ORIGINAL RESEARCH ARTICLE

Germination Response of *Striga hermonthica* Ecotypes from Western Kenya upon Exposure to Maize Root Exudates

Elvin Elizabeth Mulaa[1,2], Stephen Mwangi Githiri[1], Tesfamichael Semere Mallu[2], Damaris Achieng Odeny[2]

[1] *Department of Horticulture and Food Security, Jomo Kenyatta University of Agriculture and Technology, P.O.BOX 62000-00200, Nairobi, Kenya.*
[2] *International Crops Research Institute for the Semi-Arid Tropics–Eastern and Southern Africa, Nairobi, Kenya.*

Corresponding author email: githirim@agr.jkuat.ac.ke

ABSTRACT

Striga hermonthica, commonly known as witchweed or 'cereal killer', is a root hemiparasite of cereals, germinating only in response to host-induced molecules, strigolactones (SL). It continues to pose a serious threat to maize production in western Kenya, resulting in up to 100% crop failure. Percent germination of *Striga* is an important indicator of the virulence level of the parasite as it translates to high attachment to the host roots and varies based on the SL profile. However, specificity of *Striga* ecotypes when exposed to germination stimulants from host root exudates of differential testers of maize is unknown, hindering deployment of effective management strategies. The study sought to establish variations in virulence among *Striga* ecotypes based on crude root exudate-induced germination. We hypothesized no variation in germination responses among the *Striga* ecotypes. *In vitro* germination assays were used to determine differences in virulence among 5 *Striga* ecotypes collected from maize, sorghum, and finger millet fields in western Kenya using crude root exudates from two maize genotypes, WH403 (susceptible) and KSTP94 (resistant). *Striga* germination data was recorded by counting *Striga* seeds with a protruding radicle. All *Striga* ecotypes germinated in response to root exudates of KSTP94 and WH403. However, variations were observed among the ecotypes, ($P < 0.05$) with Alomodoi sorghum ecotype recording the highest germination in exudates of WH403 (81.9%). Bunyala sorghum ecotype recorded the lowest germination as induced by the genotypes WH403 (35%), and KSTP94 (37%). The two maize genotypes varied in their ability to *induce Striga* ecotype germination ($P < 0.05$). Results suggest that the virulence of *Striga* ecotypes could be genotype-specific. These novel outcomes necessitate using *Striga* germination response rate as a virulence factor in *Striga* virulence assays and the development of region-specific resistant genotypes. We recommend further analysis of the crude root exudates to ascertain the active compounds as well as genomic analysis of the *Striga* ecotypes.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

Keywords: *Striga* spp., Strigolactones, Root exudates, Maize, virulence.

1.0 Introduction

Striga hermonthica is a noxious root hemiparasite belonging to the genus *Orobanche.* It is a generalist parasite, able to grow on any grass family crop in endemic areas (Bellis *et al.,* 2022). The geographic distribution of *Striga hermonthica* spans Sub-Saharan Africa parasitizing hosts such as maize (*Zea mays),* sorghum (*Sorghum bicolor),* pearl millet (*Pennisetum glaucum*), and finger millet ((*Eluisine coracana*). Maize is a major staple crop and source of livelihood for many farmers in western Kenya (Buleti *et al.,* 2023). The majority of the maize producers are small-scale farmers, who practice mixed cropping under low-input rainfed systems with an average yield that is low relative to regional and world averages (FAO 2023). The low yields are partly due to abiotic and biotic stresses (Oluchi *et al.,* 2022; Yacoubou *et al.,* 2021). One of the major constraints to maize production in western Kenya is the parasitic weed, *Striga hermonthica* (hereafter referred to as *Striga)* (Ngonga *et al.,* 2024, Rich and Ejeta, 2008, Wanda *et al.,* 2019).

The lifecycle of *Striga* is highly coordinated with that of its hosts. The seeds germinate only in response to chemical cues/germination stimulants from the host roots, referred to as strigolactones (SL), often exuded into the rhizosphere of low nitrogen and phosphorous soils (Mwangangi *et al.,2021*) coupled with high temperatures and low soil moisture. The germinated seed forms a radicle that develops into a root-like organ called the haustorium in the presence of host-induced haustorium-inducing factors (HIFs). The haustorium penetrates through the roots of the plant and forms a vascular connection with the host roots, extracting nutrients and water from the host subterranean and affecting photosynthetic efficiency (Hu *et al.,* 2020). *Striga* emerges aboveground 4-7 weeks after germination where it promptly flowers and produces thousands of extremely tiny seeds that remain viable in the soil for up to 15 years, awaiting a suitable host to attach to (Mwangangi *et al.,* 2021). These large numbers of seeds with long life enable the parasite to adapt to changes in host availability, resistance, and environment, consequently making it difficult to control. The high outcrossing nature of this parasitic weed results in high diversity among its populations with observed host and geography specificities (Bozkurt *et al.,* 2014; Mbuvi *et al.,* 2017; Rodenburg *et al.,* 2017; Unachukwu *et al.,* 2017). *Striga* populations are characterized into races or biotypes based on their specificity to a host or location (Hu *et al., 2020;* Ohlson & Timko, 2020*).* In this study, an ecotype is defined as a unique combination of the host where *Striga* seed was sampled from and the specific geographic location.

Two types of host resistance have been observed in the typical *Striga*-host interactions. The first is pre-attachment resistance, which is observed as lower or lack of germination of *Striga* seeds because of low or less potent SL production by the plant, and/or reduced haustorium initiation and invasion (Fishman and Shirasu, 2021). Qualitative and quantitative differences in SL exudation among host genotypes influence their susceptibility to *Striga* (Huizinga and Bouwmeester, 2023). In sorghum, for example, the production of a less potent SL, orobanchol, as opposed to 5-deoxy

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

strigol, results in pre-attachment resistance in SRN39 (Gobena *et al.,* 2017). In maize, zeapyranolactone and zealactone have been reported to stimulate higher *Striga* germination in comparison to zealactonoic acid and zealactol (Charnikhova *et al.,* 2017; Charnikhova *et al.,* 2018; Li *et al.,* 2023). The second type of resistance is post-attachment resistance, which occurs when the plant prevents further development of already attached parasites (Fishman and Shirasu, 2021).

