Influence of Fertilizer Microdosing on Strigolactone Production and *Striga hermonthica* Parasitism in Pearl Millet

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Abstract

Parasitism by the root-parasitic plant, *Striga (Striga hermonthica L.)*, is a main threat to pearl millet production in sub-Saharan Africa and nutrient deficiency aggravates this problem, often leading to complete failure of pearl millet crops. Like many other species, pearl millet secretes germination stimulants (strigolactones) into the soil in response to mineral nutrient deficiency, which triggers *Striga* seed germination resulting in infection. A greenhouse experiment was conducted to evaluate the influence of different doses of di-ammonium phosphate (DAP) fertilizer on strigolactone production and *Striga* infection in three different African pearl millet cultivars (KBH, Sadore Local and *Striga* resistance). All the pearl millet genotypes produced varying amounts of different strigolactones like orobanchol, epi-orobanchol, orobanchyl acetate and 5-deoxystrigol, the level of which decreases with increasing doses of DAP. The control treatment (no DAP) showed maximum *Striga* germination, emergence and dry biomass production in all cultivars of pearl millet. Supply of DAP fertilizer up to 4 g per hill suppressed *Striga* germination by 69, 64 and 59%; emergence by 87, 85 and 95% and dry biomass by 91, 98 and 83% in cvs KBH, Sadore Local and *Striga* Resistance, respectively. The present findings reveal that DAP fertilizer minimizes strigolactones production and, as a result, reduces *Striga* infection in pearl millet. Low doses of DAP fertilizer is a promising strategy to lower the destructive effect of *Striga* on pearl millet. The use of small doses of DAP fertilizer combined with resistant crop cultivars, intercropping with legumes and hand pulling of *Striga* at flowering in an integrated *Striga* control strategy should be developed to help African farmers control this noxious weed. © 2014 Friends Science Publishers

Keywords: Pearl millet; Strigolactones; *Striga hermonthica*; Di ammonium phosphate

Introduction

Pearl millet (*Pennisetum glaucum* L.) has been grown in Africa since pre-historic times and is used as food and feed for people as well as animals. Currently this crop is cultivated on about 18 million hectares in Africa, which is 56% of the 32 million hectares of the world pearl millet growing area (FAO, 2011). Total millet production in Africa during 2011 was 11 million tons with an average yield of about 0.6 t ha⁻¹, much lower than its potential yield of 3.5 t ha⁻¹.

Pearl millet can withstand a number of biotic and abiotic stresses. However, in spite of this good adaptability, millet productivity in Africa is far less than needed to meet the increasing demand of the population living in the semi-arid regions. Lack of availability of improved cultivars, insects, diseases and attack of the parasitic weed *Striga hermonthica* constitute major limitations in the area (Kanampiu et al., 2003; Khan et al., 2006). Poor soil fertility, low nutrient and water use efficiency and improper use of fertilizers are considered to be major abiotic factors, which aggravate the problem of *Striga* in Africa (Showemimo et al., 2002; Dugje et al., 2006).

Generally phosphate (P) and nitrogen (N) deficiency are very common in African soils (Kim et al., 1997; Khan et al., 2008) and African farmers use only limited amounts of fertilizer in pearl millet, if at all, due to low grain prices, poverty and non-availability of costly fertilizer (Vitale and Sanders, 2005; Morris et al., 2007). Poor soil fertility due to insufficient supply of fertilizer not only affects pearl millet directly but also appears to enhance *Striga* attack, hence further reducing millet productivity (Bagayoko et al., 2011). It has been reported in a number of studies that *Striga* attack has a direct relation with the nutritional status of the soil (Cechin and Press, 1993; Jamil et al., 2011a). But the exact mechanism involved has been explored in only few
studies (Bouwmeester et al., 2007; Jamil et al., 2011a). Under mineral nutrient deficiency, the host plant releases underground signalling molecules, strigolactones, into the rhizosphere to trigger a symbiotic relation with arbuscular mycorrhizal fungi which facilitates nutrient uptake from the soil (Lopez-Raez et al., 2008). However, the seeds of parasitic weeds also use this underground signal as a host-detection mechanism and germinate upon contact with the strigolactones, after which they infect the host plant (Jamil et al., 2011a).

Mineral nutrients should be applied in African soil, to reduce and avoid *Striga* attack in cereals (Yonli et al., 2011; Jamil et al., 2012). However, the poor accessibility to fertilizer warrants studies on fertilizer application techniques that are more efficient and economical. The placement of a small dose of fertilizer in small holes close to the host plant, called “microdosing”, may be such a technique (Aune et al., 2007). It was hypothesized that application of low doses of fertilizer in close proximity of the crop might also be helpful in the reduction of *Striga* infection. Due to the quick supply of mineral nutrients, the host plant will reduce secretion of underground signalling molecules which will result in lower *Striga* seed germination and infection. In earlier work we have already studied the effect of DAP microdosing on *Striga* infection in African sorghum and found encouraging results (Jamil et al., 2013). However, the response of pearl millet with regard to underground signalling and *Striga* infection and the effect of fertilizer application have not been studied before.

