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稻馬鹿苗菌の分泌する生長促進物の性質に就きて

伊 藤 誠 哉

島 田 昌 一

ON THE NATURE OF THE GROWTH PROMOTING SUBSTANCE EXCRETED BY THE “BAKANAE” FUNGUS

By

Seiya ITO and Shoichi SHIMADA.

In 1926, E. KUROSAWA (2) published an interesting paper on the excreting substance of the causal fungus of the “*Bakanae*” which is a serious and widely distributed disease of the rice-plant in Japan. He reported that the excretion is a kind of toxic substance which is responsible for the characteristic symptom of the overgrowth of the affected rice-seedlings, and he also proved that the substance is remarkably resistant to high temperature and its deleterious action in the filtrate of the cultured solution of this fungus could be retained even after the sterilization at 100°C. for 4 hours. Since that time, T. HEMMI (1), F. SETO (1, 3, 4), and T. TAKAHASHI (5) also ascertained the characteristic effects of the filtrate on the rice-seedlings, but these authors did not refer to the intimate nature of the substance.

As the nature of the substance which is responsible for the overgrowth of the rice-seedling is the chief point of interest practically in the studies of this disease as well as of the staling of the fungus-cultures in general, the present study was undertaken to solve the problem what the stimulating substance really is.

METHODS AND MATERIALS

The original culture of the "*Bakanae*"-fungus was kindly given by J. KIMURA, who had isolated it from the diseased seedling collected in the farm of the Hokkaido Agricultural Experiment Station. As the standard nutrient solution of the fungus, a modified RICHARDS' solution of the following formula was used:

Potassium nitrate	10g.
Potassium biphosphate	5g.
Magnesium sulphate	2.5g.
Cane sugar	15g.
Ferric chloride	trace
Distilled water	1000c c.

Fifty c.c. portion of the solution were placed in ERLLENMEYER's flasks of 200c.c. capacity. After the sterilization in a KOCH's steam sterilizer, they were inoculated with the fungus, and incubated in a thermostat at 26°-28°C., during the varying length of period in different experiments. At the end of the period, the cultured solution was diluted with the KNOP's solution and then filtered through the sterilized asbestos-filter by the aid of a suction-pump. 5 c.c. of the solution were poured into the sterilized test-tube (16 × 1.5cm.) in which a funnel-shaped filter paper with a central hole prepared for the free development of the roots of the rice-seedling was placed. The-seeds of rice used in the experiments were taken from a pure line of

the variety "Bozu", which was cultivated in the Hokkaido Agricultural Experiment Station. The seeds were germinated aseptically in the Petri-dishes placed in a thermostat (26°-28°C.) or in the laboratory-room (15°-20°C.). When these seedlings attained to the length of about 2-3 cm., each one of them was transferred on the filter paper in the test-tube above stated. The plants in each series of the experiments were about 15 in number. The test-tubes thus prepared were placed in a green house. At the end of each experiment the length of the shoots and roots of the seedlings grown aseptically were measured.

EXPERIMENTAL RESULTS

1. Effects of Various Concentrations of the Filtrate

Several solutions with the different concentration of the fungus-filtrate were prepared by adding the KNOR's solution in the various amounts. The filtrates used in the present experiment were obtained from 26 days old cultures of the fungus. The experiment was carried out during Sept. 19-26, 1929. The results are shown in the following table.

Table 1.- Showing the effects of varying concentrations of the fungus-filtrate on the growth of rice-seedlings.

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Cont. m.*10%	13	7.44	8.5	5.7	5.85	8.3	3.8	Green
" 5%	10	7.95	10.8	6.0	5.33	7.3	3.0	Green
" 1%	12	7.17	10.8	4.6	5.27	7.6	3.8	Green
" 0.5%	8	7.26	9.5	5.4	4.91	6.5	2.8	Green

Filtrate,* 10%	17	8.52	10.7	7.0	Immeasurable	—	—	Pale green
" 5%	11	9.77	14.0	8.5	2.38	4.1	1.6	Pale green
" 1%	11	11.86	13.6	8.6	7.15	8.9	4.2	Green
" 0.5%	12	9.21	12.7	7.3	6.75	8.4	4.3	Green
" 0.1%	13	8.29	10.0	8.2	5.79	7.3	3.4	Green

*Control medium is prepared by the addition of the RICHARDS' solution in given percentages to the KNOPS' solution.

