



## Determination of levels of *Striga* germination Stimulants for maize gene bank accessions and elite inbred lines

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### Abstract

Parasitism by *Striga hermonthica* (Del) Benth is a severe constraint in maize production in sub-Saharan Africa. Varying levels of tolerance to *Striga* attack have been identified and exploited in breeding programs of several crops. However, the level and stability of the tolerance is generally unacceptable in field-practice. Only limited exploration has been undertaken among the farmers' landraces to find the presence of viable sources of resistance to *Striga*. The objective of this study was to examine and document the presence of the *Striga* germination stimulants from a collection of some 420 maize landraces, populations and elite inbred lines. The genotypes were variously sourced from International Maize and Wheat Improvement Center (CIMMYT), International Institute for Tropical Agriculture (IITA) and Kenya Agricultural Research Institute (KARI). The ability to effect germination as a measure of the amount of germination stimulant produced was used to assess the materials, using the standard procedures. Data were recorded on *Striga* germination by counting *Striga* seeds with protruding radicle. Highly significant ( $P=0.001$ ) differences were observed among the germplasm screened. Several landraces were found to stimulate low levels of *Striga* germination compared to the commercial checks. Landraces CRIC 51, CUBA T-31, BRAZ 1758, BRAZ 1279 and VERA 217 exhibited the lowest *Striga* germination, an indication of high level of resistance to *Striga*. The inbred lines were found to have a higher *Striga* germination percent compared to the landraces, a likelihood of a higher concentration of strigol, the stimulant causing chemical. CIMMYT lines CML 202 IR, CML 445 IR and CML 204 IR induced the least amount of *Striga* seeds to germinate. Higher levels of germination of *Striga* seeds were found in the IITA lines which are known to be

resistant, depicting a probable avoidance root architecture mode of resistance as opposed to low production of strigol. It was concluded that the landraces with low *Striga* germination percent can be used by breeders in the extraction of new *Striga* resistant inbred lines. The resistant inbred lines can be recommended for direct use in the formation of maize synthetics and hybrids resistant to *S. hermonthica*.

**Keywords:** *Striga hermonthica*; Maize landraces; Tolerance to *Striga*; Resistance to *Striga*; *Striga* germination stimulant.

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## Introduction

Maize is an important cereal crop in Africa. It constitutes the staple diet of many people in sub-Saharan Africa as evidenced by high average annual consumption levels of 79 kg per capita in the continent and 125 kg per capita in Kenya (Pingali, 2001). Productivity in grain yield in maize is a factor of its genetic make-up and its interaction with the environment such as soil, water, temperature, pests pressure, diseases and parasitic weeds such as *Striga spp* (Karaya et al., 2009). The life cycle of *S. hermonthica* is complex and comprises a series of discrete steps which are intimately tied to that of its host from seed to mature or seed producing plants. Understanding *Striga* biology is the starting point to develop mechanisms towards its control through development of resistance in maize. After dispersal, the seeds are in a state of primary dormancy for up to six months (Kuiper et al., 1996). After ripening is a second prerequisite for germination, the preconditioning of the seeds which requires a period of imbibitions of water for several weeks under humid and warm (25-35 °C) conditions (Kebreab and Murdoch, 1999; Ast, 2006). Prolonged preconditioning induces secondary dormancy which usually occurs when the *Striga* seeds have reached maximum sensitivity (Matusova et al., 2004). Germination of *S. hermonthica* is induced by stimulants exuded by roots of host and some non host plants (Ma et al., 1996). These host-derived germination stimulants are termed as xenognosins (Lynn et al., 1981; Yoder, 2001), and they have specifically been identified as sesquiterpene strigolactones (Matusova et al., 2005; Ayongwa et al., 2006).

A brief exposure of pre-conditioned *Striga* seed to a xenognosin is sufficient to initiate germination within 8-12 hours after initial exposure (Ejeta et al., 1992). The spatial relationship between host roots and *Striga* seed germination is a function of the distance from the host root (Fate et al., 1990). The germination stimulant concentration determines its ability to

elicit germination. *Striga hermonthica* being an obligate parasite must form connections with vascular system of a host plant, via the haustorium, in order to obtain water, nutrients, and carbohydrates (Ast, 2006). Seed germination and haustorial initiation cannot be elicited in the absence of specific chemical cues. The chemical elicitors of haustorial initiation are different to those moieties that stimulate germination (Maiti et al., 1984; Riopel, 1995). Chemicals shown to trigger haustorial formation include 2, 6-dimethoxy-p-benzoquinone, other phenolics include; quinones and cytokinins (Estabrook and Yoder, 1998).

