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Increasing sorghum yields by seed treatment with an aqueous extract of the plant *Eclipta alba* may involve a dual mechanism of hydropriming and suppression of fungal pathogens

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ABSTRACT

Background: Soaking of sorghum seeds for six hours in an aqueous extract of *Eclipta alba* has been shown to increase the yield of sorghum in field experiments. The effect on yield is known to depend on field location and a mechanism involving pathogen suppression has been proposed. However, it has not been clear to which extent the same effect can be obtained by soaking of seeds in pure water (hydropriming). To address this question, fifty eight field tests were conducted comparing no treatment of seeds, hydropriming and treatment with plant extract. Experiments were distributed over three years in Burkina Faso on three locations previously showing a positive yield response to the plant extract.

Results: Despite strong variation across locations and years, a mean yield increase of 19.6% was found for hydropriming compared to no treatment ($p < .018$). For the plant extract, an additional yield increase of 32.1% was found ($p < .016$) corresponding to a total increase of 51.7%. In a subset of 15 experiments, a positive, but non-significant correlation was observed between the additional effect of the plant extract and the effect of a binary pesticide, Calthio C. Significantly, however, the *E. alba* extract reduced the number of seedlings infected by seed-borne filamentous fungi ($p < .05$). A reduction of infection by more than five-fold was found for the *E. alba* extract compared to hydropriming and included potential pathogens of sorghum: *Epicoccum sorghinum* and *Curvularia* spp.

Conclusion: Using 6-hours of soaking, hydropriming was an inherent component of seed treatment with the *E. alba* extract and contributed significantly to the overall observed increase of yield and emergence. An additional yield increase was caused by factor(s) derived from the plant, *E. alba*, and may involve suppression of pathogenic fungi.

1. Introduction

Seed treatment with an aqueous extract of the plant *E. alba* has been shown to increase the yield of sorghum in Burkina Faso in several field trials (Zida et al., 2008b, 2012 and 2016). In these studies, an antifungal effect of the plant extract, protecting the seedlings, has been proposed as the mechanism primarily based on the finding of a reduced number of fungi growing on the surface of treated seeds (Zida et al., 2008b; 2012). A possible contribution to the yield increase from the aqueous solvent of the extract (known as seed hydropriming) was previously tested but was not found (Zida et al., 2012). However, a relatively long soaking time (10–20 h) was applied in that study and other studies have found that a positive effect of hydropriming in sorghum can be observed when shorter soaking times, 6–8 h, are applied (Harris, 1996; Ramamurthy et al., 2005; Aune and Ousman, 2011). Recently, an improved protocol for seed treatment with the *E. alba* extract, applying only 6 h of soaking was reported (Zida et al., 2015). Taking the previous findings of hydropriming and soaking time into account, it appeared relevant to re-test to which extent hydropriming may contribute to the increase of yield when using the shortened soaking time. Our working hypothesis would state: A part of the yield increase observed for treatment with the plant extract can be obtained by soaking of the seeds in pure solvent (water). To test this in a field trial, the present study selected three locations in which seed treatment of sorghum with the *E. alba* extract had previously shown a consistent and strong effect: Over a five-year period, a 20–40% yield increase was found at three locations in Burkina Faso: Diapangou, Ipendo and Kamboinsé, (Zida et al., 2016).The strongest effect of the plant extract

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was previously found at location, Diapangou. In a subset of new experiments at this location, the present study included a treatment with the binary pesticide, Calthio C, for comparison to both hydropriming and the plant extract. Calthio C is produced locally in Western Africa and contains the active compounds thiram (tetramethylthiuram disulfide) and Chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate) targeting fungi and insects, respectively (Elskens and Penninckx, 1995; Barron and Woodburn, 1995). The extract of *E. alba*, has previously been shown to exert inhibition of fungi *in vitro* (Saraswathy and Kumaran, 2012; Zida et al., 2008b; 2012) and to reduce symptoms of fungal infection in seedlings (Zida et al., 2015). In the present study we compared the antifungal effect of *E. alba* to hydropriming measuring for the first time the level of fungal infection directly *in planta* using both a classical method (enumeration of fungal outgrowth from plant tissue on potato-dextrose agar) and a molecular method (amplicon sequencing of fungal 18S ribosomal DNA from seedlings).

2. Materials and methods

2.1. Plant material

For each of the three years, 2014–2016, one sample of the sorghum variety Kapelga (improved variety produced by INERA, Burkina Faso) and one sample of the variety Kouria (landrace, propagated by INERA, Burkina Faso) was used for field experiments. Thus, in total, seeds from 6 sorghum seed samples were tested. Seeds were propagated at the Research Station Kamboinsé, harvested in November–December and stored for 6–7 months (dry season) before sowing in field experiments during May–June in the following season. Each year whole plants of *Eclipta alba* were collected during the months of October–December from humid areas near Ouagadougou. The *E. alba* material was shade-dried for two weeks and stored as powder, after being homogenized with a large woody pestle. A seventh seed sample of sorghum, Acc No 49.071 (also described in Zida et al., 2015) was used for the green house experiment.