Cont. m. 10% = KNOPS' solution 90% + RICHARDS' solution 10%

Filtrate 10% = KNOPS' solution 90% + Cultured solution of the fungus 10%.

From the above table we can notice that the overgrowth of the rice-seedlings was the most remarkable in the solution containing the filtrate at the rate of 1 per cent. In this series, the effect appeared not only on the leaves, but also on the roots. In the early part of the experiment the shoots in the 10% filtrate series grew up more rapidly than the others, but their growth-rate diminished gradually. Paleness of the aerial parts of the seedlings did not correlate exactly with the overgrowth.

In general, the optimum concentration of the fungus-filtrate causing the "*Bakanae*" symptom of the rice-seedlings seems to lie between 10 to 1%. Accordingly, the following experiments were performed with the solutions in these concentrations.

2. Comparison of the Effects of the Filtrates obtained

from the Cultures of the Fungus in Question and

Fusarium Lini

In order to make clear whether the stimulating action of the filtrate is or not a common nature of the genus *Fusarium*, to which the causal fungus of the "*Bakanae*" belongs, *Fusarium Lini*, a well known species of the genus, was selected as a comparing material.

Filtrates used were obtained from 35 days old cultures of both fungi. The experiment was done during Sept. 28-Oct. 5, 1929. The results are shown in the following table:

Table 2.- Showing the effects of the filtrates obtained from the cultures of the "Bakanae" fungus and *Fusarium Lini*.

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Cont. m. 10%	12	10.88	13.8	7.4	7.04	8.8	4.6	Green
" 1%	9	10.9	12.1	10.0	6.11	9.7	4.0	Green
Filtrate, 10%	13	12.95	15.0	10.2	Immeasurable	—	—	Pale green
" 1%	8	11.3	14.5	7.6	6.81	9.2	5.1	Green
Fil.(F.Lini), 10%	11	7.52	9.3	4.9	3.76	7.5	1.6	Pale green
" 1%	12	9.08	13.0	6.4	5.43	8.7	2.5	Green

The filtrate of the culture of *Fusarium Lini* did not cause any overgrowth of the aerial part of the rice-seedlings, but rather checked its growth. The color of the leaves was somewhat paler than that of the "Bakanae", and the leaves never showed such a slender appearance as in the latter. From these results, we can conclude that the substance inducing the "Bakanae" symptom is not common to the fungus-filtrates of *Fusarium*-species, at least to *F. Lini*.

3. Effects of Boiling and Incinerating on the Deleterious Action of the Fungus-Filtrate

The present series of the experiments with boiled and incinerated filtrates was conducted to prove, whether the substance inducing the "Bakanae" symptom is thermostable or not. Using the cultured solution free from the

mycelium, the following 4 cultural media together with the check medium of the rice-seedlings were prepared.

Medium No. 1. Culture solution itself.

Medium No. 2. The fungus-filtrate without any treatment.

Medium No. 3. The fungus-filtrate was boiled and reduced to about half a bulk, then added distilled water to the original volume.

Medium No. 4. The filtrate was boiled in an evaporating dish till it almost dried up, then added distilled water to the original volume.

Medium No. 5. Ashes of the filtrate were made in a crucible, then added distilled water to the original volume.

The filtrates used were obtained from 43 days old cultures. The experiment was done during Oct. 6-13, 1929. The results are shown in the following table.

Table 3.- *Showing the effects of boiled and ashed filtrates on the growth of rice-seedlings.*

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Med. No. 1, 10%	4	9.15	10.7	7.7	5.25	7.5	3.5	Green
„ 1%	7	9.39	13.0	7.5	4.16	6.3	2.2	Green
Med. No. 2, 10%	6	15.55	19.0	12.0	Immeasurable	—	—	Pale green
„ 1%	11	13.44	16.6	11.0	7.8	9.8	6.2	Green
Med. No. 3, 10%	4	14.58	16.5	10.0	2.55	3.0	2.0	Pale green
„ 1%	2	12.25	14.5	10.0	3.55	9.4	7.7	Green
Med. No. 4, 10%	9	13.41	18.0	8.5	3.04	4.2	2.3	Pale green
„ 1%	9	11.23	14.0	8.1	7.51	11.0	3.0	Green
Med. No. 5, 10%	6	9.68	14.5	6.3	6.48	10.2	2.5	Green
„ 1%	6	7.72	10.4	6.3	5.25	7.2	2.0	Green

The volatile substances from the boiling filtrate were then gathered and tested.