Therefore, this study was conducted to explore the effect of DAP microdosing on signalling molecule production and *Striga* infection in pearl millet. For this purpose, three pearl millet cultivars were selected and three fertilisation levels of di-ammonium phosphate (DAP) were tested under lab and greenhouse conditions. The outcomes of this study should provide the basis for the development of an efficient and economical package to improve pearl millet production by African farmers, while decreasing *Striga* infection.

**Materials and Methods**

**Experimental Sites and Materials**

The study was carried out at Wageningen University, the Netherlands. Three pearl millet cultivars, namely KBH (improved short duration variety), Sadore Local (local and late variety) and *Striga* Resistance (entry from ICRISAT pearl millet breeding program with low levels of *Striga* emergence) were used (courtesy Bettina Haussmann, pers. comm.). *S. hermonthica* seeds for the pot trial were collected from a sorghum field near Cinzana in Mali and *S. hermonthica* seeds used in the germination bioassays were collected from a sorghum field in Wad Medani, Sudan (courtesy of Abdel Gabar Babiker). The greenhouse conditions were 28°C day (10 h) / 25°C night (14 h) with 70% relative humidity. For the pot study in Wageningen University silver sand and soil were mixed with sand.

**Experimental Details**

For the pot study, planting was done 15th May, 2010 and harvesting on 30th July. About 5000 *Striga* seeds (25 mg) were mixed in 1 L soil to create *Striga* infected conditions. A single pearl millet plant was allowed to grow in this infected soil in a 1.5 L pot. The experiment was conducted in randomized complete block design with four replications. Three levels of DAP fertilizer (0, 2 and 4 g per plant) were placed in 3 small holes around each plant. Data on *Striga* emergence and pearl millet growth and development were collected. *Striga* emergence was observed in each pot at 48 h interval from one week after sowing up to harvesting. After harvesting, *Striga* plants were uprooted and oven dried to measure dry biomass. Similarly pearl millet shoot and root biomass was recorded at the time of final harvesting (14 weeks after planting). Some of the details are listed in Table 1.

**Strigolactone Collection**

Pearl millet seeds were placed on filter paper wetted with 3 mL sterile water in a Petri dish which was subsequently sealed with parafilm. The sealed Petri dishes were incubated at 28°C for 48 h to germinate the seeds. Two germinated seeds were transferred carefully to a pot containing about 750 mL sand. Thinning to one plant was done after seven days. Three to four holes of 5 cm depth were made around each plant in which the appropriate dose of DAP fertilizer was placed. About 250 mL, N and P deficient, half strength modified Hoagland’s was provided in each pot at two-day intervals. The pots were placed in a climate room under standard conditions (25–28°C with 10 hours photoperiod (400 µmol m⁻² s⁻¹). In the 3rd week, the root exudates were collected by passing and draining 1000 mL nutrient solution through the pots. The resulting samples were passed through a C18-Fast column (500 mg) and the strigolactones eluted with 4 mL of 100% acetone. About 2 mL of this sample was cleaned by using 0.45 µm filters before strigolactone quantification by LC-MS. The other 2 mL were used to assess the induction of *Striga* germination.

**Striga hermonthica Germination Bioassays**

*Striga* germination was assessed essentially as described previously (Jamil et al., 2011a). About 50-100 surface sterilized and preconditioned seeds of *Striga* were distributed evenly on a glass fibre filer paper disc. After strigolactones sample application in triplicate, the discs with *Striga* were placed in a parafilm sealed Petri dish. These petri dishes were covered with aluminum foil and incubated at 30°C. After two days germinated and non-germinated seeds were counted under a microscope.

**Striga Emergence**

*Striga* emergence was studied by growing pearl millet plants in pots in the greenhouse. About 25 mg (5000) *Striga* seeds were mixed in 1 L of the 50:50 soil and sand mixture for each pot. Clean soil (about 500 mL) with no *Striga* seeds was first placed on the bottom of each pot. Subsequently, about 1 L soil/ *Striga* mixture was added, followed by 200 mL clean soil on top. Two germinated pearl millet seeds per pot were planted in the center of the pot. After one week of planting, thinning was done to keep one plant per pot and then three levels (0, 2, 4 g) of DAP was applied to each pot. The pots were placed in a greenhouse at 28°C day (14 h) and 25°C night (10 h) with relative humidity 70% supplemented with light (400 μmol m⁻² s⁻¹) for another three weeks. *Striga* emergence was observed up to 10 weeks after planting. Then the *Striga* plants were up-rooted, and their dry weight determined.