There are several studies reporting differences in SLs produced by the host that lead to observed resistance or susceptibility of the host. In contrast, there is limited understanding of the qualitative and quantitative differences in the parasite that lead to observed responses in the field. *Striga* seeds have been reported to have SL receptors and binding ligands that influence their germination rates (Toh *et al.,* 2015) and the final *Striga* numbers attached to a host. Among the identified receptors, *Striga hermonthica* hyposensitive to light (ShHTL) receptor, belonging to the α/β hydrolase family and assumed to be evolutionarily derived from karrikin-insensitive2 (KAI2) receptor is the most sensitive to SL (Huizinga and Bouwmeester 2023). Given the outcrossing nature of the parasite that leads to high levels of heterogeneity in infested fields, there is need to undertake more studies to further understand the quantitative differences in the receptors and binding ligands. It is also important to understand the evolution rate of the parasite across host species and genotypes grown in different ecologies to develop long-term control strategies.

Much as local farmers are aware of the *Striga* menace, several factors, such as lack of knowledge on *Striga* seed bank, limited resources, coupled with changing and harsh weather patterns have impeded effective control of this parasite in endemic areas, mostly occupied by small-scale farmers (Kountche *et al.,* 2019, Jamil *et al.,* 2021). The key to effective control of *Striga* is the depletion of the high *Striga* seed bank, through prevention of new inoculum, avoiding the spread of inoculum seed from infested to non-infested farms, and suicidal germination of *Striga* seeds (Mussleman and Rodenburg 2023; Jamil *et al.,* 2021; Runo, 2019).

The dependency of *Striga* germination on the host-released SLs has prompted the development of "Suicidal Germination" where *Striga* seeds are germinated but do not form a vascular connection with the host resulting in the death of the germinated seeds as they cannot survive on their own seed reserves hence reduces the accumulated seed bank. Efficacy of suicidal germination depends not only on the SL analog applied but also on the application protocol, especially for rain-fed African agriculture (Jamil *et al.,* 2021). Much as several SL synthetic analogs have been developed for this control method, the utility of these compounds in realizing the suicidal germination strategy for combating *Striga* is still largely unknown partly due to variations in *Striga* populations and limited knowledge of the potency of synthetic SL analog to reduce a high percent of *Striga* in one application. Developing suitable *Striga* control strategies that take into account parasite population variations relative to the active SL compound applied is crucial to minimize *Striga* damage is an urgent need for smallholder farming. This study therefore aimed to

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

understand variations in germination rates of different *Striga* ecotypes as induced by different crude root exudates from maize differential testers. We hypothesized that there is no variation in germination responses among the *Striga* ecotypes. Germination assays were used to assess the host specificity of *Striga* ecotypes based on their perception of SL from maize root exudates.

2.0 Methodology

2.1 Plant material

Mature and dry flowers of *Striga* from natural populations of sorghum, maize, and finger millet fields in Alomodoi, Bunyala, and Mbita locations were harvested separately in November 2019. Each unique combination of field host and location where the samples were collected was treated separately and characterized as an ecotype in this study (Table 1). The seeds were threshed from the dry flowers and then sieved through 250- and 150-mm sieves. *Striga* ecotype seeds were stored in plastic Eppendorf tubes in a dry place at room temperature to retain seed viability at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)-Nairobi, Kenya (Matusova *et al.,* 2005). The three geographic locations where the seeds were collected are *Striga* prone and with diverse Agroecological zones *(Infonet Biovision, n.d.)* such that Alomodoi falls in Lower Midland 1 (LM_1), Sugarcane Zone; Bunyala in LM_4, Marginal Cotton Zone; and Mbita in LM_5 Livestock-Millet Zone. To characterize variations among the *Striga* ecotypes, two genotypes of maize based on known differential responses to *Striga* and popularity among farmers in western Kenya were used. Maize genotypes WH403 (susceptible) and KSTP94 (resistant) were obtained from Western Seed Company and the Kenya Agricultural and Livestock Research Organization (KALRO), respectively. The experiment was conducted at the ICRISAT laboratories and phytosanitary greenhouse housed at the World Agroforestry Center, Nairobi, Kenya.

Table 1: Field Host and Location of Striga ecotypes used for germination assays

Field Host	Location	Ecotype	Ecotype ID	GPS Coordinates	
Maize	Bunyala	Bunyala Maize	Bnyl Mz	0°12’81.3N	34°35’84.7E
Maize	Alomodoi	Alomodoi Maize	Almdi Mz	0°51’26.5N	34° 13’78.5E
Sorghum	Alomodoi	Alomodoi Sorghum	Almdi Sg	0°51’25.7N	34° 13’37.2E
Sorghum	Bunyala	Bunyala Sorghum	Bnyl Sg	0°12’84.3N	34° 03’56.9E
Finger millet	Mbita	Mbita Finger Millet	Mbt FM	-0° 99’31.6N	34° 51’11.6E

2.2 Germination assays

2.2.1 Crude root exudate extraction

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

Root exudates were extracted from WH403 and KSTP94 genotypes of maize and used to study germination responses of five *Striga* ecotypes following Mallu *et al.* (2021) protocol. To extract root exudates, the maize genotypes were germinated in pots measuring 13.5 cm × 11 cm × 11 cm filled with vermiculite and grown hydroponically for 7 days, at which point the first trifoliate leaves had been formed. The experiment was laid out in a Completely Randomized Design (CRD) with three replications at the ICRISAT phytosanitary greenhouse at 28°C (day) and 24°C (night), under 450 μMm^{-2} s^{-1} photoperiod and 60% relative humidity. The roots were then washed to remove vermiculite residues, rinsed using distilled water, and transferred into 50 ml boiling tubes containing 1mM N Long Ashton solution (Hudson, 1967) and anchored using cotton wool. The tubes were covered with Aluminum foil to block out light and grown hydroponically for a further 7 days under screen house conditions with one plant per boiling tube per genotype and five sets of boiling tubes per replication per genotype. The roots were then rinsed three times to remove traces of nutrients and transferred to boiling tubes containing 40 ml of deionized water (mimics low nitrogen and phosphorous soils) and covered with Aluminum foil to block out light mimicking dark soil conditions. They were then incubated in darkness for 48 hours in the greenhouse to extract crude root exudates (CRE). The final volume of water with exudates was recorded alongside the root weight of each plant to calculate the volume of normalized CRE (1ml of CRE per 1g of root weight - g/ml).