Medium No. 6. The known amount of the filtrate was boiled for about 30 minutes, and the gas from it was gathered in the equal amount of distilled water.

The filtrate used was obtained from 156 days old culture.

The experiment was done during March 20-26, 1930. The results are shown in the following table.

Table 4.- Showing the effects of the volatile substances of the filtrate on the growth of rice-seedlings

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Med. No. 1, 10%	11	12.4	14.3	9.5	7.55	9.0	6.5	Green
„ 1%	8	10.9	15.0	7.5	7.14	9.4	5.4	Green
Med. No. 2, 10%	9	14.47	18.0	10.0	Immeasurable	—	—	Pale green
„ 1%	8	14.5	17.3	6.8	7.4	10.5	6.2	Green
Med. No. 6, 10%	10	12.7	11.5	8.8	9.18	11.6	6.1	Green
„ 1%	11	13.15	15.1	11.0	8.24	10.2	5.8	Green

In the media Nos. 2, 3, and 4, the leaves of the rice-seedlings became slender, showing a characteristic feature of the “*Bakanae*”. In the medium No. 6, the overgrowth did not occur.

From the results of the present experiment, we can understand that the overgrowth of the leaves of the rice-seedlings may be caused by some thermostable substance, neither enzymic nor volatile. In the case of the medium No. 5, however, the overgrowth of the rice-seedlings was not recognized at all, and it means clearly that the toxic substance was completely destroyed by reducing the filtrate to ashes.

4. Effects of the Animal Black Treatment on the Toxic Activity of the Fungus-Filtrate

In this experiment, MERCK'S animal black was used to adsorb any of toxic substances in the filtrate and the restoration of the hydrogen-ion concentration of the filtrate was done to exclude the influence of reaction-change.

The filtrate used was prepared as in the preceding experiment. The effects of the following series of solutions upon the growth of the rice-seedlings were examined.

Medium No. 1. Culture solution itself.

Medium No. 2. The fungus-filtrate without any treatment.

Medium No. 3. The control medium (30c.c.) treated with 0.5g. of MERCK'S animal black for one day.

Medium No. 4. The fungus-filtrate treated as in the medium No. 3.

Medium No. 5. The fungus-filtrate regulated the hydrogen-ion concentration to that of the control medium with an addition of 2% HCl.

The filtrate used were obtained from 54 days old cultures. The experiment was done during Oct. 18-23, 1929. The results are shown in the following table.

Table 5.- Showing the effects of the filtrates treated with animal black on the growth of rice-seedlings.

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Med. No. 1, 10%	10	10.74	14.5	6.2	4.57	6.3	3.3	Green
„ 1%	8	9.95	15.0	7.0	4.85	7.6	2.3	Green

Med. No. 2, 10%	4	15.58	18.5	13.0	4.48	5.2	3.7	Pale green
„ 1%	5	12.32	16.2	8.5	8.46	10.9	6.8	Green
Med. No. 3, 10%	9	10.86	13.3	7.2	6.1	9.0	4.0	Green
„ 1%	6	8.2	11.3	6.3	4.73	8.2	2.0	Green
Med. No. 4, 10%	6	10.67	13.5	6.2	5.78	7.2	2.7	Green
„ 1%	3	8.1	11.6	6.3	5.07	9.4	2.0	Green
Med. No. 5, 10%	8	14.48	19.0	11.4	3.78	6.7	2.0	Pale green
„ 1%	5	11.74	16.4	9.5	7.8	11.2	4.8	Green

It is clear that the filtrate treated with animal black did not act on the growth of the rice-seedlings. The toxic substances in the filtrate, at least those concerning the overgrowth of the seedlings, were adsorbed completely by animal black. The restoration of the hydrogen-ion concentration had no effect on the toxic activity of the fungus-filtrate.

5. Effects of the Dialyse on the Toxic Activity of the Fungus-Filtrate

The experiment was done in order to determine whether the substance in problem can dialyse or not. As a semipermeable membrane a bladder-membrane of pig was used, and the filtrate was dialysed for about 20 hours in distilled water of the same amount to the filtrate.

The filtrates used were obtained from 47 days old cultures. The experiment was done during May 15-22, 1930. The results are shown in the following table.