The germinating seed produces a root like structure, the radicle. In order to attain a successful host attachment, germination must take place within 3-4 mm of the host root since *Striga* radicles have limited growth potential (Ramaiah et al., 1991). The radicle growth is directed towards the host root under the influence of gradient of chemical concentration of root exudates (chemotropism) (Williams, 1961; Ast, 2006). It is the emergence of the radicle that is used to indicate germination of the seed, which is followed by a series of physical and biochemical reactions leading to the great losses in productivity of the host plants.

This complex host-parasite interaction during early growth of the parasite is mediated by the intensity of the levels of the germination stimulants that signals initiation of the process. Thus these levels are of special interest in breeding for resistance or tolerance to *Striga*. For example, reduction in amounts or absence of germination stimulants produced by cereal host plants provides means to reduce numbers of seeds germinating at a particular point in time and space. Low or no stimulant production by cereal roots has been shown to be a mechanism of host plant resistance / tolerance to *S. hermonthica* infections (Weerasuriya et al., 1993; Heller and Wegmann, 2000; Ayongwa et al., 2006). The objective of this study was, therefore, to screen wide range of maize genotypes (420) of different classes and sources to identify the low-or non-germination stimulant producers.

## **Materials and Methods**

### *Striga and maize genotypes*

Clean *S. hermonthica* seeds were harvested from maize fields in western Kenya and prepared as germination batches following the procedure by (Berner et al., 1995). The 420 maize genotypes came from various sources,

among them International Maize and Wheat Improvement Center (CIMMYT) Gene Bank in Mexico, Kenya Agricultural Research Institute (KARI) and the International Institute of Tropical Agriculture (IITA) (Table 1).

Table 1. The list of germplasm examined to determine the presence of the germination stimulant.

Germplasm	Number	Source	Trait	Remarks
Land races	370	CIMMYT-Mexico Gene bank	Drought tolerant	M. Banziger (Personal communication)
Populations	10	IITA-Nigeria	<i>Striga</i> resistant	A. Menkir(Personal communication)
Inbred lines	20	KARI-Muguga	<i>Striga</i> resistant	J. Ininda (Personal communication)
Inbred lines	10	IITA-Nigeria	<i>Striga</i> resistant	Menkir A. (Personal communication)
Mutator lines	2	CIMMYT	<i>Striga</i> resistant	S. Hearne (Personal communication)
Herbicide resistant lines	2	CIMMYT	<i>Striga</i> resistant	Kanampiu et al., 2005
Susceptible checks	2	Seed companies	Commonly used by farmers	Kanampiu et al., 2005

#### *Striga cleaning and conditioning*

The *Striga* seeds were surface sterilized with 1% sodium hypochlorite in a beaker and rinsed with sterile water for 5 minutes. Two 9.5 cm diameter regular filter papers were moistened and placed in a sterile petri dish. A paper punch was used to cut out about 5 mm diameter disks of glass fiber filter paper in order to minimize microbial growth and a pair of forceps was used to dab up small amounts of about 10-25 *Striga* seeds on to the glass fiber disks. The disks were then placed on a moist filter paper lining the petri dish. The petri dishes were then covered using aluminium foil to create an artificial darkness and then incubated in an oven at 30 °C for 14 days for pre-conditioning (Berner et al., 1995).

The maize seeds were sown the same day the *Striga* seeds were placed in an incubator to synchronize for the maximum stimulant production which occurs during the early stage of root development. The maize plants were grown in small 20 cm diameter pots containing sterile sand, each pot carrying 5-6 plants. The seedlings were uprooted and the roots washed and macerated after 14 days of growth.

#### *Testing the maize for stimulant production*

After collecting the root exudates from the macerated maize roots and having conditioned the *Striga* seeds, small aluminum foil rings (1-2 cm

diameter and 1.5 cm height) was made and used as wells. Petri dishes were lined with moistened two pieces of regular filter paper; the rings were then placed at the center of the petridishes. One gram of the mercerated root pieces was weighed and placed into the aluminum well (Berner et al., 1995). The glass fiber disks (5 mm in diameter) with the conditioned *Striga* seeds were placed next to the aluminum foil well. Four radii of glass fiber disks radiated out from the central well (Figure 1). Three milliliters (3 ml) of sterile deionized water was added to the roots in the center well. Synthetic germination stimulant (GR24) was used as a positive control while sterile water was used as a negative control. The petri dishes containing root exudates and conditioned *Striga* seeds were returned into the incubator for 48 hours. The number of germinated *Striga* seeds on each glass fiber disk was counted under the light microscope after 48 hours.