2.2.2 Inoculum preparation

Preconditioning of *Striga* seeds to render them responsive to germination stimulants was done as described in Mbuvi et al. (2017). Briefly, 5mg of seed per ecotype were surface sterilized in 10% (v/v) Sodium hypochlorite (JIK®) solution for 10 minutes, rinsed using double-distilled water, then spread uniformly on a 9 mm glass fiber filter paper (Whatman GFA) mounted on sterile Petri dishes. To each petri dish, 5 ml of double distilled water was added, the petri-dishes sealed securely with Parafilm® to secure moisture and wrapped with Aluminum foil to block out light before incubating (thermal) at 30°C for 12 days in the dark. Each ecotype was replicated 3 times for each genotype root exudate.

2.2.3 Germination of *Striga* seed

To synchronize the germination response, CRE was extracted at the same time for all replications and used immediately to test germination. Normalized CRE, 5ml per 5 g root weight, was added to individual Petri dishes containing preconditioned *Striga* seeds. GR24, a synthetic SL analog, was used as a positive control (5 ml of 0.1 ppm), and deionized water as a negative control. Petri dishes were sealed using Parafilm®, covered with Aluminum foil, and incubated(thermal) in the dark at 30°C for 12 hours. The petri dishes were laid out in a split-plot design with genotype as the main factor and ecotype as a subfactor. Germinated *Striga* seeds were observed and photographed under a stereomicroscope model NIKON SMZ745T fitted with a NIKON DCIN12V camera (Nikon Instruments Inc.,https://www.microscope.healthcare.nikon.com/products/stereomicroscopes-

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

macroscopes/smz745-745t . Three fields of view were taken for each plate and analyzed for germination frequency using Fiji- Win64 ImageJ (http://rsb.info.nih.gov/ij).

2.3 Data collection and statistical analysis

Average *Striga* germination percent per ecotype per genotype per replication was calculated using the formula: Mean germination $=\{(^{x_1}/_{y_1} * 100) + (^{x_2}/_{y_2} * 100) + (^{x_3}/_{y_3} * 100)\}/3$, where x is the number of germinated *Striga* seeds in a specific field of view, y is the total number of *Striga* seeds in a specific field of view, sub-scripts 1, 2, and 3 are the specific fields of view, and 3 is the number of replications (Mallu et al., 2021).

Descriptive statistics of the data were run by assessing normality using the Shapiro-Wilk test in R and Levene test to asses homogeneity of variance. *Striga* germination data followed a normal distribution (W = 0.98, *p*-value = 0.62). Genotype (DF-1, Groups- 57, F value- 3.81, Pr(>F)- 0.62) and ecotype (DF- 4, Group- 54, F value- 3.04, Pr(>F)- 0.06) variances were equal. Variations among *Striga* ecotypes were analyzed by constructing analysis of variance (ANOVA) linear model with genotype as the main effect and ecotype as a random effect, together with the interaction of ecotype and genotype. Source location and source host of *Striga* ecotypes were also considered as random factors in the evaluation of variance. Post-hoc pairwise comparisons within genotype and ecotype were performed by running Tukey's Significant Difference at 5% confidence level. Boxplots of each data set with adjusted *p* values were generated in R using the ggplot2 package. To ensure that existing differences were purely ecotype dependent, variations in genotype biology were controlled for from the resulting germination frequencies by normalizing against the positive control GR24 value to obtain relative percent germination values over GR24. Normalization against GR24 was obtained by dividing the germination frequency of each ecotype genotype combination with the corresponding GR24-induced % germination per replication.

3.0 Results

3.1 Germination response of *Striga* ecotypes to CRE from KSTP94 and WH403

All the *Striga* ecotypes germinated in response to root exudates of the KSTP94 and WH403 genotypes, with germination ranging from 35.3% (Bunyala sorghum subjected to WH403 CRE) to 81.9% (Alomodoi sorghum subjected to WH403 (Figure 1, Table 3). Based on the means across each ecotype, there was a significant difference at $p < 0.05$ in germination response with Alomodoi sorghum recording the highest germination (61.3%) while Bunyala sorghum recording the lowest (44.1%) (Figure 1, Table 3). When variations in genotypes CRE were controlled for by the positive control GR24 to obtain the relative percent germination of each ecotype, similar results in ecotype variation were observed. Alomodoi sorghum is more virulent to WH403 considering the high germination percent while Bunyala sorghum is least virulent to WH403. Significant interaction ($p < 0.05$) was observed in the ecotype by genotype response as observed in the means of interaction (Table 3). This high interaction observed indicates a high degree of diversity among the *Striga* ecotypes.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

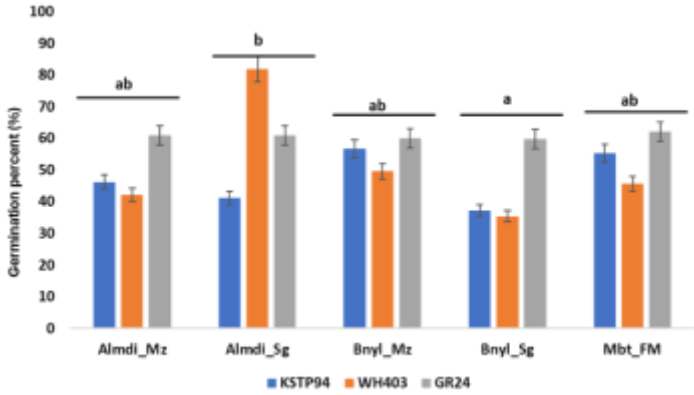

Figure 1: Overall germination response of different Striga ecotypes from Alomodoi (Almdi), Bunyala (Bnyl) and Mbita (Mbt) locations in Maize (Mz), Sorghum (Sg) and Finger millet (FM) hosts when subjected to crude root exudates of maize genotypes KSTP94 (Resistant) and WH403 (susceptible) and GR24 (positive control) at P<0.05.

