African Journal of Biotechnology Vol. 5 (10), pp. 866-870, 16 May 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Azide-based mutagenesis suppresses *Striga hermonthica* seed germination and parasitism on maize varieties

S. Kiruki*, L .A. Onek and M. Limo

Department of Biochemistry and Molecular Biology, Egerton University, P. O Box 536 Njoro, Kenya.

Accepted 11 January, 2006

Small-scale farmers currently produce more than 90% of the maize grown in Kenya. However, the parasitic weed, *Striga hermonthica* has become a menace because it is a widespread parasitic weed causing severe damages to major cereals like maize and sorghum. Mutant lines of maize were created in the laboratory using sodium azide (NaN_3) and their performance in respect to the degree of *Striga* resistance screened. The presence of active chemical stimulants in the root exudate was further investigated. This is the first report on mutagenic conversion of *Striga*-susceptible to *Striga*-resistant maize varieties (K9908, K9910 and K9911). Stable performances of the new varieties have been demonstrated in *Striga*-infested fields in western Kenya by agronomic researchers.

Key words: Azide-based mutagenesis, biochemical characterisation, *Striga hermonthica*.

INTRODUCTION

The genus S*triga* in the family S*crophulariaceae* is composed of some 50 species, all of which are holoparasites of tropical cereals or legumes. *Striga hermonthica* (Del.) Benth and *S. asiatica* (L.) Kuntze are the species which cause the most economically significant damage to cereals (Butler, 1995). *S. hermonthica* infests an estimated 46 000 hectares of land in the traditional food producing areas in western and coastal Kenya (Kiriro, 1991). It attacks staples such as maize, sorghum, millet, sugar cane and rice resulting in total failure or halving of yields. The existing *Striga* control methods have given no conclusive and consistent feasible results for the peasant farmers, due to its high fecundity and the mismatch between technologies and the farmers' socio-economic conditions. So far no cereal variety has shown total resistance to *Striga* in the field. The major challenge therefore is to develop methods/varieties that will help small-scale farmers control *Striga* effectively within a sustainable and profitable farming system (Doggett, 1988).

In resource-limited subsistence agriculture prevalent in Africa, development and use of genotypes that can withstand *Striga* parasitism holds the greatest promise in its control. Such genotypes could be typified by resistance, where the *Striga* does not invade the plant or tolerance where the plant withstands the negative effects of invasion. Tolerance is partly a problem as the soil seed load continues to build up with each cropping season. Moreover, attempts to produce high yielding locally acceptable resistant cultivars have had limited success (Ogborn, 1987; Parker and Riches, 1993; Ramaiah, 1987). In view of the relative failure of these breeding programmes, there is an urgent need for varieties with very high resistance potential.

Gamma irradiation mutation is known to alter both morphological and physiological processes in crops (Sax, 1963). Sharon and Muralidharan (1978) found that doses of 3 and 5 krad increased the percentage germination of sorghum. The treatment described here for sodium azide can be used for most dry seeds (Diedrick et al., 1990). Other mutagens such as nitrosomethylurea (NMU) and ethyl methane sulphonate (EMS) have been used with barley, *Arabidopsis,* peas and maize (Diedrick et al., 1990).

The present paper reports on mutagenic conversion of *Striga*-susceptible to *Striga*-resistance maize mutants (K9908, K9910 and K9911). We have developed and identified non-stimulating maize varieties among the maize mutants. These 'non-stimulants' could be used in breeding programmes for the improvement of maize resistant to *Striga hermonthica*.

*Corresponding authors E-mail:kirusila@yahoo.com

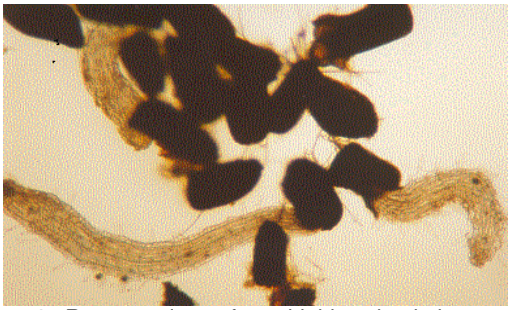

Figure 1. Root exudates from highly stimulating maize varieties (TZB-Str) induced high percentage of *Striga* seed germination as indicated by radical protrusion.