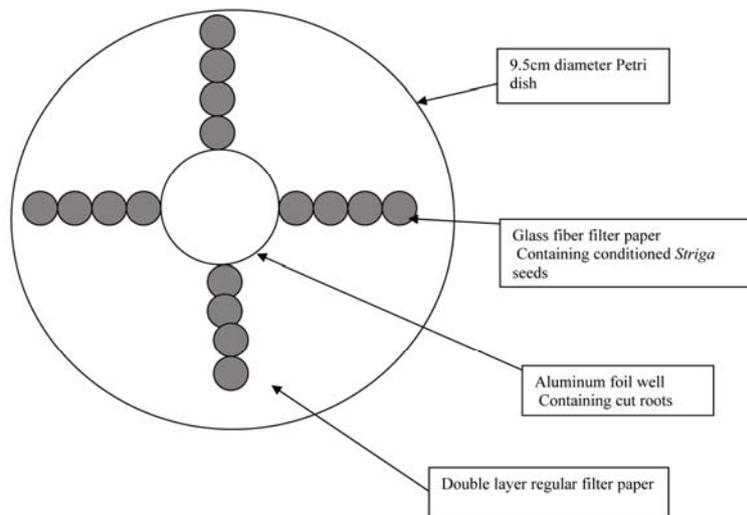


Figure 1. Testing for *Striga* germination due to Strigol stimuli exuded by maize roots in the laboratory.

#### Data collection

The assessment of *Striga* germination was done under a dissecting microscope by counting the number of *Striga* seeds that had started to germinate or germinated, 2 days after receiving the stimuli according to standard procedures by Berner et al. (1995). A seed was scored as

germinated if the root tip (radicle) was seen having protruded through the seed coat (Figure 2). The number of germinating seeds was expressed as a percentage of the total number that received the germination stimulant per disk, per radial position and per petri dish.

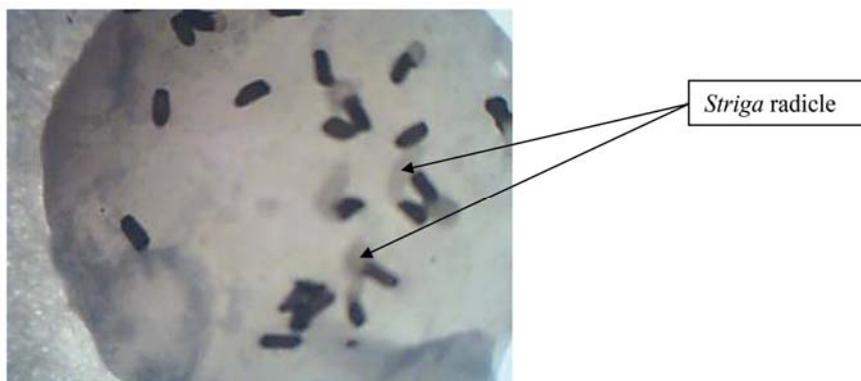


Figure 2. *Striga* radicle observed under a dissecting microscope due to stimulant production by maize roots.

#### Data analysis

The data was subjected to analysis of variance (ANOVA) procedure using the Statistical Analysis System (SAS 9.2) (SAS, 2003). The means were separated using  $LSD_{0.05}$ . Statistical analysis for percent *Striga* germination data was performed after arcsine  $\sqrt{Y}$  transformation of the actual data. This was done as shown in the formula below:

$$Y' = \sin^{-1} \sqrt{Y}$$

Where Y= the square root of the percent *Striga* germination.

The correlation coefficient of the *Striga* germination percent to the distance was also calculated.

#### Results and Discussion

All maize genotypes, categorized as inbred lines, landraces, open pollinated varieties and hybrids, germinated very well in the pots. Commercial checks stimulated high levels of *Striga* seed germination compared to the land races. Highly significant ( $P=0.001$ ) differences in

*Striga* germination were observed among the maize genotypes (Table 2). *Striga* seeds germinated at different levels along the radial position in the petri dish in all maize genotypes, indicating presence of diversified levels of germination stimulants (Ma et al., 1996). As expected there was no *Striga* germination in the negative control while the positive control GR24 exhibited a high, 58.7% *Striga* germination. The results according to the genotypes classes are discussed below.