Table 3. Striga germination Percentage for each ecotype when subjected to WH403 and KSTP94

ECOTYPE	KSTP94	WH403				MEAN (*p*)
	Means (%)	Means (%)	*SE*	*SED*	*%CV*	
Bnyl_Mz	56.60	49.50	*6.06*	*8.57*	*12.24*	53.0 ab *(0.99ns)*
Bnyl_Sg	41.07	35.30	*6.06*	*8.57*	*17.16*	36.2 a *(1.00ns)*
p (within)	*0.004 ** *					
Almdi_Mz	46.17	46.17	*6.06*	*8.57*	*14.44*	44.1 ab *(1.00ns)*
Almdi_Sg	41.07	81.90	*6.64*	*8.57*	*7.4*	61.5 b *(0.001**)*
p (within)	*0.10 ns*					
Mbt_FM	55.20	45.60	*6.06*	*8.57*	*13.29*	50.4 ab *(0.98ns)*

Mean values of genotypes KSTP94 and WH403 the interaction between the specific genotype and specific ecotype obtained from ANOVA post hoc analysis run with Tukey's LSD at 95% confidence interval and the Average MEAN per ecotype across the genotypes. p-values obtained from post hoc analysis with pairwise comparisons highlight significant differences within the same region. SE- Standard Error, SED- Standard Error of Difference, and & %CV- Percent Coefficient of Variation.

3.2. Variation within each maize genotype for *Striga* germination induction

No significant variations ($p < 0.05$) were observed between the maize genotypes for their ability to induce *Striga* ecotype germination. However, WH403 CRE induced a high germination % overall

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

based on means across ecotypes in comparison to KSTP94 (Figure 2A & B). A comparison of germination % within each ecotype revealed no significant difference in the germination of Alomodoi maize, Bunyala maize, and Bunyala sorghum irrespective of whether CRE or GR24 was used (Figure 2 A & B). For Alomodoi sorghum and Mbita finger millet, there was no significant difference between germination % induced by GR24 and CRE from the resistant genotype, KSTP94 (Figure 2A). However, we observed significant differences when CRE from the susceptible genotype WH403 was used. For Alomodoi sorghum, significantly ($p < 0.05$) higher germination % was observed while for Mbita FM, significantly ($p < 0.05$) lower germination rate was reported in comparison to GR24 and CRE from KSTP94 (Figure A). When CRE from maize genotypes was controlled for by GR24, similar results were obtained except that there was no significant difference in Mbita finger millet (Figure 2B).

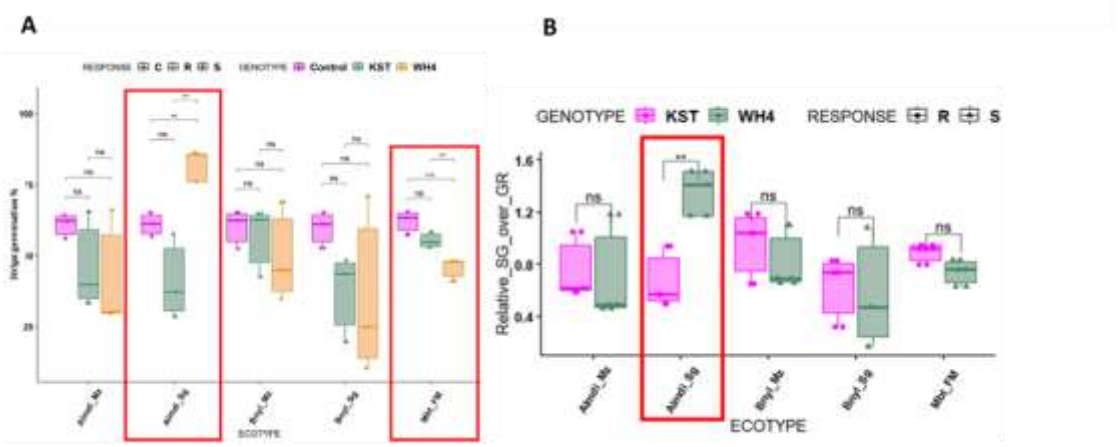

Figure 2: Boxplots showing the overall distribution of germination response of different Striga ecotypes from Alomodoi (Almdi), Bunyala (Bnyl) and Mbita (Mbt) locations in Maize(Mz), Sorghum (Sg) and Finger millet (FM) hosts when subjected to crude root exudates of maize genotypes KSTP94 (Resistant -R) and WH403 (susceptible - S) and GR24 (positive control -C) at P<0.05. A. Overall differences in germination % observed for each ecotype relative to the positive control GR24. B. Overall differences in germination % observed for each ecotype when CRE of maize genotypes is controlled for by GR24.

3.3. Effect of source location and host species on *Striga* ecotype germination rate

In Table 3 above, the *p*-values between the ecotypes highlight where significant differences exist within the same region per genotype. For instance, the significant *p*-value (0.004**) between Bunyala maize and Bunyala sorghum ecotypes suggests a notable variation between the ecotypes. In contrast, Alomodoi maize and Alomodoi sorghum show no significant differences ($p > 0.05$) suggesting a similarity between the ecotypes. Variations observed within Bunyala ecotypes with ecotypes from maize host resulted in higher germination than those from sorghum genotypes

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

(Figure 3A) with no interaction between the ecotypes. Contrarily, in Alomodoi, the ecotypes had exhibited a positive divergent interaction such that when CRE from the susceptible genotype WH403 was used in the Alomodoi sorghum ecotype, there was a high positive increase in germination (Figure 3B).