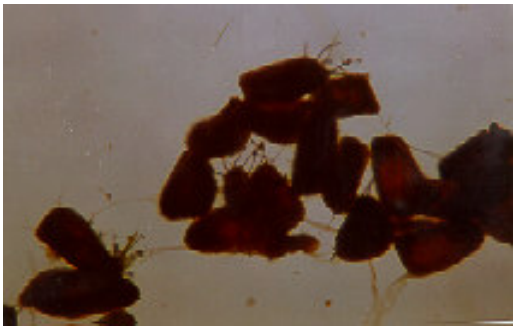

Figure 2. Non-stimulating maize varieties (K9908 and K9910) induced no *Striga* seed germination.

MATERIALS AND METHODS

Surface sterilization of seeds

Seeds of *S .hermonthica* (Del.) Benth were collected and surface-sterilized for 3 minutes with a 5% (v/v) solution of sodium hypochlorite (10-14%, w/v available chlorine), followed by several washes with sterile H_2O through a millipore filter unit. Seeds were then pre-conditioned by incubation on two layers of Whatman No 1 filter paper saturated with sterile distilled H_2O in 9 cm petri dishes at 25 mg per petri dish. Petri dishes were wrapped in aluminium foil to create a dark environment and placed in incubator at 33°C for 7 days.

Production and collection of root exudates

Stimulant production and collection was done as described by Weerasuriya et al. (1993). For each host species, the root systems of two maize plants was submerged together in 15 ml of sterile distilled water and incubated at 30°C in the dark for 24 h. The solution was diluted twice and used as natural germination stimulant. Root exudates from each pot was collected daily over a period of seven days and stored in a refrigerator.

Striga seed bioassay

Striga seed bioassay was done as described by Ariga (1996). Test solutions (200 µl) and conditioned *Striga* seeds (12 per well) were added to a flat -bottomed polystyrene micro-titre plates (Nunclon, Denmark) at time zero and incubated in the dark at 33°C. Each experiment was performed using five wells for each treatment. Experiments were repeated five times. Germination counts based on radical protrusion were made at intervals up to one week after sowing and the number of germinated *Striga* seeds recorded.

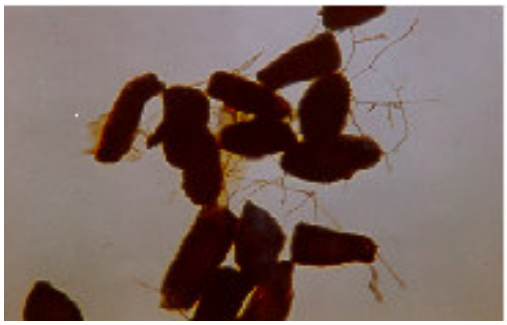

Figure 3. Distilled water (control) induced no *Striga* seed germination.

Analysis of root exudates by HPLC

Maize root exudates collected over 7 days was pooled and extracted with ethyl acetate. The ethyl acetate from the extraction of volume 100 ml of root exudates was evaporated and residue taken up in 1 ml of methanol : H_2O (1:1, v/v). A Hewlett-Packard (Waldbrown Analytical Division system G1030AX) HPLC was used with Titanium pump and high-pressure gradient mixing. Separation occurred on a Spherisorb S5 ODS-2 column, which was maintained at 25°C. The methanol/water extract of stimulant from maize cultivars (20 µl) of each sample was injected onto the column via an AS100 T auto-injector and eluted with hexane at a flow rate of 0.4 ml/min. The elution gradient was 90% hexane to 10% Solvent B (Acetonitrile : THF : Isopropanol in the ratio of 5:4:1) in 50 min. The UV absorption of the root exudates was monitored at 240 nm (Butler, 1995). Data analysis was carried out using Hewlett-Packard HP Ultra VGA series 1280 software.

