African Crop Science Journal, Vol. 2, No. 2, pp. 173–177, 1994
Printed in Kenya.

1021-9730/94 $ 10.00 + 0.00

SCREENING OF MAIZE GENOTYPES FOR LOW *STRIGA ASIATICA* STIMULANT PRODUCTION USING THE 'AGAR GEL TECHNIQUE'

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(*Received* 14 *June* 1993; *accepted* 29 *November* 1993)

ABSTRACT

One of the best characterized mechanisms of resistance to the parasitic weed (*Striga*) is low germination stimulant exudation by host plants. Using a simple and rapid agar gel assay, 112 maize genotypes were tested for low stimulant production and haustorium initiation of *Striga asiatica* (L.) Kuntze. Most of the genotypes induced high level of germination of Striga seeds at distances more than 1.3 cm, from the crop seedling root to the furthest germinating Striga seed. Germination distance highly correlated with rate of germination and was used as a quick way of determining the level of haustorial germination induced by the test genotypes. Some breeding lines such as PR 91A 496-13 and PR 91A 496-25 induced moderately low germination. B 37.91-952-1 induced the least germination distance (0.87 cm). This is comparable to that of highly resistant sorghum varieties. The commercial varieties induced high level of germination. A similar procedure was followed to observe the rate of haustorium initiation. Despite the tendency to stimulate considerably high germination, some entries permitted very low haustorium initiation. The best three lines in terms of low haustorium initiation were PR 496-45, PR 91B 5323 33 x 34 and PR 91A 496-17. There was no relationship between germination distance and haustorium initiation.

Key Words: Exudates, parasitic weed, root growth

RÉSUMÉ

Un des meilleurs méchanismes charactérisant la résistance contre le parasite des mauvaises herbes est la sécrétion par les plantes hôtes d'un stimulant de germination lente. Par l' usage d'un test simple et rapide, celui du gel d'agarose, 112 genotypes de maïs ont été testés pour la production de stimulant de germination lente et le déclenchement de la formation de l'haustorium de *Striga asiatica* (L.) Kuntze. La plupart des génotypes ont induit une germination élevée des semences de *Striga* à plus de 1.3 cm, des radicules des plantules en culture à la germule de *Striga* la plus éloignée. Une corrélation élevée entre distance de germination et taux de germination a été notée et a servi à déterminer le niveau de germination hausteriale induite par les génotypes. Certaines lignées de production telles que PR 91A 496-13 et PR 91A 496 -25 ont induit une germination lente modérée. B 37-91-952-1 á été à l'origine de la plus courte distance de germination (0,87 cm). Celle-ci était comparable à celle des variétés les plus résistantes de sorgho. Les variétés commerciales ont induit une germination élevée. De la même manière, le taux d'induction d'haustorium à été suivi. En dépit de la tendance à stimuler de façon considérable une haute germination, certaines variétés ont été très peu favorables à la

formation d'haustorium Les trois meilleures lignées en termes d'inhibition de la formation d'haustorium ont été PR 496-45, PR 911353 23 33 x 34 et PR 91A 496-17. Aucune relation entre distance de germination et induction d'haustorium.

Mots Clés: **Exudats, parasites de mauvaises herbes, croissance de racine.**

INTRODUCTION

Striga spp. (Scrophulariaceae) are very devastating parasitic weeds in Africa. Mboob (1986) estimated that *Striga* is affecting the lives of about 100 million people in sub-Saharan Africa. Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench) are parhaps the crops most threatened, and maize yield can be reduced by as high as 100% if infection occurs at an early stage (Kim *et al.*, 1986). Current available control measures require costly inputs. Therefore, resistant varieties are likely to continue to be the most feasible approaches to control *Striga*. However, lack of fast and reliable screening techniques has slowed progress in breeding for resistance.

Vasudeva Rao (1987) described various screening methods for low stimulant production, one of the best characterized resistance mechanisms of host plants to *Striga*. More recently, Hess *et al.* (1992) developed an agar gel technique and showed that field reaction of sorghum genotypes to *Striga* can be reproduced using this method. These workers demonstrated that sorghum genotypes which caused germination of a large percentage of the *Striga asiatica* seeds also stimulated the germination of seeds that were further away from the host root than did sorghum genotypes that caused only a few *Striga* seeds to germinate. A high positive correlation (0.93) was observed between percent germination of *Striga* seeds and the distance from the host root to the furthermost germinated *Striga* seed. Hence, one important advantage of this technique is that quite a large number of entries can be evaluated at a time because measuring the distance from the host root to the furthermost germinated *Striga* seed can be done much more rapidly than determining the percent germination. Reported here are results of the use of the agar gel technique for screening maize genotypes for low *Striga* stimulant production.

MATERIALS AND METHODS

Surface sterilizing of maize seeds. Maize seeds (20 per sample placed in a 25 ml flask) were sprayed with ethanol (75%) and soaked in 1% NaOCl solution for 25–30 minutes, then rinsed with water three times. Each sample was then soaked overnight in double deionized water (ddH_2O) containing 10% Captan (N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) 48.88% wettable powder. Seeds were rinsed with double distilled water three times, then incubated in petri dishes on moist filter paper at 27°C. After 48 hr, germinating seeds were placed onto agar plates.