**Statistical Analysis**

A randomized complete design, with four replicates was used. A standard procedure was adapted to record data on *Striga* infection and pearl millet growth and development. The data collected were subjected to analysis of variance (ANOVA) through GenStat package release 9.2 (PC/Windows XP) (VSN international Ltd, UK).

**Results**

**Strigolactone Production**

Four strigolactones, orobanchol, *epi*-orobanchol, orobanchyl acetate and 5-deoxystrigol, were detected in pearl millet cultivars (KBH, Sadore Local and *Striga* Resistance). The pearl millet cv Sadore Local secreted maximum levels of orobanchyl acetate where no DAP fertilizer level was applied. The other cv *Striga* Resistance showed maximum production of orobanchol, *epi*-orobanchol and 5-deoxystrigol under DAP deficient condition (Fig. 1). The cv KBH produced the comparatively less production of orobanchol, *epi*-orobanchol and orobanchyl acetate as compared with Sadore Local and *Striga* Resistance. All the pearl millet cultivars showed reduction in secretion of strigolactones in response to enhanced levels of application of DAP fertilizer (Fig. 1).

**Striga Infection**

*Striga Germination:* Application of DAP fertilizer decreased *Striga* germination, with strikingly similar responses for the three cultivars (Fig. 2) and indeed maximum *Striga* germination was observed in the nutrient deficient control treatment (no DAP per pot) in all three pearl millet cultivars (Fig. 2). The pearl millet cultivar

**Table 1:** Experimental details at Wageningen University, Netherlands

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wageningen Univ. Netherlands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars</td>
<td>KBH, Sadore Local, <em>Striga</em> Resistance</td>
</tr>
<tr>
<td>Sowing date</td>
<td>May 15, 2010</td>
</tr>
<tr>
<td>Harvesting date</td>
<td>August 30, 2010</td>
</tr>
<tr>
<td>Experimental design</td>
<td>RCD</td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
</tr>
<tr>
<td>Pot size</td>
<td>1.5 L</td>
</tr>
<tr>
<td>Plant spacing</td>
<td>Single per pot</td>
</tr>
<tr>
<td>N and P-source DAP</td>
<td>(18% N:46% P₂O₅)</td>
</tr>
<tr>
<td>DAP levels</td>
<td>0, 2, 4 g per plant</td>
</tr>
<tr>
<td><em>Striga</em> infection</td>
<td>25 mg per pot (5000 seeds)</td>
</tr>
</tbody>
</table>

**Table 2:** Influence of fertilizer micro dosing on shoot and root biomass of pearl millet cultivars under greenhouse conditions at Wageningen University

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DAP</th>
<th>Shoot dry biomass (g)</th>
<th>Root dry biomass (g)</th>
<th>Total dry biomass (g)</th>
<th>Root:shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBH</td>
<td>0</td>
<td>30.1±0.5†</td>
<td>16.0±2.1†</td>
<td>46.1±1.9†</td>
<td>0.50±0.07</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>40.3±0.4†</td>
<td>12.1±0.5</td>
<td>52.4±0.3</td>
<td>0.30±0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40.0±0.7†</td>
<td>5.5±0.2</td>
<td>45.5±0.6</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Sadore Local</td>
<td>0</td>
<td>34.0±1.1†</td>
<td>9.1±0.4†</td>
<td>43.0±0.9†</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>40.2±0.9</td>
<td>6.3±0.5</td>
<td>46.5±0.5</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>48.4±0.9</td>
<td>4.3±0.3</td>
<td>52.6±0.9</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td><em>Striga</em> Resistance</td>
<td>0</td>
<td>32.9±0.7†</td>
<td>11.6±0.4†</td>
<td>44.5±1.0†</td>
<td>0.35±0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>31.5±0.7</td>
<td>9.3±0.4</td>
<td>40.8±0.7</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>34.3±0.6</td>
<td>8.0±0.3</td>
<td>42.3±0.2</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>Cultivar (P)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>DAP (P)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cultivar x DAP (P)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>*** (+ve)</td>
<td>** (-ve)</td>
<td>*** (+ve)</td>
<td>*** (-ve)</td>
<td>0.4</td>
</tr>
<tr>
<td>S.E.M‡</td>
<td>1.0</td>
<td>1.4</td>
<td>1.8</td>
<td>4.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

† Mean±standard error n=4.
‡ Standard error of difference of means.
§ Least significant differences of means at P = 0.05 by ANOVA test "**P<0.01; "***P<0.001

Sadore Local showed maximum *Striga* germination (35%) in the control treatment and supply of DAP fertilizer reduced germination to 15%. Cv KBH showed less *Striga* germination compared with the other pearl millet cultivars, which was also reduced significantly by application of 4 g DAP fertilizer (Fig. 2).