Mutagenesis and analyses of maize inbred lines

This experiment investigated the effects of azide-based mutagenesis on seeds of grain maize with reference to the ability of maize root exudates to induce *Striga* seed germination. Seeds were soaked overnight in water at 4°C and then aerated vigorously for 8 h at 20°C. Incubation with sodium azide (3 mM) was carried out as described by Hibberd and Green (1982) in 0.1 M phosphate buffers (pH 3.0) for 2 h. The seeds were washed in 2 volumes of distilled water and running tap water in the cold for 30 min, followed by drying and planted in the field. The seeds were grown through a complete generation to produce the M2 seed that was used for the selection of mutants. The mutant (M2) seeds were soaked for 15 min in 1% sodium hypochlorite solution for surface sterilization, washed thoroughly and imbibed for 8 h with distilled water at 35°C before placing in sterilized petri dishes containing moistened glass fibre filter and left overnight to germinate at room temperature, 35°C (Baggonneaud-Berthome, 1993). The germinated seeds were then transferred to circular plastic pots, which had several holes made at the bottom and filled to about 6 cm from the rim with sterilised coarse sand.

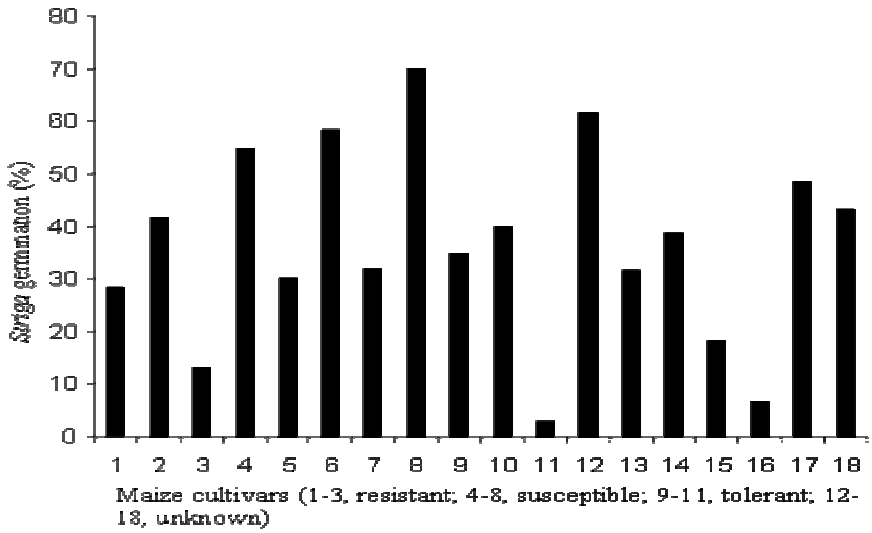

Figure 4. *Striga* germination as induced by root exudates from existing maize cultivars.

