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Presowing Hardening of the Host with Phenolic Acids Reduces Induction of Seed Germination in the Root Parasite Striga asiatica

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Presowing Hardening of the Host with Phenolic Acids Reduces Induction of Seed Germination in the Root Parasite *Striga asiatica*

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Summary. *Striga asiatica* (L.) Kuntze, a root parasite, causes severe loss of yield in sorghum and several other crops. The seeds of the parasite are induced to germinate by a stimulant in the host root exudate. Presowing hardening of the host with vanillic acid, caffeic acid and ferulic acid (25 ppm) reduces the induction of seed germination in the parasite by the host root exudate. The treatment causes a slight improvement in the dry matter production in the host and in addition, increases the phenolics level in the host root exudate. The latter effect might be responsible for reducing germination in *Striga*. If the treatment remains effective under field conditions also, it reduces significantly the incidence of *Striga* in cultivated fields.

Introduction

Striga asiatica (L.) Kuntze is a root parasite on sorghum and several other crops, seriously affecting the yield. Many control measures have been tried (Hosmani, 1978) but the problem has remained largely unsolved.

Hardening wheat (*Triticum aestivum* L. var. UP 301) with phenolic acids is known to impart resistance in the seedlings against the allelopathic action of weeds (Cowsik and Jayachandra, 1979). Hence a study was undertaken to see if the treatment was in any way useful against the parasite, *Striga*.

Materials and methods

Sorghum (*Sorghum bicolor* var. CSH 1) was selected from amongst the hosts of *Striga*, for the study and the seeds were obtained from the National Seeds Corporation, Bangalore, India. The seeds of *Striga asiatica* were collected during August, 1977 from sorghum fields around Kikkeri, Mandya district, Karnataka, India. Of the phenolic acids, anisic acid was crystallised from the root leachate of *Parthenium hysterophorus* L. in the authors' laboratory, ferulic acid and caffeic acid were from Koch-Light Laboratory, England and vanillic acid was from Fluka-Buchs, Switzerland.

Sorghum grains were hardened with distilled water and 25 ppm anisic acid, ferulic acid, caffeic acid and vanillic acid. The hardening treatment consisted of soaking the grains in the respective media for 4 h followed by drying them to their original weight. This was repeated thrice. During hardening the grains were spread in a single layer in 'Corning' beakers and the soaking medium was just enough to cover the grains. The containers were periodically shaken during the soaking period. The whole treatment was given under 5000–6000 lux from fluorescent lamps. The temperature varied from 22 to 26°C and the relative humidity ranged between 55 and 85%.

The hardened and untreated sorghum grains were sown in 150 g coarse sand held in double paper cups of 6.5 cm diameter and 8 cm depth, in five replications. The inner cups had a perforated bottom that was covered with a filter paper lining before filling with sand. Each container was supplied with 15 ml distilled water daily. All the containers were arranged on a rack (35 cm square) in a completely randomised design. The whole set, in duplicate, was subjected to diurnal cycles of a ten hour photoperiod (5000–6000 lux from fluorescent lamps) followed by darkness. Temperature and relative humidity variations were the same as for the hardening treatment. Ten-day-old

seedlings of one set, with five seedlings per container, were used for determining dry weight and of the other, with fifteen seedlings per container, were used for collecting root exudate under suction following Parker *et al.* (1977).

Striga germination test

Seeds of *Striga* were placed on 5 mm diameter glass fibre filter paper discs, 25 on each and pretreated at 37°C in the dark for ten days. Four such discs with pretreated *Striga* seeds were placed on a filter paper lining moistened with 2.5 ml of sorghum root exudate in 9 cm diameter petri plates, in three replications. The set was covered with polythene bags to check evaporation further and incubated at 37°C in the dark. Germination counts were taken after 48 h.

Phenolic content

Total phenolic content in the root exudate from the ten-day-old sorghum seedlings was determined in five replications, using Folin-Denis reagent (Ribereau-Gayon, 1972).

