Helminthosporium sativumの培養ろ液がコムギ苗に及ぼす刺激的,保護的効果

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Stimulatory and Protective Effects of Culture Filtrate of *Helminthosporium sativum* on Wheat Seedlings*

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A.M. Das and D.N. Srivastava : *Helminthosporium sativum* の培養ろ液がコムギ苗に及ぼす刺激的，保護的効果

Abstract

Using root elongation as index, the stimulatory and protective effects of culture filtrate of *H. sativum* on wheat seedlings were assessed.

Seeds presoaked for 24 hours in 1/50–1/500 dilutions of culture filtrate of the fungus or in similar dilutions of extract of inoculated soil maize medium, showed significantly greater root length over the control when germinated and grown in water.

Seeds presoaked in dilutions of 1/100–1/500 of the culture filtrate and grown in inoculated soil had significantly greater root length, maximum being at 1/100 dilution where it was 40 percent more than in the control. This is interpreted as protective effect of the culture filtrate of the pathogen against the pathogen itself.

(Received September 17, 1970)

Introduction

Stimulation of wheat seedlings inoculated with *Helminthosporium sativum* P., K. and B. under certain conditions was first reported by Sallans[7]. He suggested that the toxin or other influence produced by the fungus in the host tissue increased the intake of mineral ions into the plant and as the conditions became favourable for growth, the presence of large amounts of minerals, thus accumulated in the plant increased the size of subsequently formed leaves. Sallans[8,9] further reported that such increase in leaf area in the inoculated plants produced statistically larger yields than the controls only when the growth conditions for the host were good. He contended that the stimulation was brought about by the toxin itself when its concentration was reduced under conditions favourable for growth of the plant. Stimulation of barley seedlings in soil with less than 2 percent infestation level of *H. sativum* was also reported by Ludwig *et al.*[4], which was considered to be the reason for stimulated yields obtained at times as a result of artificial inoculation. Prasad[6], working on the toxic effect of serial dilutions of culture filtrate of the fungus on root elongation of wheat seedlings, noticed stimulatory effects at dilutions beyond 1/50. Subramanian[10] reported similar finding by another worker at Jodhpur, and postulated that the stimulatory effect was probably due to a growth factor.

Stimulatory effect of piricularin, the toxin produced by the rice blast fungus *Piricularia oryzae*, in high dilutions (about 1/10,000,000) on the growth of rice plants was recorded by Tamari and Kaji[11]

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in Japan. In this respect, piricularin resembled the giberellins produced by *Fusarium moniliforme*[1].

Zoja[12] from Italy reported "immunizing" property of extract of *H. sativum* culture and sap of diseased plants against attack by the fungus itslef. Leeman[3] in Germany found that wheat seeds soaked in water extract of agar culture of the fungus for 21 hours and dried for 14 days showed very weak infection when inoculated with the funguns, while seeds sown immediately after soaking became heavily infected. Similarly, extract of soil in which diseased plants had grown, when added to the soil, increased the susceptibility of seedlings to subsequent inoculation with the fungus, but when seeds were soaked in the extract, only slight infection followed. Both these workers found the "immunizing" substance to be water soluble and thermostable. Prasad[6] observed that when wheat germlings were allowed to grow in dilutions beyond 1/50, upto a certain level, of the culture filtrate, their root growth was considerably stimulated. Additional literature is scanty on this interesting aspect.

In the light of the above reports, culture filtrate of *H. sativum* was tested for its stimulatory and protective effects on wheat seedlings and the results are presented in this paper.

Materials and methods

A culture of *H. sativum* isolated from roots of foot rot affected wheat plants which was highly pathogenic and which was found to produce high concentrations of the toxin in culture[2] was used. It was maintained on oat agar slants.

The culture media employed for the studies were:

1) Potato dextrose solution

Potato slices	250 g
Dextrose	20 g
Water	1 *l*

2) Soil maize medium

Air dried sieved soil	20 g
Maize meal	10 g
Water	30 m*l*

The first medium was autoclaved at 15 lb pressure for 20 minutes after dispensing 20 m*l* in 100 m*l* Erlenmeyer flasks, and the second at 15 lb pressure for 1 hour on 3 consecutive days.

The inoculum used for growing the fungus consisted of a concentrated spore suspension in distilled sterile water. It was added in the ratio of 0.5 m*l* to 20 m*l* of potato dextrose solution or to 100 g of the soil maize medium.

Culture filtrate was prepared by inoculating the fungus into flasks containing 20 m*l* of potato dextrose solution and incubating at 25°C for 7 days. The filtrate was freed from mycelial bits and spores by filtering through Whatman No.1 filter paper and was stored at 2°C for future use.

