an effective sorghum breeding strategy for *Striga*-affected farmers in Ethiopia.

# Abbreviations

AATF: Africa Agricultural Technology Foundation

ISM: Integrated *Striga* management

SBM: *Striga* Biomass

SC: *Striga* Count

SL: *Striga* Length

SSA: Sub Saharan Africa

# Declarations

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### Competing interests

The authors declare no relevant financial or non-financial interests to release.

### Ethical approval

Ethical approval has been given for the software, as well as previous studies we used to refer to and discuss in this publication, and the manuscript acknowledges all the works.

**Informed consent:** Not applicable

### Data availability

The dataset generated during the experiment is available from the corresponding author on reasonable request. The analyzed data supporting the texts and figures of this manuscript are included in supplementary information files.

### Authors’ contribution

Conceptualization *and design of the experiment were performed by Abiy Legesse, Habte Nida, and Alemu Tirfesa. Material preparation, execution of pot experiments, and data collection were executed by Abiy Legesse, Hewan Demissie, and Taye Tesema. Data interpretation of results and preparation of the manuscript were done by Abiy Legesse. The first draft of the manuscript was written by Abiy Legesse, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.*

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# Figures

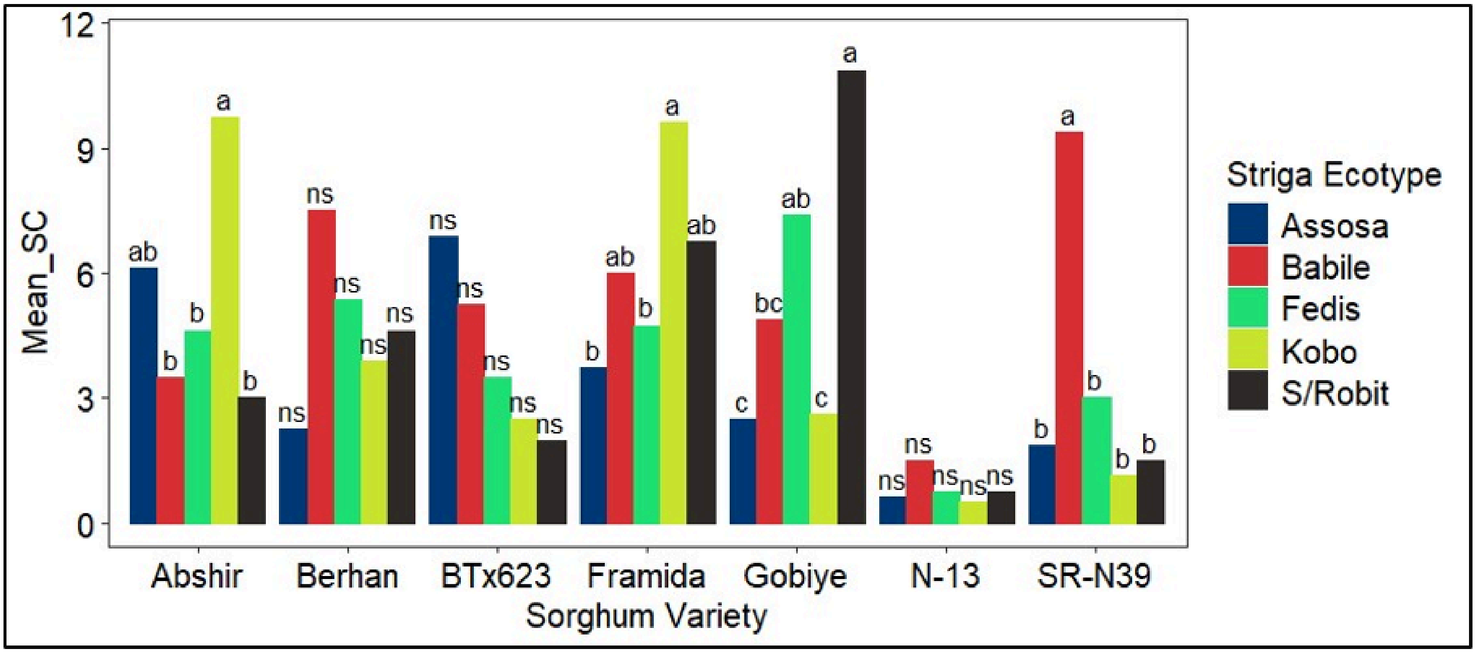


**Figure 1**

Ecotypes mean SC inside each level of a variety

*Means with the same letter are not significantly different at 5% probability level, and ns: non-significant*