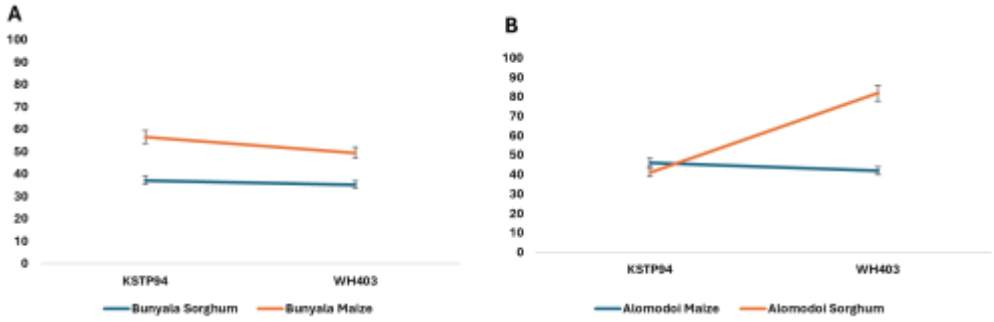

Figure 3: The germination rates of different Striga ecotypes per source location when subjected to CRE of maize genotypes KSTP94 (Resistant) and WH403 (susceptible) at $p < 0.05$. A. Overall differences in germination % observed for Bunyala ecotypes. B. Overal differences in germination % observed for Alomodoi ecotypes

Regarding the source host, no variations were observed across the maize ecotypes. However, the Bunyala maize ecotype recorded higher germination as induced by both KSTP94 and WH403 (Figure 4A), but this did not result in an interaction between the ecotypes. Sorghum ecotypes recorded significant mean germination differences ($p < 0.05$) with the Alomodoi sorghum ecotype recording the highest germination (81.9%) while Bunyala sorghum recorded the lowest germination of 35.3% (Table 4, Figure 4B).

Table 4: Means and probabilities of germination of Source host p-values obtained from post hoc analysis with pairwise comparisons between the ecotypes highlight where significant differences exist within the same region per genotype.

	KSTP94	WH403	MEAN	*p between ecotypes*
Bnyl_Mz	56.60	49.50	53.0 ab	
Almdi_Mz	46.17	46.17	44.1 ab	0.57
p (between genotypes)	*1*	*0.99*		
Bnyl_Sg	41.07	35.30	36.2 a	
Almdi_Sg	41.07	81.90	61.5 b	0.004***
p (between genotypes)	*1*	*0.0002 ****		

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

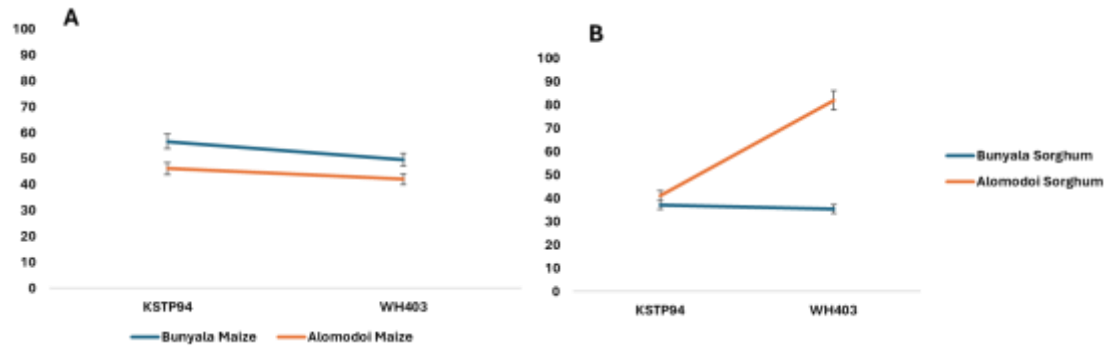

Figure 4: The germination rates of different Striga ecotypes per source host when subjected to CRE of maize genotypes KSTP94 (Resistant) and WH403 (susceptible) at $p < 0.05$. A. Overall differences in germination % observed for maize host ecotypes. B. Overall differences in germination % observed for sorghum host ecotypes.

4.0 Discussion

The study results show *Striga* ecotype populations varied in their ability to germinate, indicating variations in virulence possibly attributed to variations in the size of SL receptor binding pocket in *Striga* seed populations and/or SL profile in genotypes (Conn *et al.,* 2015). Matusova *et al. (*2004) observed that *Striga* seeds increase sensitivity toward SL after preconditioning at 30°C for a certain period, after which this sensitivity gradually decreases until the seeds enter secondary dormancy. In this study, the *Striga* ecotypes were harvested at the same time, stored in similar conditions, and preconditioned at 30°C for 12 days before inducing germination with crude root exudates, providing uniformity for germination assays such that any variation observed would be as a result of innate ecotype differences.

In this study, the 5 *Striga* populations were sampled from different hosts and different locations possibly resulting in the observed variations in the means percent germination rates. Regarding host specificity, molecular markers used to study variations in *Striga* populations sampled from different hosts have indicated host specificity in *Striga* populations (Bellis *et al.,* 2022). Qui *et al.* (2022) observed a variation in virulence among *Striga* populations sampled from maize, sorghum, and finger millet within the same location much as the variation was plastic. This plastic nature of virulence could explain the high interaction observed between ecotypes and genotypes in mean percent germination.

Regarding geography specificity, *Striga* seed populations used in this study were sampled from three different agroecological zones, Alomodoi (Lower Midland 1 (LM_1), Sugarcane Zone); Bunyala (LM_4, Marginal Cotton Zone); and Mbita (LM_5 Livestock-Millet Zone) with varying temperatures and average rainfall *(Infonet Biovision, n.d.)*. Suitable environment for *Striga* growth and multiplication includes low rainfall, high temperatures, and poor soil nutrition evidenced by the

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

soil's physical and chemical properties (Mwangangi *et al.,* 2021; Sainju *et al.,* 2022). Pathogen virulence evolution based on temperature and humidity has been observed in other plant pathogens and in *Striga* where *Striga* populations from different zones vary in their virulence and host specificity (Bozkurt *et al.,* 2014; Estep *et al.,* 2011; Rodenburg *et al.,* 2017; Unachukwu *et al.* 2017; Wu *et al.*, 2016; Zarattini *et al.,* 2021). Mbuvi *et al.* (2017) observed that *Striga* population from Kibos (LM_3 with lower relative humidity) was more virulent to sorghum genotypes in *Striga* count, length, and weight when compared with *Striga* from Alupe and Mbita. In our study, the Bunyala maize ecotype, falling on LM_4, with lower relative humidity, recorded an overall high germination percent as induced by GR24, WH403, and KSTP94 relative to other ecotypes. Variations within the Alomodoi would be a result of genetic drift while gene flow would result in lack of variation within Bunyala.