Assay of maize seedlings for germination stimulant production at different growth stages. Maize seeds were prepared using the above described procedure. Pre-germinated seeds were placed on moist glass wool in small plastic cups with perforated bottom and incubated at 27°C for five days and beyond. Root exudate was collected through suction starting from the second day. The assay was conducted on multi-wel plates into which conditioned *Striga* seeds were pipetted. Observations on induced level of germination was made 24 and 48 hours later.

Surface sterilization and conditioning of *Striga* seeds. *Striga asiatica* seeds were obtained from Dr. Robert Eplee of Whiteville Methods Development Center, Whiteville, N.C. 28472, and were handled under quarantine producedures approved by USDA/APHIS and the Indiana Department of Natural Resources. *S. asiatica* seeds (0.11 g) were placed in 50 ml flasks and rinsed three times in 10 ml of distilled water into which 3–5 drops of Tween 20 (polyoxy-ethylene sorbitan monolaureate) was added. The seeds were sonicated for three minutes during the first rinse. Seeds were further sonicated for three minutes after 10 ml Sporicidin (Phenol 7.05%) solution was added into each flask. Seeds were then washed three times with distilled water to remove traces of the fungicide. Keeping *Striga* seeds in Sporicidin longer tends to reduce germination. Seeds were conditioned in 14 ml distilled water to which was added 1 ml of 0.015% solution of Benomyl (methyl 1-butyl carbonyl-2-benzimidazole carbamate). Seeds were conditioned for 14

days at 27°C. During the conditioning period the Benomyl solution was changed 2-3 times.

Assay set up. Conditioned *Striga* seeds (50 µl) were pipetted into 9 cm diameter petri dishes. Thirty millitres of autoclaved water agar (1.05 gm of bacto agar) in 150 ml of double distilled water was poured onto the *Striga* seeds in each dish just before it became cool enough to solidify. A germinating maize seed was submerged in the solidifying agar near one edge of the plate, with the root tip pointing across the plate. Plates were incubated at 27°C for 48 hr before assessment of induced germination, and for a further 24 hr (total-72 hr) for reading haustoria initiation. Germination of *Striga* seed was observed with a dissecting microscope focussed through the bottom of the dish. The index for germination and haustorium initiation was, in both cases, the furthest distance from the root of maize seedling at which *Striga* was found germinating and forming haustoria initials. The experiment was run on 112 maize genotypes.

RESULTS AND DISCUSSION

Germination stimulant. Generally seeds of the improved varieties and hybrids tested germinated readily and stimulated high level of *Striga* seed germination. The inbred lines germinated poorly and sometimes difficulty was encountered in obtaining uniformly growing seedlings. However, significant differences in germination distance were observed between most of the test entries. The least germination distance (GD) of 0.6 cm was recorded on inbred lines B37 91-952-1. The line PR-91A 496-25 also induced low distance germination. On the other hand, inbred line PR 91A 311-16 induced germination up to 3.4 cm away from its root. Similarly, hybrids Zimmerman-327, Varis-V2544 and Agra Tech-825 (B) had significantly high *Striga* germination indexes (Table 1).

TABLE 1. Effect of root exudate of maize genotypes on *Striga asiatica* seed germination

Line/ Variety	Germination distance[a]
H 51 500557	2.65 A[b]
PR 91A 496-24	2.62 AB
PR 90B 5492-78	2.62 AB
WF 9 500646	2.60 ABC
Voris, V 2573	2.60 ABC
Agra Tech. 825	2.60 ABC
H 29 500540	1.46 DEFG
PR 91A 496-33	1.45 DEFG
H 46 500552	1.45 EFG
H 28 500537	1.36 FGH
PR 91A 496-25	1.25 GH
PR 91A 496-13	1.25 GH
B 37 91-952-1	0.87 H

[a]Distance in cm at which the furthest *Striga* seed was found germinating from the host root; data are means of four replicates.
[b]Means with similar letters are not significantly different ($P \leq 0.05$).

Germination was particularly high around the older part of the host root, which suggests that more of the stimulant is produced in this zone, and declined gradually towards the tip. This is contrary to the report by Okonkwo (1991) which states that stimulant is not produced by the older part of maize root.

In many instances *Striga* seeds germinated on and around the seed and shoot initial of the host. It is suspected that active stimulants were also possibly being produced in this part of the host seedlings.

Stimulant production at different seedling ages. Host seedlings of different ages were compared for exudate production. Significant differences were observed between entries, and highest germination frequencies were observed at five days (Fig. 1). Germination percentage of *S. asiatica*

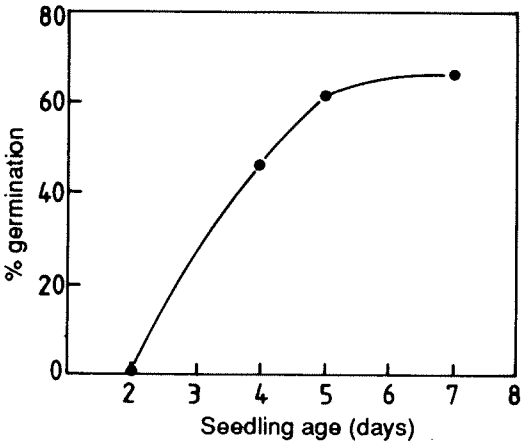

Figure 1. Germination response of *Striga asiatica* treated with root exudates of maize seedling at different ages.