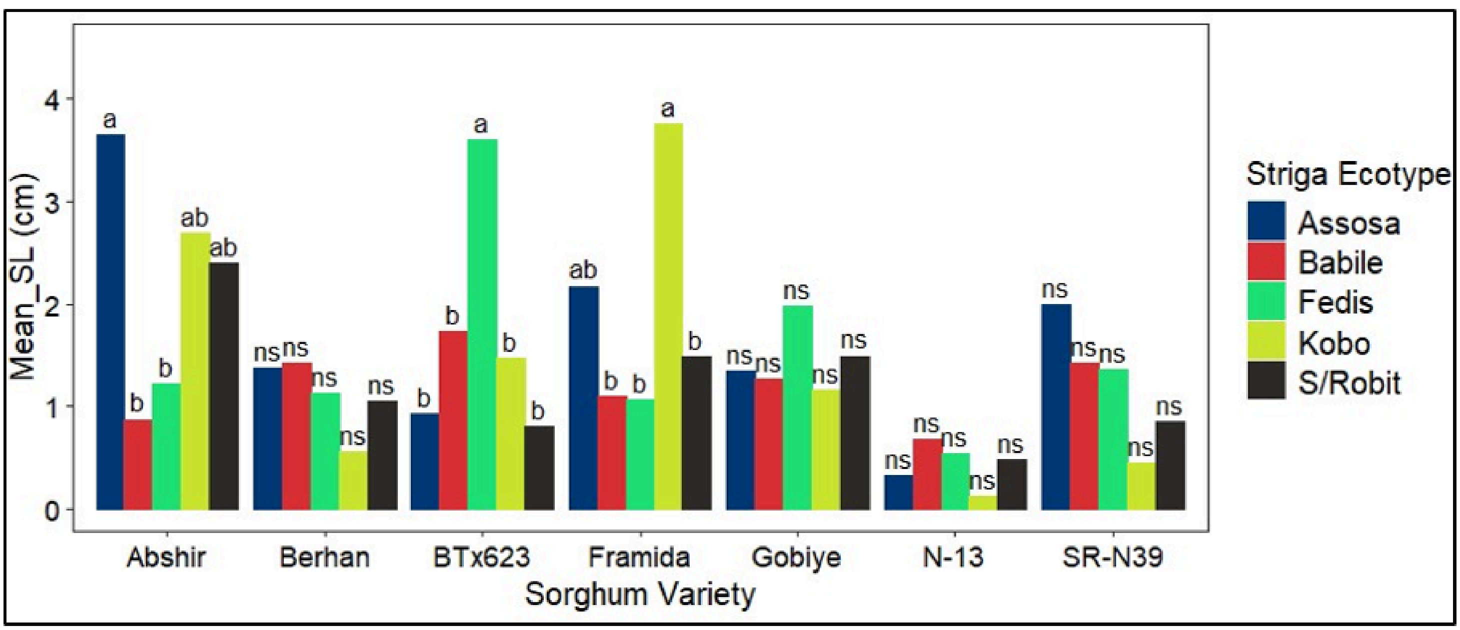


**Figure 2**

Ecotypes mean SL inside each level of a variety