Recent advances in sequencing technologies and bioinformatics have enabled the characterization of the protein-specific family in parasite seed that perceives and hydrolyzes SL from hosts to initiate the germination process. SL perception and hydrolysis processes in *Striga* are controlled by the protein family KAI2d in the D14 family. The diameter of the KAI2d receptor ligand varies in size among *Striga* populations causing distinct sensitivity to SL profiles perceived from the host crude root exudates, which translates to variation in the germination response of *Striga* populations. (Bürger & Chory, 2020; Huizinga and Bouwmeester, 2023; Toh *et al.,* 2015). These variations in the SL receptor pocket also play a role in host adaptation and specificity (Conn *et al.,* 2015). The results of this study agree with Bürger & Chory (2020), who modeled the germination of various *Striga* populations using 20 different ligand pockets and observed that they perceived varying SL profiles differently. Toh *et al.* (2015) also characterized 11 SL receptors in *Striga* and observed one to have a relatively large binding pocket able to perceive Pico molar concentrations of SL, explaining the ability of each *Striga* population to germinate in response to the crude root exudates. Bellis *et al.* (2022) reported little phenotypic and genomic evidence of host-specific SL receptors in a diverse set of *Striga,* with only 9% of parasite virulence transcripts accounting for host specificity, suggesting plastic host preference in *Striga* populations. This could explain the ability of all the *Striga* populations from different regions and hosts used in this study to germinate. Irafaha *et al. (*2023) observed that SL perception is followed by a complex network of hormonal cross-talk that either enhances or represses germination rates, resulting in variations observed which could explain the variations observed among the *Striga* populations in this study.

Structural makeup and concentrations of SL vary across hosts and determine the germination percentages of *Striga* populations and the susceptibility of genotypes (Huizinga & Bouwmeester, 2023). In this study, KSTP94, though a resistant genotype, did not consistently induce lower germinations across all the ecotypes even when controlled for by GR24, relative to WH403, a susceptible genotype, suggesting a higher effect of ecotype on the percent germinations observed. This is contrary to Yoneyama *et al.* (2015) who observed that KSTP94 induced lower germination rates in *Striga* relative to a susceptible cultivar, Pioneer 3253, by exuding sorgomol, a

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

low-germination stimulant of *Striga*. However, in his study, only a single population of *Striga* was used. In a study to understand the potential of *Striga* germination as induced by crude root exudates from a collection of maize landraces, inbred lines, and populations, Karaya *et al.* (2012) observed that landraces induced lower germination of *Striga* seeds compared to inbred lines, which induced higher germination irrespective of their susceptibility, suggesting a probable avoidance of root architecture mode of resistance as opposed to low SL production. Studies have indicated that the low-germination stimulant orobanchol can be potent in one *Striga* population and less potent in another *Striga* population. Precisely, *Striga* populations from Mali and Niger have recorded poor germination induced by orobanchol while Kenyan populations induced the highest germination (Gobena *et al.,* 2017; Haussmann *et al.,* 2004). Considering that the specific pre- and post-attachment resistance mechanisms for WH403 are not recorded, further studies need to be conducted to verify the specific SL exuded by WH403 and the post-attachment resistance mechanisms that render its classification as a susceptible genotype. Variations in the SL profile present in the host root exudate and variations in the SL receptor sizes in the *Striga* ecotypes would contribute to the variations observed in the germination percentages. Overall, the *Striga* population and host genotype played a significant role in germination percentages relative to the susceptibility of each genotype, alluding to high genetic diversity in the *Striga* populations. With the advent of suicidal germination technique to manage *Striga,* these results allude to the fact that potent SL analogs used in suicidal germination can only be identified when tested alongside varying parasite populations. *Striga* germination translates to *Striga* attachments hence eventual damage on host crops. These results indicate host-specific virulence of *Striga* hence useful knowledge for farmers to select varieties that support *Striga* in their specific locality.

5.0 Conclusion and recommendation

Results show that virulence of the *Striga* ecotypes with respect to SL perception resulting in different germination rates. Alomodoi sorghum recorded the highest mean germination percentage. Ecotypes did not cluster based on source host or region, indicating high diversity within the same region. WH403 showed more variation, especially in the Alomodoi sorghum ecotype, where its performance significantly exceeds that of KSTP94 and the least germination in Bunyala sorghum. This suggests that WH403 may be more susceptible in certain ecotypes making it an unsuitable variety for farmers relative to a more stable KSTP94 that showed minimal variability. KSTP94 showed minimal variability across ecotypes, with mean percentages ranging from 41.07% to 56.60%. The consistent performance of KSTP94 suggests a stable host-pathogen interaction, with no significant susceptibility to the specific ecotype.

Since *Striga* germination percent response is a function of *Striga* population and SL profile in genotypes and in some cases interaction of both, we recommended further chemical analysis of the SL in the crude root exudates of WH403 and KSTP94 to determine the specific type and concentration of SL coupled with molecular analysis of the SL receptors in the *Striga* ecotypes to verify the exact cause of high germination percentages. Pot and field experiments to ascertain *in*

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

vitro assay results are also recommended alongside studies to understand the precise effect of host root mass on the SL profile.

6.0 Acknowledgements

6.1 Ethical consideration

None

6.2 Conflict of Interest

The authors declare no conflict of interest.