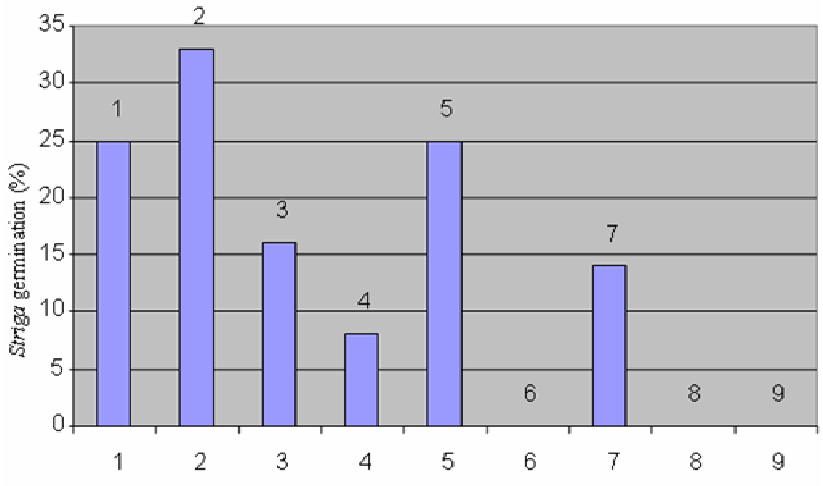

Figure 5. *Striga* percentage germination induced by root exudates from azide-mutated maize varieties.

The plastic containers were then placed in a glasshouse (temperature of 25-35°C), irrigated with 30 ml of distilled water on the first day and 20 ml on succeeding days for 10 days before the root exudates of the maize seedlings were separately extracted from each container using a suction pump. This exudate was then tested against pre-conditioned (sensitized to germinate) *Striga* seeds.

RESULTS AND DISCUSSION

Striga seed bioassay

Resistant and tolerant maize varieties gave low percentage *Striga* germination while susceptible varieties gave high percentage *Striga* seed germination (Figures 1 and 2). The low percentage stimulation observed from resistant cultivars could be due to high versatility or occurrence of chemical stimulants at low concentrations. Distilled water (Figure 3, negative control) gave zero germination thus indicating that exogenous chemicals exuded by maize plants stimulated germination of *Striga* seeds (Weerasuriya et al., 1993).

Chidley and Drennan (1987) screened a wide range of sorghum cultivars for stimulant production in the laboratory and observed two groups, low-and high stimulant types with very few intermediates. However, in this study we have further developed and identified non-stimulating maize varieties among the maize mutants (K9908, K9910 and K9911). Stable performances of

these new varieties have been demonstrated in *Striga*-infested fields of western Kenya, including, Kibos, Kisumu and Kakamega by agronomic researchers from Egerton University and Kenya Agricultural Research Institute. These 'non-stimulants' could also be used in breeding programmes for the improvement of maize resistance to *S. hermonthica*. These varieties could also be used as resistant control in studies on the genetics and the mechanisms of resistance to *S. hermonthica*.

Mutagenesis of maize inbred lines

The present findings suggest that *Striga* seed germination and the production of germination chemical stimulant were adversely affected by the azide-based mutagenesis. On average, the mutagenesis reduced the release of chemical stimulants by the host plants to zero in some maize grains depending on the effect of the mutagen (Figures 4 and 5). This observation was in conformity with the Bebawi (1984) findings and also supports an assertion that even though natural mutations are known to induce resistance to *Striga*, the process can be accelerated in the laboratory through azide-based mutagenesis. In some maize varieties the mutagenesis stimulated their growth. The stimulating effect of azide-based mutagenesis on maize growth at establishment phase is consistent with those of Sharon and Muralidharan (1978) on growth of sorghum.

High pressure liquid chromatography (HPLC)

HPLC studies on the root exudate of various maize varieties indicate that their HPLC elution profiles are different. It was clear that even though root exudates from the resistant and susceptible varieties stimulated *Striga* seed germination the active chemicals varied. However, it is interesting to note that some of the prominent peaks observed in the susceptible were absent in the non-stimulating varieties. A low germination stimulant activity against *S. hermonthica* was observed in resistant maize root exudates. However, the chromatographic properties of the resistant stimulants differed from those of the non-stimulating varieties. The most prominent peak in non-stimulating varieties had the same retention time on HPLC as that of the experimental control.

Conclusions and recommendations

Present investigation has demonstrated the high resistance of a few maize varieties developed in the laboratory. It is authentic that the non-stimulating varieties are the most resistant developed in this study.

These varieties could be used in breeding programmes for the improvement of maize for resistant to *S. hermonthica*. Among them, the varieties, K9908, K9910 and K9911 showed a particularly high degree of resistance and their use as resistant gene source in maize improvement programmes should be considered.