2.2. Seed treatment

As described by Zida et al. (2015). Briefly: Hydropriming was conducted by soaking seeds for 6 h in pure water at ambient temperature (close to 25 °C). The *E. alba* extract was prepared by passive extraction of 10 g dry powder per 100 ml water by incubation over night at ambient temperature (close to 25 °C) without shaking. After incubation, particulate matter was removed by gently pressing the extract through a piece of cotton cloth. Seeds were then soaked in the extract for 6 h at ambient temperature (close to 25 °C). Following soaking in either water or plant extract, seeds were air-dried ON (16–20 h) before sowing. Seeds treated with pesticide, Calthio C (Saphyto, Bobo Dioulasso, Burkina Faso) were powdered with 4 g per 1000 g of seeds. The pesticide was formulated as 25% w/w Thiram and 25% w/w Chlorpyrifos-ethyl by the company. In all experiments non-treated (NoT) seeds were included as a control. In the tables and text below, the terms H_2O, Ea10 and Cal are used to describe, hydropriming, treatment with *E. alba* extract (10%) and Calthio C, respectively.

2.3. Field plots

Field plots were prepared and managed as described by Zida et al. (2016). Briefly, sowing was done by hand using 4–6 seeds per seed hole obtaining an overall seeding density of approximately 156.000 seeds/ha. At sowing, 100 kg/ha mineral fertilizer (NPK 14-23-14) was applied, followed by 50 kg/ha urea after 3 weeks. No irrigation was applied. Emergence was enumerated as the number of seed holes populated with plants 15 days after sowing at which time point plants were thinned to a maximum of 4 seedlings per seed hole. At crop maturity (October–November), grains were harvested and sun-dried for 2 weeks before determination of weight. The minimum plot-size was 25 rows (spaced by 80 cm) x 5 m length. The distance between seed holes was 40 cm. During the period from May to July, daily rainfall (mm) was recorded on two climate stations located at two of the three locations (Kamboinsé and Diapangou, respectively) and the average of the two recordings was calculated.

2.4. Seedling experiment in greenhouse

A growth experiment was set up twice using 96 sorghum seeds per treatment (seed accession 49.071 from the INERA Research Centre in Burkina Faso) as follows. Four seed treatments were compared: Three of the treatments tested in field trials and described above (NoT, H_2O, Ea10) were included together with a fourth treatment: heat treatment (55 °C water bath/40 min) as described in Stokholm et al. (2016). Following treatments, seeds were dried overnight and sown in freshly autoclaved soil (75% Pindstrup sphagnum mixed with 25% sand) and incubated in a climate chamber at 25 °C with a light/dark ratio of 14/10 h, as previously described (Stokholm et al., 2016) After seven days, seedlings (roots and shoots) were harvested by rinsing in tap water. Fresh weight of shoots was determined. Roots and stems from seedlings with a shoot-fresh-weight > 20 mg were surface sterilized, as in Stokholm et al. (2016), with 1.5% sodium hypochlorite, followed by cutting into ~1 cm long pieces. For each seedling the pieces (root and stem, separately) were divided in two groups, in order to assess the mycoflora by two independent methods: 1) Counting of fungal outgrowth from each individual seedling (morphological analysis) 2) Measurement of fungal DNA in pooled material from each treatment (DNA analysis).

2.5. Morphological analysis of mycoflora in seedlings

For each seedling, stem and root pieces (cut at 1 cm length) were placed on potato dextrose agar in Petri dishes, followed by incubation under UV-light for 5 days. The number of seedlings with filamentous fungal outgrowth was determined and fungi were morphologically identified as either *Epicoccum* sp (as *Phoma sorghina* by Mathur and Kongsdal (2003)). or other filamentous fungi.

2.6. DNA analysis of mycoflora in seedlings

For each treatment, samples (root and shoots, separately) were freeze dried, ground in liquid nitrogen and DNA was extracted as described in Stokholm et al. (2016). The 18S rDNA was targeted for PCR amplification and meta-barcoding using the 18S rDNA primers and methods previously described in Stokholm et al. (2016), with the following modifications: The PCR reaction was split in two steps, with the first step being 15 cycles at annealing temperature 50 °C using untagged primers (Table 1), followed by 5 cycles at 50 °C and 10 cycles at 65 °C using 5 µl of from the first round as template (total volume of 50 µl) and fusion primers tagged for barcoding (Table 1). The resulting amplicons (400–500 bp) were assessed by gel-electrophoresis, excised and purified, as described in Stokholm et al. (2016). Equimolar concentrations of the root and stem amplicons were mixed and amplicon sequenced using the Pacific Biosciences (PacBio) SEQUEL Technology Platform at the Uppsala Genome Centre, SciLifeLab, at Uppsala University, in Sweden. The resulting amplicon sequence data was processed through the open source SCATA pipeline (Sequence Clustering and Analysis of Tagged Amplicons (http://scata.mykopat.slu.se); Clemmensen et al., 2016) as previously described (Stokholm et al., 2016). For the QC part, sequences containing a match > 70% to both the forward primer sequence and the 14 bp insertion of the reverse primers (Table 1) were accepted, followed by removal of the primer sequences. Reads with a 3′-5′ direction were reverse complemented. Sequences shorter than 170 bp, or sequences of low quality with a mean quality score < 20 or bases