6.3 Funding

We acknowledge the support received from Crop TRUST through the project "Safeguarding crop diversity for food security: Pre-breeding complemented with Innovative Finance", which was funded by the Templeton World Charity Foundation, Inc. (TWCF0400), and the initiative "Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives", supported by the Government of Norway (QZA-14/0005).

7.0 References

Bellis, E. S., Münchow, C. S. von, Kronberger, A., Odero, C. O., Kelly, E. A., Xia, T., Huang, X., Wicke, S., Runo, S. M., dePamphilis, C. W., & Lasky, J. R. (2022). Genomic signatures of host-specific selection in a parasitic plant [Preprint]. *BioRxiv*, 2022.02.01.478712. https://www.biorxiv.org/content/10.1101/2022.02.01.478712v2%0A

Bozkurt, M. L., Muth, P., Parzies, H. K., & Haussmann, B. I. G. (2014). Genetic diversity of East and West African *Striga hermonthica* populations and virulence effects on a contrasting set of sorghum cultivars. *Weed Research*, *55*, 71–81. https://doi.org/10.1111/wre.12117

Buleti, S. I., Kuyah, S., Olagoke, A., Gichua, M., Were, S., Chidawanyika, F., & Martin, E. A. (2023). Farmers' perceived pathways for further intensification of push-pull systems in Western Kenya. *Sustainable Food Systems*, *7*(1191038), 1–14. https://doi.org/10.3389/fsufs.2023.1191038

Bürger, M., & Chory, J. (2020). In-silico analysis of the strigolactone ligand-receptor system. Plant Direct, 4(9), 1–10. https://doi.org/10.1002/pld3.263

Charnikhova, T. V., Gaus, K., Lumbroso, A., Sanders, M., Vincken, J. P., De Mesmaeker, A., Ruyter-Spira, C. P., Screpanti, C., & Bouwmeester, H. J. (2017). Zealactones. Novel natural strigolactones from maize. *Phytochemistry*, *137*, 123–131. https://doi.org/10.1016/j.phytochem.2017.02.010

Charnikhova, T.V., Katharina Gaus, Alexandre Lumbroso, Mark Sanders, Jean-Paul Vincken, Alain De Mesmaeker, Carolien P. Ruyter-Spira, Claudio Screpanti, & Harro J. Bouwmeester. (2018). Zeapyranolactone – A novel strigolactone from maize. *Phytochemistry Letters*, *25*, 172–178. https://doi.org/10.1016/j.phytol.2018.01.003

Conn, C. E., Bythell-Douglas, R., Neumann, D., Yoshida, S., Whittington, B., Westwood, J. H., Shirasu, K., Bond, C. S., Dyer, K. A., & Nelson, D. C. (2015). Convergent evolution of strigolactone perception enabled host detection in parasitic plants. *Plant Evolution*, *349* (6247). https://www.science.org

Estep, M. C., Mourik, T. A. Van, Muth, P., Weltzien, E., & Bennetzen, J. L. (2011). Genetic diversity of a parasitic weed, *Striga hermonthica*, on sorghum and pearl millet in Mali. *Tropical Plant Biology*, *4*, 91–98. https://doi.org/10.1007/s12042-011-9073-6

FAO. (2023). World Food and Agriculture: Statistical Yearbook 2023. https://doi.org/10.4060/cc8166en

Fishman, M. R., & Shirasu, K. (2021). How to resist parasitic plants: pre- and post-attachment strategies. *Current opinion in plant biology*, *62*, 102004. https://doi.org/10.1016/j.pbi.2021.102004

Gobena, D., Shimels, M., Rich, P. J., Ruyter-spira, C., Bouwmeester, H., & Kanuganti, S. (2017). *Mutation in sorghum LOW GERMINATION STIMULANT 1 alters strigolactones and causes Striga resistance*. *114*(17), 4471–4476. https://doi.org/10.1073/pnas.1618965114

Haussmann, B. I. G., Hess, D. E., Omanya, G. O., Folkertsma, R. T., Reddy, B. V. S., Kayentao, M., Welz, H. G., & Geiger, H. H. (2004). Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. *Theoretical Applied Genetics*, *109*, 1005–1016. https://doi.org/10.1007/s00122-004-1706-9

Hu, L., Wang, J., Yang, C., Islam, F., & Bouwmeester, H. J. (2020). The effect of virulence and resistance mechanisms on the interactions between parasitic plants and their hosts. *International Journal of Molecular Sciences*, *21*(9013), 1–27. https://doi.org/10.3390/ijms21239013

Hudson, J. P. (1967). Sand and water culture methods used in the study of plant nutrition. *Experimental Agriculture*, *3*(2), 104–104. https://doi:10.1017/S0014479700021852

Huizinga, S., & Bouwmeester, H. J. (2023). Role of strigolactones in the host specificity of broomrapes and witchweeds. *Plant & cell physiology*, *64*(9), 936–954. https://doi.org/10.1093/pcp/pcad058

Infonet Biovision. (n.d.). *Agro-Ecological Zones (AEZs) FAO System*. Infonet Biovision. Retrieved August 15, 2024, from https://www.infonet-biovision.org/agro-ecological-zones/aezs-fao-system

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

Irafaha, G., Mutinda, S., Mobegi, F., Hale, B., Omwenga, G., Wijeratne, A. J., Wicke, S., Bellis, E. S. *, & Runo, S. (2022). A transcriptome atlas of *Striga hermonthica* germination. *BioRxiv - Plant Biology*, 1–21. https://doi.org/10.1101/2022.12.14.520245

Jamil, M., Kountche, B. A., & Al-babili, S. (2021). Current progress in *Striga* management. Plant Physiology, 185, 1339–1352. https://doi.org/10.1093/plphys/kiab040

Karaya, H., Njoroge, K., Mugo, S., Ariga, E. S., Kanampiu, F., & Nderitu, J. H. (2012). Determination of levels of *Striga* germination Stimulants for maize gene bank accessions and elite inbred lines. *International Journal of Plant Production*, *6*(2), 1735. www.SID.ir