*Striga Emergence and Dry Biomass Production:* *Striga* emerged in highest numbers in the control treatment (44 in KBH, 43 in Sadore Local and 27 in *Striga* Resistance) and application of DAP reduced *Striga* emergence in all three cultivars by up to 69% (Fig. 3A). Similarly, in the nutrient deficient control treatment maximum *Striga* dry biomass occurred due to highest *Striga* infestation (Fig 3B). The pearl millet cv Sadore Local showed maximum *Striga* biomass production in control treatment (2.6 g). With increased levels of of application of DAP fertilizer, a strong
reduction of Striga dry biomass (down to 0.1 g), was seen in all three cultivars (Fig. 3B).

**Plant Growth and Development**

An increase in pearl millet biomass was observed with the supply of fertilizer whereas an inverse trend was recorded in root mass and root to shoot ratio (Table 2). A significant interaction between levels of DAP fertiliser application and pearl millet cultivar was observed for plant biomass and root/shoot ratio. The shoot dry biomass was low in the control treatment in all cultivars possibly due to Striga infection and nutrient stress and increased with the application of DAP fertilizer the shoot biomass increased. On the other hand, under Striga infection, nutrient stress in the control treatment caused
the pearl millet plant to produce maximum root biomass and this increase in root biomass resulted in a high root: shoot ratio.

**Discussion**

*Striga* is known as an indicator of low soil fertility and it appears that its occurrence on crops has a direct relation with the nutrient status of the soil (Lopez-Raez et al., 2008). The direct or indirect suppressing effect of fertilizer on *Striga* infection has been reported previously (Kim et al., 1997; Pageau et al., 2003; Jamil et al., 2012). Recently the relationship between underground signalling molecules, the strigolactones, with *Striga* germination in response to fertilizer levels has been explored (Jamil et al., 2011a). It was found that host plants secret underground signalling molecules according to levels of mineral nutrients in the soil. Maximum secretion was observed under low P levels which reduced gradually with increasing amount of P in the soil (Jamil et al., 2011a). Due to poverty and poor availability of fertilizer, it is almost impossible for African farmers to maintain optimum soil fertility levels in their fields. If a more efficient and economical method of fertilizer application would be available, this would be an attractive method to increase yields and reduce *Striga* attack. Here we show that a low level of DAP fertilization near the roots of pearl millet reduces secretion of strigolactones. As a result of lower concentrations of signalling molecules in the rhizosphere, *Striga* seed germination and infection is reduced, as was also demonstrated for a similar study with fertiliser application in maize and sorghum (Jamil et al., 2012, 2013).

The LC-MS analysis in the present study showed that all three pearl millet cultivars had maximum production of four strigolactones under no DAP fertilizer while a gradual increase in the DAP application from 2 g to 4 g led to a reduction of the production of these germination stimulants in the rhizosphere (Fig. 1). Moreover, the maximum amount of orobanchol was observed in pearl millet cultivar *Striga* Resistance while orobanchyl acetate was highest in Sadore Local in the control treatment. The pearl millet host plant reduces its strigolactone secretion in the rhizosphere after receiving mineral nutrients through micro dosing which resulted in lower *Striga* germination and this trend was similar for all three cultivars (Fig. 2). The *Striga* emergence and dry biomass production also negatively responded to DAP supply (Fig. 3). These results validate our previous findings in a similar study on three cultivars of sorghum, which showed the same trend of decreasing strigolactone secretion and *Striga* infection with increasing microdosing of DAP fertilizer (Jamil et al., 2013). The three different pearl millet cultivars exhibited a different response with regard to amount and type of strigolactone under varying dose of DAP fertilizer. Sadore Local was more susceptible due to its high production of strigolactones (orobanchyl acetate) while cv Striga Resistance and, to a lesser extent,
KBH showed low Striga infection (Fig. 3). These cultivars also secreted less strigolactones than Sadore Local (Fig. 1). The genetic variation in strigolactone production might be helpful to select less affected Striga crop varieties (Ejeta, 2007; Jamil et al., 2011b). Although the present findings seem promising and effective to reduce damage of Striga, still a number of factors should be considered before its practical application. The low purchasing power of African farmers, access to fertilizer and credit, application cost and labour availability might affect this strategy. Studies on how to further lower fertiliser application rates could make it even more efficient and affordable for the farmers.

The present findings support our hypothesis that a low doses of DAP fertilizer application in the vicinity of pearl millet host roots reduces its rhizosphere signalling, which results in a reduction in Striga seed germination and subsequent infection of the host plant. Since very low doses of DAP fertilizer resulted in a significant reduction in Striga germination and infection in all cultivars of pearl millet, this may be an efficient and economical way to reduce the Striga problem in African countries, which appears independent of pearl millet variety. Based on these findings, a combination of control options in an integrated Striga control strategy could possibly be developed for African farmers to reduce damage of the noxious parasitic Striga weed in their cereal crops.

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