Table 6.- Showing the effects of the dialysed filtrates on the growth of rice-seedlings.

Solution used		Number of plants tested	Length of shoots (in cm)			Length of roots (in cm.)			Color of Shoots
			Aver.	Max.	Min.	Aver.	Max.	Min.	
Cont. m.	10%	3	13.17	14.6	10.7	8.0	8.7	7.3	Green
"	1%	4	9.55	11.2	8.0	5.95	9.3	4.5	Green
Filtrate,	10%	6	17.97	22.0	12.5	5.95	8.2	4.5	Pale green
"	1%	5	10.62	12.0	10.0	6.84	9.7	5.0	Green
Fil. (Dialysed),	10%	3	17.33	19.5	13.5	7.23	8.2	5.5	Green
"	1%	4	11.98	12.5	11.3	8.25	9.2	7.0	Green

From the result obtained, it was proved that the substance in problem is a well dialyzable matter. In the case of the dialysed filtrate, the color of shoots is not so pale as in the series containing the untreated fungus-filtrate. This is probably due to the dilution in the course of dialysis.

6. Effects of the Fungus-Filtrates on the Growth of Rice-seedlings, when some Constituents of the RICHARDS' solution were omitted and the Fungus was cultured in them

The following cultural series for the fungus was set up by the omission of the constituents of the normal RICHARDS' solution.

Medium No. 1. Normal RICHARDS' solution.

Medium No. 2. RICHARDS' solution without KNO_3 .

Medium No. 3. RICHARDS' solution without KH_2PO_4 .

Medium No. 4. RICHARDS' solution without MgSO_4 .

Medium No. 5. RICHARDS' solution without cane sugar.

At the end of the cultural periods, each cultural solution was filtered,

and at the same time the growth of the fungus, the change of the H-ion concentration and the appearances of the culture solution were observed.

Experiment 1.

Cultural period.....33 days

Table 7.- Showing the results of culture of the fungus in the modified Richards' solution.

Media	Development of mycelium	Development of aerial mycelium	Color of the cultured media	PH	
				Initial	Final
No. 1	++++	++	Amber Yellow	4.5	6.4
No. 2	+	—	Hyaline	4.5	4.5
No. 3	+	—	Mustard Yellow	4.5	6.4
No. 4	+	±	Massicot Yellow	4.5	—
No. 5	±	—	Hyaline	4.5	4.6

The experiment on the rice-seedlings with the fungus-filtrate of each culture was done during Nov. 17-23, 1929. The results are shown in the following table.

Table 8.- Showing the effects of the fungus-filtrates obtained from the cultures of the fungus in the variously modified Richards' solutions on the growth of rice-seedlings.

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Med.No.1,Cont. 10%	5	13.78	15.8	12.2	7.02	9.8	4.7	Green
„ 1%	4	13.78	16.4	10.6	7.7	8.6	6.4	Green
Med.No.2,Cont. 10%	11	12.36	15.2	8.8	6.31	8.4	4.2	Green
„ 1%	2	12.75	13.8	11.7	6.65	10.1	3.2	Green
Med.No.3,Cont. 10%	1	14.9	—	—	5.9	—	—	Green
„ 1%	7	13.53	16.2	11.7	6.71	9.2	5.2	Green

Med.No.4, Cont. 10%	9	15.12	17.6	12.8	8.54	11.5	4.7	Green
„ 1%	4	14.2	16.0	12.9	6.28	6.8	5.6	Green
Med.No.5, Cont. 10%	9	10.18	12.8	7.0	3.87	5.3	2.0	Green
„ 1%	2	13.5	13.7	13.3	5.05	5.3	4.8	Green
Med. No.1, Fil. 10%	7	18.24	20.3	16.0	4.5	5.8	3.2	Pale green
„ 1%	9	15.93	20.8	12.2	7.21	11.7	5.8	Green
Ned. No.2, Fil. 10%	11	13.07	15.4	10.8	6.22	11.9	4.8	Green
„ 1%	5	13.22	14.7	10.8	6.3	7.3	5.3	Green
Med. No.3, Fil. 10%	3	5.0	5.6	3.9	Immea- surable	—	—	Pale green
„ 1%	0	—	—	—	—	—	—	—
Med. No.4, Fil. 10%	3	6.13	6.7	5.0	Immea- surable	—	—	Pale green
„ 1%	6	7.85	10.7	5.6	Immea- surable	—	—	Pale green
Med. No.5, Fil. 10%	5	12.12	14.4	9.0	5.43	6.4	4.5	Green
„ 1%	7	12.72	15.3	11.0	6.0	8.9	4.3	Green

Experiment 2.