Kountche, B. A., Jamil, M., Yonli, D., Daniel, P. N., Ania, B., Asami, T., Zwanenburg, B., & Al, S. (2019). Suicidal germination as a control strategy for *Striga hermonthica* (Benth.) in smallholder farms of sub - Saharan Africa. 107–118. https://doi.org/10.1002/ppp3.32

Li, C., Dong, L., Durairaj, J., Guan, J., Yoshimura, M., Quinodoz, P., Horber, R., Gaus, K., Li, J., Setotaw, Y. B., Qi, J., de Groote, H., Wang, Y., Thiombiano, B., Floková, K., Walmsley, A., Charnikhova, T. V., Chojnacka, A., Correia de Lemos, S., Bouwmeester, H. J. (2023). Maize resistance to witchweed through changes in strigolactone biosynthesis. *Plant Science*, *379*, 94–99. http:///doi/10.1126/

Mallu, T. S., Mutinda, S., Githiri, S. M., Achieng Odeny, D., & Runo, S. (2021). New pre-attachment *Striga* resistant sorghum adapted to African agro-ecologies. *Pest Management Science*, *77*(6), 2894-2902. https://doi.org/10.1002/ps.6325

Matusova, R., Rani, K., Verstappen, F. W. A., Franssen, M. C. R., Beale, M. H., & Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiology, 139*(920–934). https://doi.10.1104/pp.105.061382.920

Mbuvi, D. A., Masiga, C. W., Kuria, E., Masanga, J., Wamalwa, M., Mohamed, A., Odeny, D. A., Hamza, N., & Timko, M. P. (2017). Novel Sources of Witchweed (*Striga*) Resistance from Wild Sorghum Accessions. *Frontiers in Plant Science*, *8*(116). https://doi.org/10.3389/fpls.2017.00116

Mussleman, J. L., & Rodenburg, J. (2023). Parasitic Plants in African Agriculture: Witchweed. In *CABI* (pp. 72–88). *CABI: Biological Sciences Faculty Books*. https://doi.org/10.1079/9781789247657.0007

Mwangangi, I. M., Büchi, L., Haefele, S. M., Bastiaans, L., Runo, S., & Rodenburg, J. (2021). Combining host plant defense with targeted nutrition: key to durable control of hemiparasitic *Striga* in cereals in sub-Saharan Africa. *The New phytologist*, *230*(6), 2164–2178. https://doi.org/10.1111/nph.17271

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

Ohlson, Erik. W., & Timko, Michael. P. (2020). Race Structure of cowpea witchweed (*Striga gesnerioides*) in West Africa and its implications for *Striga* resistance breeding of cowpea. *Weed Science Society of America*, *68*(2), 125–133. https://doi.org/10.1017/wsc.2020.3

Oluchi, G. D., Ayangbenro, A. S., Odhiambo, J. J. O., & Babalola, O. O. (2022). *Striga hermonthica:* A highly destructive pathogen in maize production. *Environmental Challenges*. *8*(100590). https://doi.org/10.1016/j.envc.2022.100590

Rich, P. J., & Ejeta, G. (2008). Towards effective resistance to *Striga* in African maize. *Plant Signaling and Behavior*, *3*(9), 618–621. https://doi.org/10.4161/psb.3.9.5750

Rodenburg, J., Cissoko, M., Kayongo, N., Dieng, I., Bisikwa, J., Irakiza, R., Masoka, I., Midega, C. A. O., & Scholes, J. D. (2017). Genetic variation and host-parasite specificity of *Striga* resistance and tolerance in rice: the need for predictive breeding. *New Phytologist*, *214*, 1267–1280. https://doi.org/10.1111/nph.14451

Runo, S. (2019). Modern breeding approaches against the parasitic plant *Striga*. *Afrika Focus*, *32*(2), 109–115. https://doi.org/10.1163/2031356X-03202009

Sainju, U.M., Liptzin, D., Jabro, J.D. (2022). Relating soil physical properties to other soil properties and crop yields. *Scientific Reports* 12, 22025. https://doi.org/10.1038/s41598-022-26619-8

Toh, S., Ehrhardt, D. W., Biol, N. C., Holbrook-Smith, D., Stogios, P. J., Onopriyenko, O., Lumba, S., Tsuchiya, Y., Savchenko, A., & Mccourt, P. (2015). Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Sciencemag Research Reports,* 350(6257), 203–208. https://doi.org/10.1126/science.aac7446

Unachukwu, N. N., Menkir, A., Rabbi, I. Y., Oluoch, M., Muranaka, S., Elzein, A., Odhiambo, G., Farombi, E. O., & Gedil, M. (2017). Genetic diversity and population structure of Striga *hermonthica* populations from Kenya and Nigeria. *Weed Research*, *57*(5), 293–302. https://doi.org/10.1111/wre.12260

Wu, E. J., Yang, L. N., Zhu, W., Chen, X. M., Shang, L. P., & Zhan, J. (2016). Diverse mechanisms shape the evolution of virulence factors in the potato late blight pathogen Phytophthora infestans sampled from China. *Scientific Reports*. 6. Nature Publishing Group. https://doi.org/10.1038/srep26182

Yacoubou, A. M., Zoumarou Wallis, N., Menkir, A., Zinsou, V. A., Onzo, A., Garcia-Oliveira, A. L., Meseka, S., Wende, M., Gedil, M., & Agre, P. (2021). Breeding maize (Zea mays) for *Striga* resistance: past, current and prospects in sub-Saharan Africa. *Plant Breeding*. *140*(2), 195–210. https://doi.org/10.1111/pbr.12896

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1

Yoneyama, K., Arakawa, R., Ishimoto, K., Kim, H. il, Kisugi, T., 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 Phytologist*, *206*(3), 983–989. https://doi.org/10.1111/nph.13375

Zarattini, M., Farjad, M., Launay, A., Cannella, D., Souli, M. C., Bernacchia, G., & Fagard, M. (2021). Every cloud has a silver lining: how abiotic stresses affect gene expression in plant-pathogen interactions. *Journal of Experimental Botany*. *72*(4), 1020–1033. https://doi.org/10.1093/jxb/eraa531

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v24i1.1