Wheat seeds of variety NP 798 were used for the experiments. They were surface sterilized in 0.1 percent solution of mercuric chloride for 10 minutes followed by rinsing in 4 changes of distilled sterile water. Germination tests were made on sterile moist blotting papers in Petri plates at 25°C for 27 hours. After this period, a majority of the seeds produced the desired root length of 5 mm. Germinated seeds have been referred to as "germlings" hereafter.

Luke and Wheeler[5] working with Victoria blight of oats incited by *H. victoriae* observed that inhibition of elongation of the total root system of an inoculated oat seedling was correlated with the inhibition in elongation of the longest root. The latter could be used as an index of the disease for experimental purposes. Prasad[6] observed the same to be true for the foot rot of wheat incited by

H. sativum and employed this criterion for assaying the pathogenic effects of the fungus under different conditions. The same method was used in these experiments for measuring the stimulative and protective effects of the culture filtrate.

The data of all the experiments were subjected to statistical analysis and the inferences drawn are based on highly significant differences between various treatments and controls.

To avoid any confusion to the readers, the remainder of the procedures followed have been stated alongwith the results under each experiment.

Results

Stimulatory effect of culture filtrates on wheat root elongation

It was considered important to determine if the seeds presoaked in higher dilutions of the toxin, retained a stimulatory effect on root elongation when germinated subsequently in water. Surface sterilized seeds were soaked in culture filtrate at dilutions of 1, 1/5, 1/50, 1/100, 1/200 and 1/500 for 24 hours at 25°C. They were washed and germinated in water. Ten germlings of each treatment were subsequently placed in Petri plates containing water and incubated for further root elongation at 25°C. Each treatment was replicated 6 times. Measurement of the longest root of each seedling was taken at 48 hours and the average root length for each treatment was determined. Seeds soaked in corresponding dilutions of uninoculated potato dextrose medium, germinated and grown similarly served as the control. A separate lot of seeds soaked in water, germinated and grown in the same way served as a second control. The results are presented in Fig. 1.

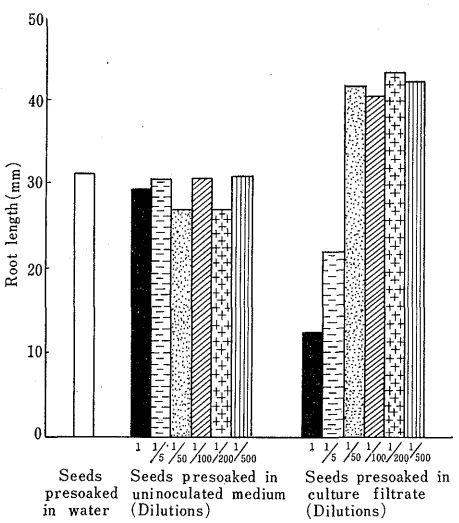

Fig. 1. Stimulation in root elongation of wheat seedlings from seeds presoaked in culture filtrate of *Helminthosporium sativum.*

Culture filtrate at dilutions of 1/50 to 1/500 afforded significant stimulation of root elongation when compared with the corresponding uninoculated medium or the water control. At 1/200 dilution of the culture filtrate, an increase of 61.2 percent of root length over the corresponding dilution of the uninoculated medium control was obtained.

Stimulatory effect of extract of inoculated soil on wheat root elongation

To every 50 g of the soil maize medium, which originally contained 15 m*l* of water, 60 m*l* of

water was added after 10 days growth of the fungus and stirred well. The suspension was filtered through Whatman No. 1 filter paper. The filtrate thus collected was roughly considered to be 1/5 dilution of the normal soil extract. Further dilutions of 1/20, 1/50, 1/100, 1/200 and 1/500 of this filtrate were made and seeds were soaked in these for 24 hours. Root elongation of the seedlings was studied in the same way as in the previous experiment. The results are presented in Fig. 2.

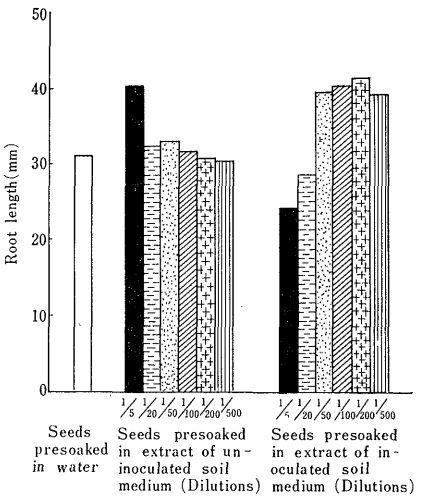

Fig. 2. Stimulation in root elongation of wheat seedlings from seeds presoaked in extract of soil inoculated with *Helminthosporium sativum.*