TABLE 2. Germination of *Striga asiatica* seeds treated with root exudates of some maize genotypes[a]

Line/ Variety	Germination distance (cm)	Percent germination
Zimmerman, 327	2.6	60.0 A
Voris, V 2544 2.6	59.2 A	
PR 91A 496-27	2.1	59.1 A
PR 91A 496-13	1.3	55.5 A
PR 91A 496-25	1.3	8.5 B

[a]Data are means of four replicates and means with a different letter are significantly different ($P \leq 0.05$)

TABLE 3. Response of *Striga asiatica* seed to root exudates of maize

	Germination distance	
LIne/Variety	24 hours	48 hours
Voris, V 2544	40.9	59.2
Zimmerman, Z 27	51.3	60.0
PR 91A, 496-33	54.3	60.6
PR 91A 496-13	48.9	55.6
PR 91A 496-27	51.0	59.2

[a]Data are means of four replicates and means with a different letter are significatly different ($P \leq 0.05$).

TABLE 4.Effect of root exudates of maize genotypes on haustorium initiation

LIne/Variety	Haustorium distance (HD)[a]	Haustorium initiation remarks
PR 91A 496-6	1.87 A	High
PR 91A 496-30	1.43 A	High
WF 9 500646	1.00 AB	High
Zimmerman, z 27	1.00 AB	High
PR 91A 496-27	0.35 CDEFG	Low
PR 91A 496-35	0.35 CDEFG	Low
Pioneer 3154 (A)	0.35 DEFG	Low
A 675 500659	0.43 EFG	Low
PR 91A 496-25	0.28 FG	Low
PR 91A 496-15	0.45 GH	Low
PR 91B 5353 41 x 42	0.30 GH	Low
PR 91A 496-17	0.28 GH	Low
PR 91B 5323 33 x 34	0.10 HI	Very low
PR 91A 496-45	0.0 I	Very low

[a]Distance at which the furthest *Striga* seedling was found forming haustorium initial; data are means of four replicates.
[b]Data were transformed into log (x + 0.001) for analysis. Means followed by the same letter are not significantly different ($P = 0.05$).

TABLE 5. Effect of maize genotypes on haustoria initiation in *Striga asiatica*[a]

Line/Variety	Hautoria distance (cm)	Percent haustoria[b] initiation (mean/4 reps)
PR 91A 496-6	1.87	16.7 A
PR 91A 496-30	1.40	10.8 AB
PR 91A 496-45	0.0	6.4 B

[a]Data are means of four replicates.
[b]Percentage of *Striga* seedlings forming haustoria initials, and means followed by the same letter are not significantly different ($P \leq 0.05$).

seed in responses to root exudate of PR91A 496–25 (GD = 1.4 cm) was 8.6% at full strength and nil at x 5 dilution. This may partly be due to the slow growth of seedlings of this line. Assessed after two days the percentage rose to 31% of that of varieties such as Zimmerman-z 27 (Table 2). An anomaly was observed with respect to PR91A 496-13. It recorded a low GD (1.3 cm) and yet stimulated a considerably high rate (55%) of *Striga* germination.

Haustorium factor. The germination induced by most of the maize genotypes tested was considerably higher than that which is normally observed in sorghum. Hautorium initiation, however, was low for many genotypes. The distance from host root to the furthest *Striga* seedling forming haustoria initials (HD) ranged from 0 to 2.3 cm. Lines with the lowest distance index (less than 0.3 cm) were PR 91A 496-45, PR 91B 5323 33x34, Pr 91A 496-17 and PR 91B 5353 41 x 42 (Table 4). Profuse production of the stimulant was observed on Pr 91A 496-30 and PR 91A 496-6, and haustoria initiation took place up to 1.87 cm away from their roots.

In determining the percentage of *Striga* seedlings forming haustoria initials, it was confirmed that genotypes with lower haustorium distance indexes were the ones that induced significantly lower percentage of haustorium formation (Table 5). PR 91A 496-p 45 (HD = 0) has 6.4% which was significantly lower than that of PR 91A 496-6 (HD = 1.87) with 16.7%, although the difference was not as high as expected because of the unusually low *Striga* seed germination in that assay. Further experiments (field or in pots) will be carried out on promising lines to determine if low

haustoria initiation indicates resistance. It is also possible that some chemicals are being produced which are converted into active haustorium signals in the soil or the stimulant was inactive in medium like the agar gel. There was no relationship between the level of germination and haustorium production in a given genotype.

ACKNOWLEDGMENT

We gratefully acknowledge the financial support by the Canadian International Development Agency (CIDA), and thank INTSORMIL for allowing use of laboratory facilities. The technical assistance of Mr. Mark Alkire of Biochemistry Department, Purdue University is deeply appreciated.

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