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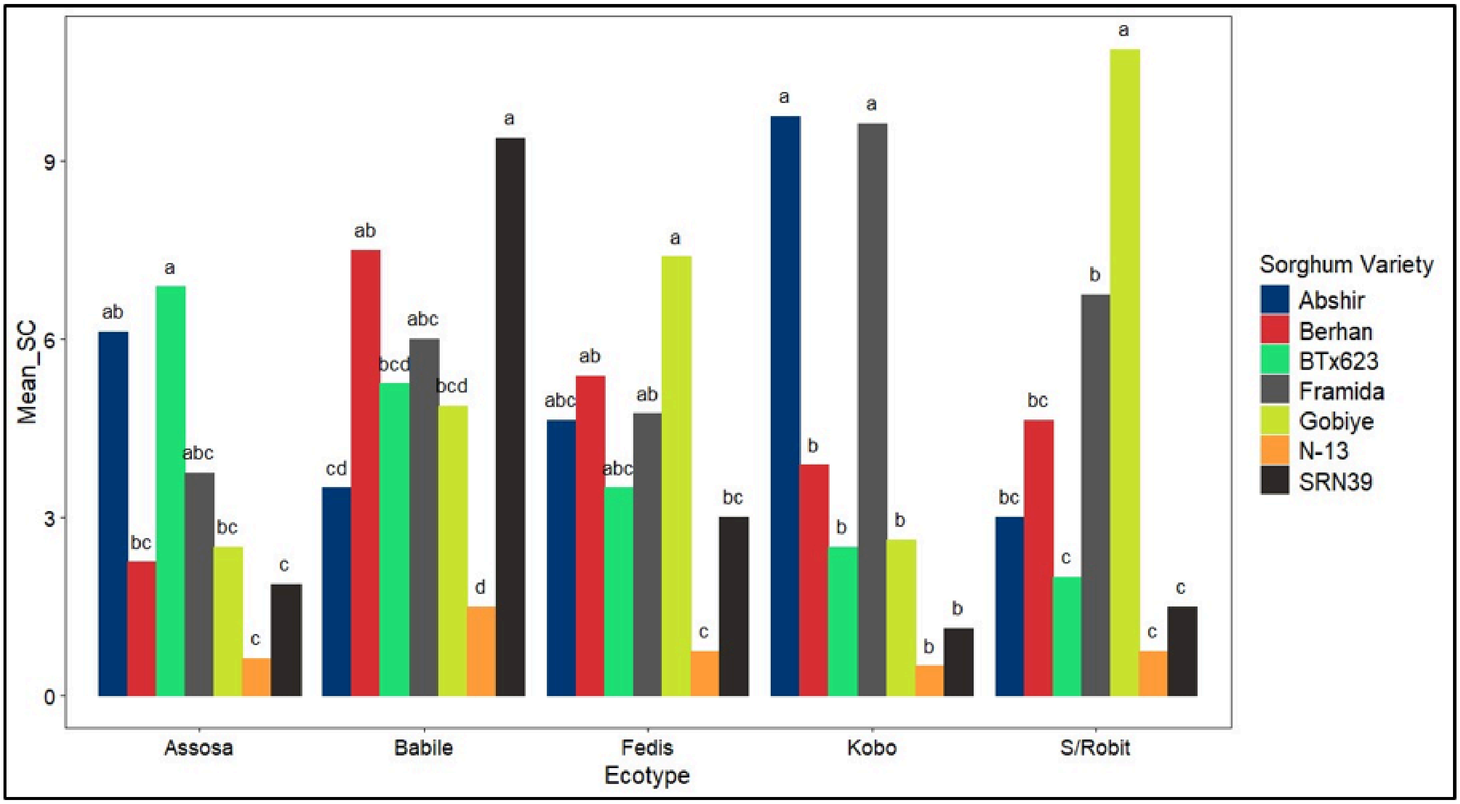
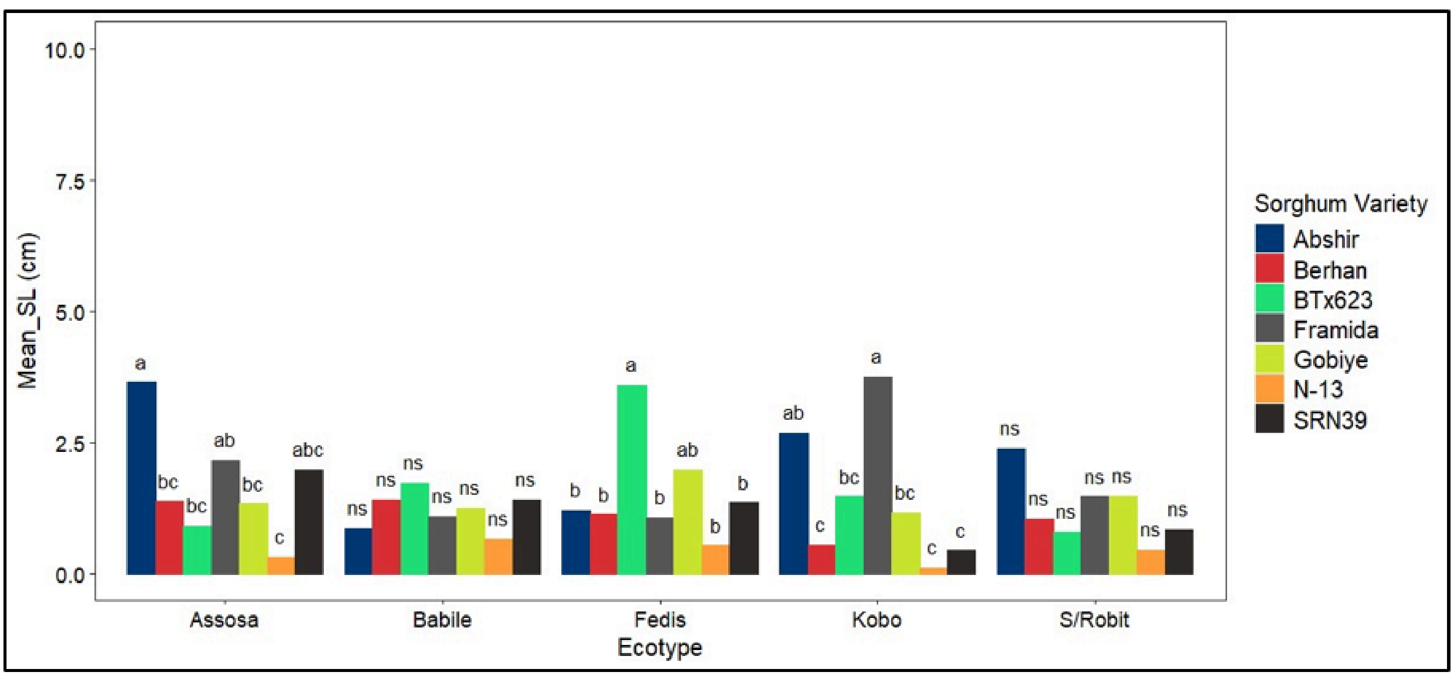