Root exudate from the ten-day-old sorghum seedlings was extracted in twice the volume of petroleum ether and ethyl acetate sequentially. Both these fractions were concentrated until their volumes were reduced to 0.5 ml and they were then spotted on thin layer silica gel plates. After developing the chromatogrammes with benzene:ethyl acetate (55:45) and chloroform:acetic acid (90:10) following Harborne (1975), the phenolic spots were visualised by u.v-fluorescence and in iodine chambers.

Experimental data were statistically analysed using the *t*-test.

Results and discussion

Data in Fig. 1 show that presowing hardening of sorghum grains with phenolic acids brought down the ability of the host to induce seed germination in *Striga,* the decline being 96.1, 87.6, 71.0, 15.9 and 11.8% in treatments with vanillic acid, caffeic acid, ferulic acid, anisic acid and distilled water, respectively. As anisic acid was only slightly effective, the treatment was not considered for further analytical studies.

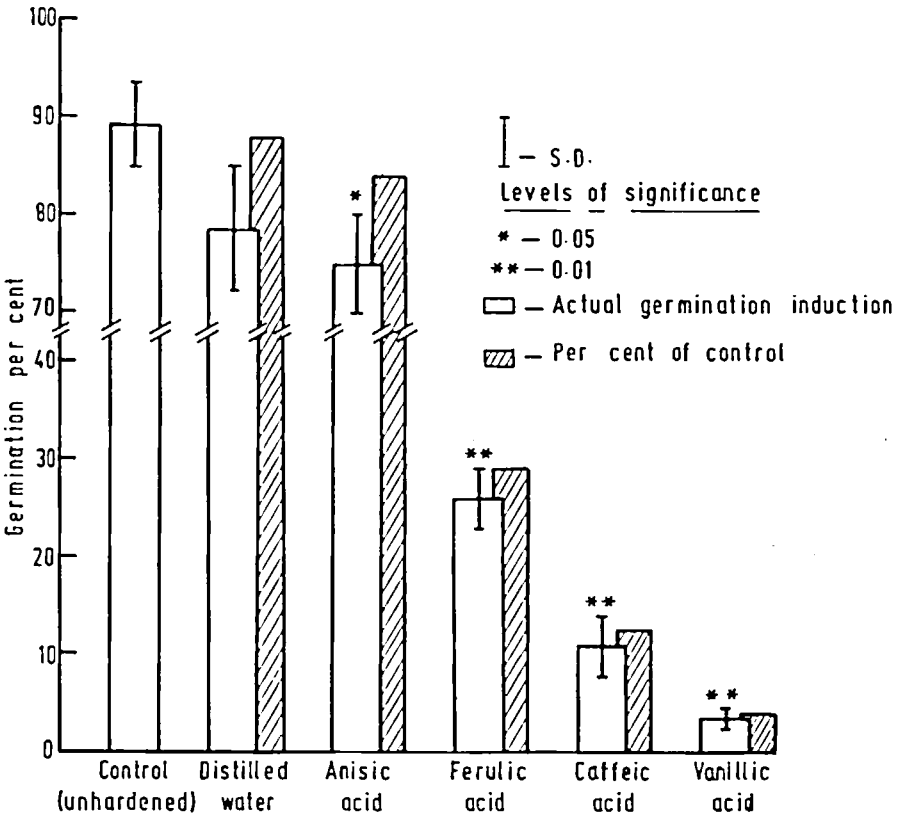

Fig. 1. Effect of presowing hardening of *Sorghum bicolor* var. CSH 1 with phenolic acids (25 ppm) on the ability of the root exudate to induce seed germination in *Striga asiatica.*

TABLE 1. DRY WEIGHT OF TEN-DAY-OLD SEEDLINGS OF *SORGHUM BICOLOR* VAR. CSH 1 AS INFLUENCED BY PRESOWING HARDENING

Treatment	Dry weight (mg)	
	Shoot	Root
Control (unhardened)	8.5 (±0.8)	2.8 (±0.2)
Hardened with:		
Distilled water	8.7 (±0.3)	3.1* (±0.3)
Anisic acid (25 ppm)	10.3* (±1.2)	3.5* (±0.6)
Ferulic acid (25 ppm)	8.6 (±0.3)	2.9 (±0.0)
Caffeic acid (25 ppm)	10.3* (±1.3)	3.5* (±0.2)
Vanillic acid (25 ppm)	10.1* (±0.8)	3.2* (±0.1)

Figures in parentheses refer to standard deviations.
*Significant at 0.05 level.