Table 2. *Striga* germination Percent (%) of the top, middle and lower 20 including two commercial checks and positive and negative controls.

	Rank	Entry	Genotypes	<i>Striga</i> germination percent (%)
Top 20	1	106	CRIC 51	3.71
	2	321	CUBA T-31	4.35
	3	167	BRAZ 1758	4.55
	4	151	BRAZ 1279	5.22
	5	105	VERA 217	5.99
	6	170	BRAZ 1832	6.82
	7	337	ARZM 14105	7.68
	8	165	BRAZ 1738	7.73
	9	107	CRIC 52	7.94
	10	189	BRAZ 2151	7.94
	11	314	CHIS 743	8.00
	12	143	BRAZ 917	8.35
	13	153	BRAZ 1384	8.43
	14	146	BRAZ 1114	8.56
	15	79	PARA GP3	8.91
	16	150	BRAZ 1188	9.09
	17	128	PARA 151	9.18
	18	173	BRAZ 1863	9.28
	20	166	BRAZ 1757	9.57
	Middle 20	21	265	BRAZ 1059
22		90	CAUC 381	22.03
23		99	VERA 177	22.13
24		262	PERU 636	22.19
25		353	PAZM 14107	22.21
26		327	PAZM 10043	22.42
27		120	HAIT 19	22.54
28		217	BRAZ 1403	22.66
29		121	HAIT 21	22.71
30		354	PAZM 14119	22.71
31		23	HAIT GP6	22.76
32		211	BRAZ 2093	22.79

Continue Table 2.

	Rank	Entry	Genotypes	<i>Striga</i> germination percent (%)
	33	247	BRAZ 2258	22.79
	34	230	URUG 116	22.90
	35	114	CUBA 73	22.93
	36	169	BRAZ 1831	23.42
	37	116	CUBA 85	23.49
	38	214	BRVI 100	23.50
	39	52	CUBA 156	23.78
	40	220	BRAZ 1477	23.79
	41	39	SNLP 104	42.18
	42	228	URUG 696	42.18
	43	277	BOLI 461	42.32
	44	368	BRAZ 1731	42.56
	45	340	PAZM 4039	42.63
	46	276	PUEB 101	42.65
	47	93	VALL 385	43.37
	48	275	PUEB 82	44.23
	49	288	NAYA 130	45.20
Bottom 20	50	361	PAZM 14096	46.07
	51	255	ECUA 433	46.37
	52	283	GUAN 36	46.71
	53	317	CUBA 316	46.75
	54	36	GUAT 79	46.96
	55	359	PAZM 2019	48.19
	56	420	PHB3253	49.51
	57	360	PAZM 2036	49.67
	58	296	ARZM 16021	51.62
	59	278	CHIS 39	51.68
	60	363	OAXA 553	53.40
	61	423	Positive control GR24	58.78
	62	424	Distilled water	0.00
Checks		MEAN		25.20
		CV		26.83
		LSD		9.15
		SIG		***

## a) Land races

Highly significant differences ( $P=0.001$ ) were observed among the land races in their ability to effect *Striga* germination with a range of 3.7-53.4%. The least *Striga* germination was recorded from the land race CRIC 51

(3.7%). Relative to the positive control, the rate was lower than the synthetic Strigol (GR24-58.7%). *Striga* germination as induced by the landraces has indicated that the germplasms could be categorized into three distinct groups: the top 20, the middle 20 and the bottom 20 (Figure 3).

The top 20 landraces caused less than 10% *Striga* germination while the commercial checks KSTP94 and PHB3253 effected 38% and 50%, respectively. The five landraces with the lowest *Striga* germination were CRIC51, CUBA T31, BRAZ1758, BRAZ 1279 and VERA 217. These landraces together with the other 15 can be regarded as resistant to *Striga*. Among the 129 land races carrying prefix BRAZ out of the 420, 49 were among the landraces with the lowest *Striga* germination. Similarly, constituting 60% of the first 20 genotypes with the lowest *Striga* germination percent belonged to this BRAZ group. Low *Striga* germination percent depicted ability to effect low production of germination stimulant, the best known mechanism in *Striga* resistance research (Vasudeva, 1987). There were no significant differences observed between the two commercial checks although KSTP94 has been classified as a tolerant variety (Gethi, 2003) as opposed to the other check, PHB3253. Variations among the land races were also observed whereby some land races exhibited lower *Striga* germination and others high.