## Figure 3

Variety mean SC inside each level of the ecotypes

*Means with the same letter are not significantly different at 5% probability level*




## Figure 4

Variety mean SL inside each level of the ecotypes

*Means with the same letter are not significantly different at 5% probability level, and ns: non-significant*

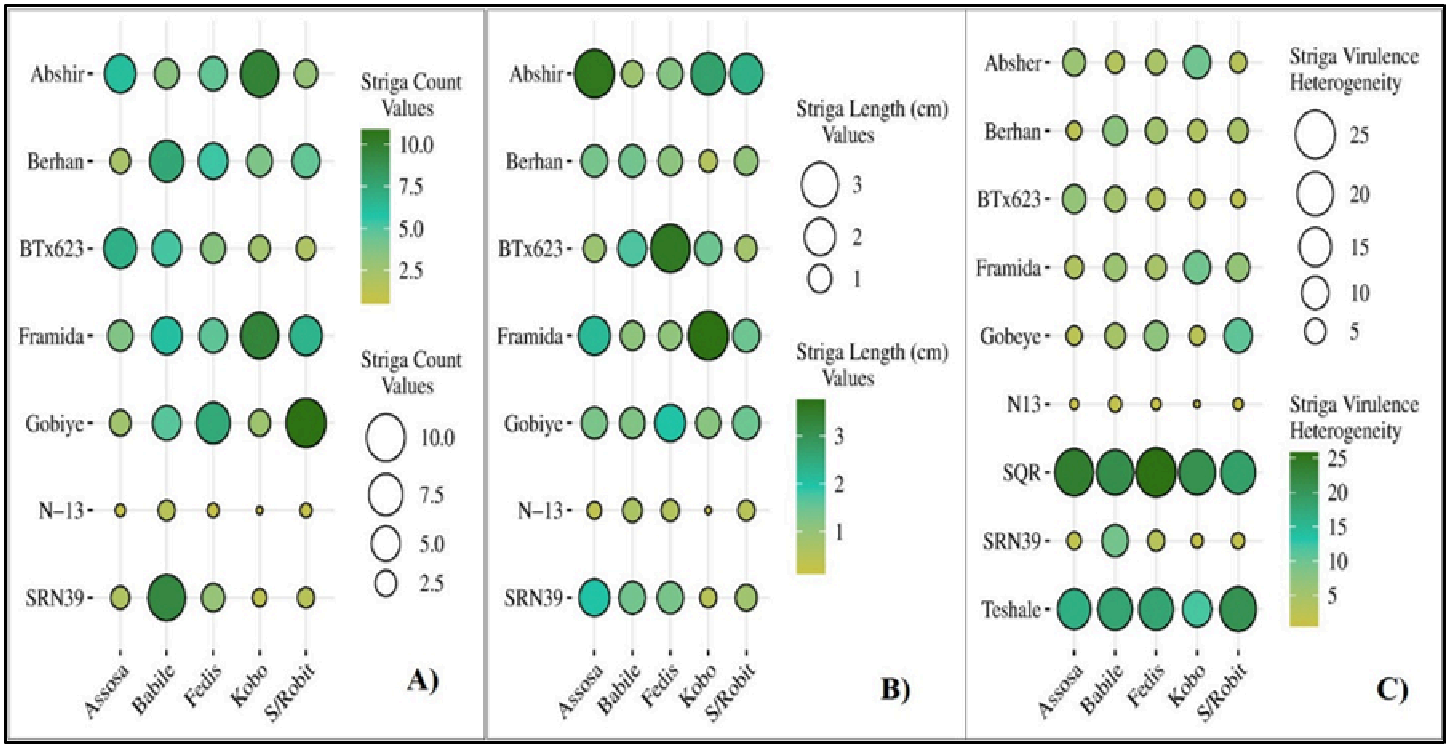



**Figure 5**

Summary on variations in the virulence level of major Ethiopian *Striga*ecotypes. **(A)** SC of seven resistance sorghum varieties, **(B)** SL (cm) of seven resistance sorghum varieties, and **(C)**SC of seven resistance sorghum varieties and two susceptible checks.

# Supplementary Files

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- SupplementaryTable.docx