Cultural period.....156 days

Table 9.- Showing the results of the culture of the fungus in the modified Richards' solutions.

Media	Development of mycelium	Development of aerial mycelium	Color of the cultured media	PH	
				Initial	Final
No. 1	++++	++	Barium Yellow	4.5	6.8
No. 2	+	±	Pale Ochraceous- Buff	4.5	4.4
No. 3	+	—	Mustard Yellow	4.5	6.4
No. 4	++	+	Raw Seinna	4.5	8.0
No. 5	±	—	Hyaline	4.5	4.6

The experiment on the rice-seedlings with the filtrate of each culture was done during March 20-26, 1930. The results are shown in the following table.

Table 10.- Showing the effects of the filtrates obtained from the cultures of the fungus in the variously modified Richards' solutions on the growth of rice-seedlings.

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Med No.1, Cont. 10%	11	12.4	14.2	9.5	7.55	9.0	6.5	Green
„ 1%	8	10.9	15.0	7.5	7.14	9.4	5.4	Green
Med. No.1, Fil. 10%	9	14.47	18.0	10.0	Immeasurable	—	—	Pale green
„ 1%	8	14.5	17.3	6.8	7.4	10.5	6.2	Green
Med. No.2, Fil. 10%	12	10.53	14.2	7.7	Poor growth	—	—	Pale green
„ 1%	11	12.76	15.2	9.3	6.57	9.7	4.3	Green
Med. No.3, Fil. 10%	5	Immeasurable	—	—	Immeasurable	—	—	—
„ 1%	11	4.25	5.5	2.2	Immeasurable	—	—	Pale green
Med. No.4, Fil. 10%	7	4.37	6.1	2.8	Immeasurable	—	—	Pale green
„ 1%	12	5.93	9.0	3.1	Immeasurable	—	—	Pale green
Med. No.5, Fil. 10%	13	11.71	13.2	9.2	7.52	8.2	6.0	Green
„ 1%	11	12.3	14.3	8.5	7.79	9.1	6.5	Green

It is interesting to notice that the remarkable contrasts in the results took place between the medium No. 1 and No. 3 or No. 4. In Nos. 3 and 4, the filtrate caused neither overgrowth nor slender appearance of the seedlings, although the normal growth of the shoots and roots was greatly checked in these solutions. The solutions containing the filtrates of Nos. 2 and 5 did not cause so distinct an overgrowth as in the medium No. 1. It is probably due to the poor development of the fungus in the culture media No. 2 and

No. 5.

Reviewing the results of the foregoing experiments, the following cultural experiment was conducted supplementally.

Medium No. 6. RICHARDS' solution without KH_2PO_4 and MgSO_4 .

Experiment 3.

Cultural period..... 47 days

Table 11.- Showing the results of the culture of the fungus in the modified Richards' solution.

Media	Development of mycelium	Development of aerial mycelium	Color of the cultured media	PH	
				Initial	Final
No. 1	++++	++	Naphthalene Yellow	—	6.8
No. 6	+	—	Pale Yellow	—	7.0

The experiment on the rice-seedlings with the filtrate of the culture was done during May 15-22, 1930. The results are shown in the following table.

Table 12.- Showing the effects of the filtrates obtained from the cultures of the fungus in the modified Richards' solution on the growth of rice-seedlings.

Solution used	Number of plants tested	Length of shoots (in cm.)			Length of roots (in cm.)			Color of shoots
		Aver.	Max.	Min.	Aver.	Max.	Min.	
Med No.1, Cont. 10%	3	13.17	14.6	10.7	8.0	8.7	7.3	Green
„ 1%	4	9.55	11.2	8.0	5.95	9.3	4.5	Green
Med. No.1, Fil. 10%	6	17.97	22.0	12.5	5.95	8.2	4.5	Pale green
„ 1%	5	10.62	12.0	10.0	6.4	9.7	5.0	Green
Med.No.6, Cont. 10%	3	8.13	9.0	7.7	5.03	7.4	3.5	Green
„ 1%	4	9.6	10.4	8.5	4.98	5.8	3.3	Green
Med. No.6, Fil. 10%	4	3.68	4.0	3.0	Immeasurable	—	—	Pale green
„ 1%	5	5.12	9.0	3.1	Immeasurable	—	—	Pale green

As shown in the above table, the growth of the rice-seedlings was greatly checked in the filtrate of the medium No. 6. Accordingly, we came to the conclusion that the KH_2PO_4 and MgSO_4 play an important rôle for the production of the substance in problem in the metabolism of the causal fungus of the "*Bakanae*".