While 1/5 dilution of the extract of the inoculated soil caused 22.4 percent inhibition of root elongation over the water control, the corresponding dilution of the extract of the uninoculated soil medium stimulated root elongation by 28.8 percent over the water control. Dilutions of 1/50, 1/100 and 1/200 of the extract of the inoculated soil showed progressive stimulatory effect over the water control. Similar dilutions of the extract from the uninoculated medium did not differ in their response from the water control. The high degree of stimulation encountered in 1/5 dilution of the extract of uninoculated soil could probably be attributed to the nutritive value of the maize meal; this effect was lost in higher dilutions of 1/20 and above. The stimulatory property of the extract from inoculated soil was evidently suppressed by the inhibitory action of the toxin at dilutions of 1/5 and 1/20, but the stimulatory effect of subsequent dilutions (1/50, 1/100, 1/200 and 1/500) was significantly higher than the corresponding dilutions from the uninoculated soil and the water control.

Protective effects of the culture filtrate

The ability of the culture filtrate in soaked seeds to protect root elongation i) against the toxic effect of the culture filtrate itself and ii) against the fungus in inoculated soil was investigated.

i) Against the toxic effect of the culture filtrate: Surface sterilized seeds were soaked in 1, 1/5, 1/50, 1/100, 1/200 and 1/500 dilution of the culture filtrate for 24 hours at 25°C. Later, they were washed and germinated in water. The germlings of each treatment were subsequently exposed to the culture filtrate at dilution of 1 and 1/10 for further root elongatoin at 25°C for 48 hours. Wheat seeds immersed in corresponding dilutions of the uninoculated potato dextrose medium and subsequently germinated and exposed to the culture filtrate in the same way served as control. A separate lot of seeds immersed in water and later treated similarly served as a second control. The number of seedlings in each treatment was 60.

The results did not show any protection against the toxic effect of the culture filtrate on root elongation of the seedlings raised from seeds presoaked in various dilutions of the culture filtrate.

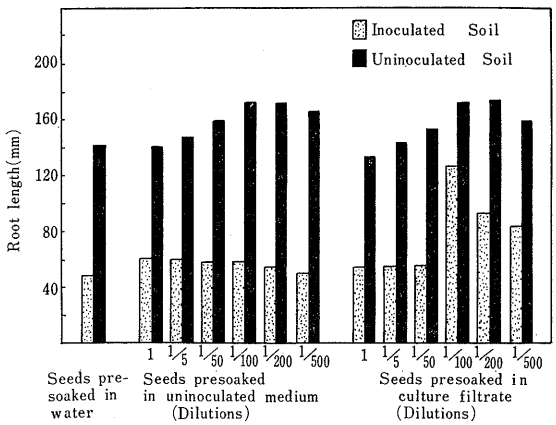

Fig. 3. Protection of wheat seedlings of seeds presoaked in culture filtrate of *Helminthosporium sativum* against foot rot disease.

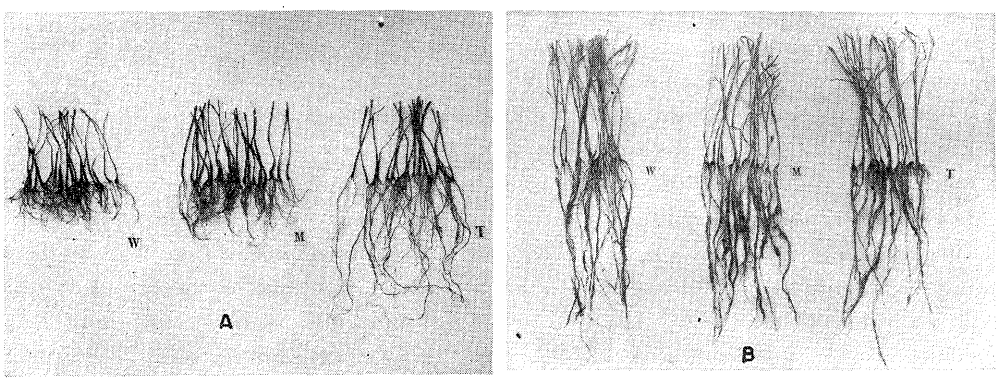

Fig. 4. Protection of wheat seedlings of seeds presoaked in culture filtrate of *Helminthosporium sativum* against foot rot disease.
A. Root development in inoculated soil.
B. Root development in uninoculated soil.
W: Seeds presoaked in water.
M: Seeds presoaked in 1/100 dilution of uninoculated medium.
T: Seeds presoaked in 1/100 dilution of culture filtrate.

ii) Against the fungus in inoculated soil: Soaking of seeds in various dilutions of the culture filtrate, in the uninoculated medium and water was accomplished in the same way as in the preceding experiment. Sixteen seeds treated in each of the 6 dilutions of the culture filtrate were sown in potted soil inoculated with the fungus. The test was replicated three times. Equal numbers of seeds soaked in the same dilutions of the culture filtrate were sown in uninoculated soil to serve as control. Similar number of seeds presoaked in corresponding dilutions of the uninoculated medium and also in water alone were sown in both inoculated and uninoculated soils for further comparison. The pots were kept in the open and the seeds were allowed to grow for 30 days after which observations on the longest root of each seedling in the various treatments were recorded. The results are presented in Figs. 3 and 4.