As is evident from the data in Table 1, hardening with phenolic acids caused an increase in the dry weight of ...hum seedlings by over 1–22% in the shoot and 3–25% in the roots. Presowing hardening of wheat (*T. aestivum* ... UP 301) with phenolic acids (1–5 ppm) is reported to cause a 20–25% increase in the shoot dry weight of ten-... old seedlings (Cowsik and Jayachandra, unpublished). In the present study, the increase in dry matter due to the ...tment is quite significant in the ten-day-old sorghum seedlings, which were quite normal and healthy (Fig. 2). ... reduced ability of the host to induce seed germination in *Striga* cannot therefore be considered as due to any ... ging effect of the treatment on the host and considering the trend in wheat, the treatment is unlikely to cause ...dverse effect on sorghum plants at later stages of development.

The reduced ability of the sorghum seedlings of the hardened set to induce germination in *Striga,* might be ... to significant changes in the composition of their root exudate, qualitatively and/or quantitatively. Data in ... e 2 show that the treatment with caffeic acid and vanillic acid effected a significant increase in the total ...olics level in the root exudate of sorghum. Chromatogrammes of the phenolics of sorghum root exudate did not ... any spot that was characteristic of the treatment. Hence, the increase was probably, only quantitative. As ...olics are germination inhibitors (Mayer and Poljakoff-Mayber, 1976) an increase in their level in the host root ...te must have contributed to the lowered induction of germination in *Striga.* Seed germination in *Striga* is ... to be induced by the stimulant in the root exudate of the host (Parker, 1965). An increase in the phenolics

Fig. 2. Photograph showing that presowing hardening with phenolic acids did not have any adverse effect on ten-day-old seedlings of *Sorghum bicolor* var. CSH 1. A unhardened, B–E hardened with distilled water and 25 ppm of ferulic acid, caffeic acid and vanillic acid, respectively.

TABLE 2. INFLUENCE OF HARDENING ON PHENOLICS LEVEL IN THE ROOT EXUDATE OF TEN-DAY-OLD SEEDLINGS OF *SORGHUM BICOLOR* VAR. CSH 1

Treatment	Phenolics (mg/g dry weight of root)
Control (unhardened)	2.34 (±0.1)
Hardened with:	
Distilled water	2.01 (±0.2)
Ferulic acid	1.85 *(±0.1)
Caffeic acid	2.45 *(±0.1)
Vanillic acid	2.49 *(±0.1)

Figures in parentheses refer to standard deviations.
*Significant at 0.05 level.

level must obviously have decreased the stimulant to inhibitor ratio in the sorghum root exudate, consequently reducing its ability to induce germination in *Striga*. The importance of such a reduction in the ratio of the stimulant to the inhibitor in the host root exudate in reducing broomrape infestation in agricultural situations has been pointed out by Whitney (1979)

In the treatments with distilled water and ferulic acid, although the phenolics level in the root exudate was lower than in the control, the ability of the host to induce *Striga* germination was lowered significantly. This shows that changes other than in the level of phenolics might affect the ability of the host root exudate to induce germination in *Striga*. The hardening treatment altering the nature of the stimulant(s) or lowering their level in the root exudate and consequently, the lowered ratio of stimulants to inhibitors having reduced the induction of germination in *Striga*, are not unlikely. Investigations on the influence of the hardening treatment on changes in the stimulant to inhibitor ratio in sorghum seedlings would be quite interesting.

Presowing hardening with caffeic acid and vanillic acid increases the phenolic level in the sorghum root exudate and this might in turn have reduced the ability of the host to induce germination in *Striga*. In the case of the distilled water and ferulic acid treatments the mechanism might be different. The treaments might have affected the nature and/or level of the germination stimulants in the host root exudate. These findings are significant in that if these effects persist under field conditions, the simple presowing hardening of the host crop with phenolic acids would, in addition to inducing resistance to allelopathic action (Cowsik and Jayachandra, 1979), be useful in reducing the incidence of *Striga* without affecting the crop adversely.

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