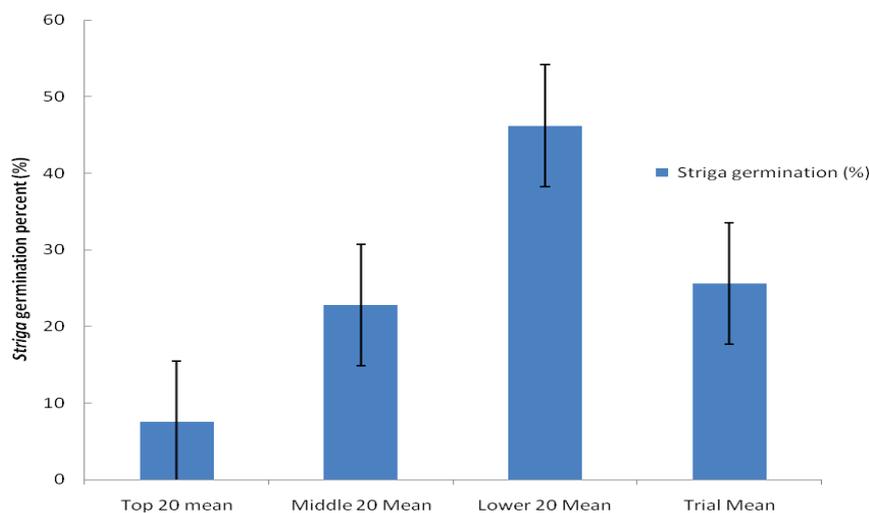


Figure 3. *Striga* germination percent of the landraces with low, medium and high *Striga* germination percent compared with the trial mean.

### b) Inbred lines

The inbred lines in the study stimulated a higher *Striga* germination percent with a mean of 38.8% compared to that of the landraces 25.2% (Table 3). The CIMMYT inbred lines were among the top five inbred lines with low *Striga* germination percent ranging from 14.3% to 29.7%. CML 444IR with 49.3% had the highest *Striga* germination percent among inbred lines. Except for CML 204IR, all other CIMMYT lines exhibited significantly higher ( $P=0.001$ ) resistance than all other inbred lines used in this study. This observation has implications for research on *Striga*. Whereas none of the CIMMYT lines was bred specifically to combat *Striga*, they were developed either to tolerate common African field stresses, ability to remain stable across various growth conditions and to possess high grain yield potential. It would thus seem that breeding for these characteristics would inadvertently confer an appreciable degree of tolerance to *Striga*, which also exists as a weed-stress in a growing crop. Among the CIMMYT lines were imidazolinone resistant (IR) ones representing a novel *Striga* control technology that has been recommended for farmers in the Lake Victoria Region (Kanampiu and Friesen, 2003). In this technology, IR maize seed is treated with herbicide, usually in form of low doses of imazapyr (30 g/ha) applied as a seed coat. This kills germinating *Striga* within the vicinity of the seed. Seeds of these inbred lines were not coated with the herbicide in this study, which shows that other than being herbicide resistant these lines either inherently or due to the IR gene also stimulated low *Striga* seed germination. A phenomenon that should be investigated further, as this is in contrast to research from other workers which have shown the susceptibility of IR lines without herbicide coating (Kanampiu et al., 1999).

The KARI Muguga inbred lines formed the second group with low *Striga* germination percent with a range of 30% to 36%. The inbred line EARLY-N-POP-7-13-5-1 in this group stimulated the highest *Striga* seeds germination at 50%. Five inbred lines were among the top ten with the lowest *Striga* seed germination. Surprisingly, the transposon induced mutant technology mutator lines were not ranked at the top although they were also placed very highly compared to many other lines.

Table 3. Different Levels of *Striga* germination percent exhibited by the inbred lines.

Rank	Entry	Genotypes	<i>Striga</i> germination percent (%)
1	411	CML202IR	14.34
2	417	CML444	22.37
3	415	CML445-IR	22.59
4	416	CML395	23.7
5	394	CML206//56/44-6-3-7-1	29.72
6	388	JI-30-18	33.03
7	399	F1-14-79-4-1-3	34.32
8	382	JI-30-7	34.56
9	386	JI-30-16	35.18
10	400	OSU231//56/44-6-4-17-3	35.79
11	412	CML204IR	35.92
12	383	JI-30-7	36.91
13	372	TESTR 133	38.46
14	380	TESTR 156	39.02
15	391	JI-30-21	40.46
16	387	JI-30-17	40.82
17	397	F1-14-14-24-4-5-4	40.92
18	398	DT//56/4-6-1-15-2	41.27
19	381	JI-30--4	42.03
20	375	TESTR 149	42.9
21	374	TESTR 139	43.01
22	385	JI-30--3	44.38
23	396	MGA19-4-1	45.12
24	377	TESTR 151	48.35
25	395	E11-133/7/44-6-3-17-3-2	49.11
26	414	CML444-IR	49.26
27	384	JI-30-8	49.87
28	376	TESTR 150	50.03
29	373	TESTR 136	50.04
30	393	EARLY-N-POP-7-13-5-1	50.14
31	379	TESTR 153	56.55
32	423	Positive control GR24	58.71
33	424	Distilled water	0.00
	MEAN		38.75
	CV		18.44
	LSD		9.78
	SIG.		***