From the present findings it is apparent that both resistant and susceptible maize cultivars stimulated *Striga* seed germination, though at varying degrees, indicating that host resistance is as a result of other physiological factors. The results of this study were surprising in that azide-mutated maize varieties (H511 and 8338-1) lead to decline in production of germination chemical stimulant and stimulated growth of host plant. However, more work is necessary for the full isolation and characterization of different genes for non-stimulating varieties and their use in genetic manipulation to reinforce resistance to *Striga.* Elucidating the mode of action of the chemical stimulants is also essential.

ACKNOWLEDGEMENTS

We are grateful to Mr Francis Mwangi, Chief technician, Department of Biochemistry and Molecular Biology, Egerton University and Mr C.D. Ochieng, Chief chemist, Pyrethrum Board of Kenya for their competent technical assistance.

REFERENCES

Ariga ES (1996) Isolation and bioassay of *Striga hermonthica* seed germination stimulants from non-host crop plants parts and field testing for control efficacy. Ph.D thesis. University of Nairobi, Kenya.

Baggonneaud-Berthome V (1993). Recherche de mole'cules herbicides utilisabes pour la lutte selective contre les striga dans les cultures vivrieres(Cereales, Legumineuses) er Afique. Memoire de Recherche-Diplome d' Etudes Approfondies de Biologie Cellulaire, Universite' de Nantes.

Bebawi FF (1984). Effects of gamma Irradiation on *Sorghum bicolor–Striga hermonthica* Relation Environmental and Experimental Botany 24:123-129.

Butler LG (1995). Chemical communication between parasitic weed *Striga* and its crop host. A new dimension in allelochemistry Agricultural Experiment Station Purdue University: West Lafayette, IN 47907. pp. 156-166.

Chidley VL, Drennan DSH (1987). *In vitro* culture of *Striga asiatica* (L.) Kuntze (*Scrophulariaceae*). Proceedings of the fourth international symposium on parasitic flowering plants. Marbug Philips University.

Diedrick TJ, Frish DA, Gengenbach C (1990). Tissue culture isolation of a second mutant locus for increased Threonine accumulation in Azide-mutated maize. Theor. Appl. Genet. 75: 209-217.

Doggett H (1988). Witchweed (*Striga*) In: G. Wirgley (ed.) Sorghum. Second ed. Longman Scientific and Technical, London. pp. 368-404.

Hibberd KA, Green CE (1982). Inheritance and expression of Lysine-plus Threonine resistance selected in maize tissue Culture. Proc. Natl. Acad. Sci. USA 79:559-563.

Kiriro FH (1991). The *Striga* problem in Kenya. In: Kim S. K. (ed.) Combating *Striga* in Africa. Proceeding of the International Workshop organized by IITA, Ibadan, Nigeria. Pages 15-17.

Ogborn JEA (1987). *Striga* control under present farming conditions. In L.J. Musselman (ed.) Parasitic weeds in agriculture. CRC Press Inc., Boca Raton, Florida. 1:145-158.

Parker C, Riches CR (1993). Parasitic Weeds of the World: Biology and Control. CAB Int., Walliford. U.K.

Ramaiah KV (1987). Breeding cereal grains for resistance to witch-

weed. In: LJ Musselman (ed.). Parasitic weeds in Agriculture vol. 1.CRC press, Boca Raton, F L. pp. 227-242.
Sax K (1963). The Stimulation of plant growth by ionizing.
Sharon M, Muralidharan K (1978). Effect of γ-irradiation on the growth of Sorghum vulgare. India Journal of Plant physiology. 21: 156-161.
Weerasuriya Y, Hess D, Ejeta G, Butler LG (1993). Influence of S*triga* germination stimulant exuded by roots of several host crops. J. Agric. Food Chem. radiation.*Radiation Botany* 3: 179-186.