SUMMARY

1. Hoping that the experimental results may aid the solution of the problem as to what the stimulating substance in the filtrate of the cultured solution of the "*Bakanae*"-fungus really is, the experiments were conducted from six different phases.
2. The optimum concentration of the filtrate resulting the "*Bakanae*" symptom seems to be between 10 and 1%.
3. The function producing the symptom is not common to the species of *Fusarium*, at least to *Fusarium Lini*.
4. The growth promoting substance is thermostable, neither enzymic nor volatile.
5. The substance is adsorbed completely by animal black.
6. The substance is diffusible through the semipermeable membrane.
7. By the difference of the cultural solution of the fungus, the filtrate does not cause the "*Bakanae*" symptom, for example the filtrates obtained from the cultures in the RICHARDS' solution omitted KH_2PO_4 or MgSO_4 or both of them do not cause any overgrowth of the rice-seedlings but checked its normal growth.

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EXPLANATION OF PLATE

Fig. 1. Rice-seedlings treated with the fungus-filtrates boiled or incinerated.

- A. Seedlings cultured in the KNOP'S solution with 10% sterile cultural medium. (Control)
- B. Seedlings cultured in the KNOP'S solution with 10% incinerated filtrate.
- C. Seedlings cultured in the KNOP'S solution with 10% heated and dried filtrate.
- D. Seedlings cultured in the KNOP'S solution with 10% boiled filtrate.
- E. Seedlings cultured in the KNOP'S solution with 10% untreated filtrate.

Fig. 2. Rice-seedlings cultured in the KNOP'S solution with the fungus filtrate treated with animal black.

- A. Seedlings cultured in the KNOP'S solution with 10% sterile culture medium. (Control)
- B. Seedlings cultured in the KNOP'S solution with 10% sterile culture medium treated with animal black.
- C. Seedlings cultured in the KNOP'S solution with 10% filtrate treated with animal black.
- D. Seedlings cultured in the KNOP'S solution with 10% filtrate regulated PH-value to that of control medium.
- E. Seedlings cultured in the KNOP'S solution with 10% untreated filtrate.

Plate.

Fig. 1.

Fig. 2.

摘 要

稻馬鹿苗病の特有病徴たる徒長現象が其の病原菌の培養濾液中に含まるゝ毒素に關係ある事は既に證明せられたる處なるも、その毒素の性質に就きては熱に對する影響を調査せる黒澤氏の報告あるに過ぎず。

本實驗は馬鹿苗病徴に關係ある一種の刺戟的物質即馬鹿苗病徴發源素の性質につきて行ひたるものなり。本實驗に於て使用せる菌の培養液はリチャーズ氏液にして、その濾液に對し一定の處理を施せる後、これにクノツブ氏液を加入して一定の濃度に稀釋し、該液を用ひて稻苗を培養し、生育狀態を調査し其の影響の如何によりて該物質の性質を考察せり。斯くして得たる結果を要約せば次の如し。

1. 馬鹿苗病徴を生ぜしむべき培養濾液の最適濃度は 10% 或は 1% の中間にあり。
2. 他のフザリウム菌少くとも亞麻の立枯病菌たる *Fusarium Lini* には如斯物質を分泌する性質なし。
3. 該物質は熱に對し頗る安定にして濾液を沸騰せしめ蒸發乾固に致らしむるも尙その特性を現はすも、灰となす時は分解せられて最早馬鹿苗病徴を起さしめず。
4. 該物質はメルク製獸炭によりて完全に吸着せらる。
5. 該物質は半透膜、例へば豚の膀胱膜を通過す。
6. 該物質の病原菌による分泌はその培養液の成分により影響を受くる事大にして、リチャーズ氏液の場合には酸性磷酸加里或は硫酸マグネシウムの孰れか一方或は兩者共存せざる場合には、其の培養濾液は馬鹿苗病徴を生ぜしめず却つて稻の生長を抑制す。