The soaking of seeds in culture filtrate at the dilution of 1/50 afforded no protection against the root stunting effect of the pathogen in inoculated soil, but little stunting occurred at dilutions of 1/100, 1/200 and 1/500. The maximum protection was at 1/100 where the root length was greater by more than 100 percent than in the corresponding dilution of the medium and the water control.

In the uninoculated soil, the seeds soaked in the culture filtrate at 1/100, 1/200 and 1/500 dilutions and those in 1/100 and 1/200 dilutions of the uninoculated medium showed significantly higher root length over the water control, indicating the beneficial effect of these treatments.

The root length obtained by treating the seeds with 1/100 dilution of the culture filtrate in inoculated soil was 127.2 mm and 58.7 mm for the uninoculated medium control. In the uninoculated soil the root length obtained by 1/100 dilution of the culture filtrate was 173.1 mm and 174.2 mm for the uninoculated medium control. This means that the inhibition in the root length of seedlings from the uninoculated medium-treated seeds was 115.5 mm i.e. 66.3 percent, whereas in the seeds treated with the culture filtrate it was 45.9 mm i.e. 26.5 percent. Thus, presoaking the seeds in 1/100 dilution of the culture filtrate, though did not impart complete immunity against the pathogen, afforded protection to the extent of 40 percent.

Discussion

Distinct stimulation in root elongation was observed when seedlings were grown in water from seeds which were presoaked in dilutions of 1/50 to 1/500 of the culture filtrate. This effect was not seen when such seeds were grown in uninoculated soil. However, seeds presoaked in the culture filtrate at dilution of 1/100 and sown in inoculated soil showed root length about 40 percent greater than that of seeds presoaked in corresponding dilution of the uninoculated medium under similar conditions. The failure to obtain any stimulation in root elongation by the culture filtrate in the uninoculated soil appears to be a logical consequence of the normal uptake of nutrients by the plant from the soil without any physiological disturbances. On the other hand, in the inoculated soil, the toxin of the pathogen which is expected to exert a detrimental effect on the metabolism of the plant was counteracted to a significant extent by the culture filtrate absorbed in the seeds. The inability of lower dilutions of the culture filtrate to impart any such effect could be due to intake of greater quantities of the toxic principle itself into the seeds, thus counteracting the beneficial effects of the higher dilutions.

Prasad[6] has shown that pathogenic isolates of *H. sativum* differed in their ability to elaborated the toxic principle in culture filtrate. Further the medium and the temperature also determined its concentration. Hence the most effective dilution of the filtrate to obtain the desired protection against the pathogen would vary with the isolates of the fungus, the substrate, temperature and duration of growth.

These findings lend support to the earlier observations of Zoja[12] and Leeman[3], but differ from that of the latter in that the 14 days gap between soaking the seeds in the culture filtrate and testing them in the soil was not necessary. The gap period in this experiment was less than 48 hours during which the seeds had dried up before being sown in the inoculated soil.

The mechanisms of stimulation and protection offer interesting avenues for future investigation. The protective effects are important from the applied angle and should be examined in areas endemic to foot rot of wheat incited by *H. sativum*.

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和文摘要

Helminthosporium sativum の培養ろ液がコムギ苗に及ぼす刺激的，保護的効果

A. M. Das and D. N. Srivastava

根の伸長を指標として，*Helminthosporium sativum* の培養ろ液がコムギ苗に及ぼす刺激的，保護的効果を検討した。種子を本菌のジャガイモ煎汁培養ろ液，または本菌を培養した土壌・トウモロコシ粉培地の浸出液の 50–500 培希釈液中に24時間浸漬したのち，水中で発芽させると，根長は明らかに対照区よりもすぐれていた。

種子を培養ろ液の 100–500 倍希釈液中に浸漬したのち，病土に播種して発育させた場合にも，根の伸長は明らかに良好であったが，100 倍希釈液処理がもっとも良好で，対照区の 40% 増であった。これは，培養ろ液が病原菌の侵害から苗を保護したものと解釈される。