The IITA inbred lines exhibited higher germination percent compared to other inbred lines although they have been shown to be *Striga* resistant in the field (Abebe, personal communication). Their ability to stimulate germination ranged 39-57%. If present, the resistance mechanism of these

lines is likely to be through other mechanisms such as avoidance by having less branched root architecture which resists attachments of the nearby germinated *Striga*. Others could be a kind of incompatibility that does not support normal growth of the attached parasites as was observed with the inbred line ZD05 developed from *Zea diploperennis* (Amusan et al., 2008).

The level of *Striga* germination and the distances from which stimulants were released has been presented in Table 4. Germination was particularly high around the source of stimulant, which suggested that the higher the concentration of the stimulant the higher the *Striga* germination was. The germination stimulant is mainly exuded in a distance of 3-6 mm radius from the root apex (Hess et al., 1991). In this study, the highest germination percent was recorded in the disks which were next to the source of stimulant compared to the rest. Highly significant ( $P=0.001$ ) and positive correlation coefficients were observed between *Striga* germination and the distances from the source of the stimulant. An indication that the closer the *Striga* seeds to the source of stimulant the higher the amount of seeds elicited to germinate and vice versa. This spatial relationship between host roots and *Striga* seed germination as a function of the distance from the host root to where germination stimulant is still active in large enough concentrations to elicit germination was also reported by several workers (Riopel and Baird, 1987; Fate et al., 1990). Interaction between maize genotypes and the distance from the source of stimulant was not observed. This is in agreement with previous work done by Ariga (personal communication) on non-host *Striga* germination stimulants such as cotton.

Table 4. Correlation between *Striga* seed germination and the distance from the source of *Striga* germination Stimulant.

Distance	Germination percent			
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>
D <sub>1</sub>				
D <sub>2</sub>	0.71***			
D <sub>3</sub>	0.69***	0.75***		
D <sub>4</sub>	0.62***	0.71***	0.78***	1

D= Glass fibre disc.

### Conclusions and Recommendations

The land races used in the present study had low levels of *Striga* germination stimulant production compared to commercial checks, and hence could serve as useful sources to select for resistance to *Striga* in

maize. The best land races in this score were CRIC 51, CUBA T-31, BRAZ 1758, BRAZ1279 and VERA 217. The land races with the prefix BRAZ were found to be among the best in terms of low *Striga* germination production. These constituted over 60% in the top 20 land races with the lowest *Striga* germination group. These landraces being drought tolerant can therefore be evaluated further in the development of *Striga* resistant materials which can be grown in *Striga* prone areas which are known to receive erratic rains. The inbred lines induced a higher germination of *Striga* seeds as opposed to the landraces, likelihood that the inbred lines produced higher concentrations of germination stimulant.

Five CIMMYT inbred lines exhibited the lowest germination percent below 23%. These were particularly low, especially the IR inbred lines CML 202 IR, CML 445 IR and CML 204 IR. This suggested that the IR lines may possess good levels of resistance to *Striga* in addition to being herbicide resistant.

The KARI-Muguga sourced inbred lines exhibited moderate levels of *Striga* germination percent which is an indication of good resistance levels to *Striga*. Higher levels of germination percent were also observed from the IITA inbred lines known to be resistant to *Striga*, and may probably show resistance through avoidance by growing deep root architecture rather than through low production of *Striga* germination stimulant. This mechanism could be of importance to breeders if used in combination with the ability to produce low stimulants. These types of materials would lead to suicidal *Striga* germination that in the long turn will result in reduced *Striga* seed bank in the soil. Inbred lines with low levels of *Striga* germination percent can be used by maize breeders for further evaluation and also for the development of new maize varieties resistant to *Striga*. The mechanism of resistance found in the IITA inbred lines needs to be studied further as it could be more beneficial in long run.

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