稻馬鹿苗菌の分泌する生長促進物の 性質に就きて (第二報)

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FURTHER STUDIES ON THE NATURE OF THE GROWTH PROMOTING SUBSTANCE EXCRETED BY THE "BAKANAE" FUNGUS

By

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Many phytopathogenic organisms are known to excrete some toxic substance which are responsible for the symptoms of the disease. This fact was well demonstrated by HUTCHINSON (5) and JOHNSON and MURWIN (7) in bacterial diseases, and by BRANDES (1), BROOKS and BRENCHLEY (2), HAYMAKER (3), NEAL (9), ROSEN (11) and WHITE (15) in fungus diseases. While such toxic substances mostly cause the hindrance of the normal growth of the host plant, the toxin excreted by the "*Bakanae*" fungus (*Gibberella Fujikuroi*) results in the overgrowth of the seedlings of *Oryza sativa* (4, 6, 8, 12, 13 & 14). Since the writer with Prof. S. ITO (6) reported first on the nature of the growth promoting substance in question, further studies on it, especially on its extraction with some solvents and its effect on the growth of some other plants, not the proper host in nature, were carried out.

The experiment was conducted under the direction of Prof. S. ITO, to whom I am greatly indebted for his valuable suggestions and encouragement. I am also indebted to Profs. Y. TOCHINAI and T. FUKUSHI for their kind advices throughout the work. Thanks are also due to the members of the staff of the Phytopathological laboratory.

I. EXPERIMENTS ON THE SEPARATION OF GROWTH PROMOTING SUBSTANCE

In the first step of the determination of the chemical nature of the toxic substance, it is of prime importance to separate the substance in pure state. For this purpose, separation experiments were carried out with several sol-

vents. Though the real chemical nature is not yet determined, the results of the experiments will be stated.

The materials used in the experiments are the same as those of the previous experiments (6). The fungus was cultured in the ERLHENMYER'S flasks of 200 c.c. capacity, containing 100 c.c. of a modified RICHARD'S solution (6), and the cultured solution was collected through a sterilized asbestos filter after the desired duration of culture. Then the filtrate was treated in various ways. After treatment, the substance in question was extracted using the following solvents; 95% alcohol, ether, acetone, distilled water and chloroform, and after the solvent was evaporated, the remainder substance was dissolved in distilled water with KNOP'S solution. The solution thus obtained was used as the nutrient medium of rice-seedlings. The seedlings were obtained by sowing sterilized seeds in sand giving KNOP'S solution. When the seedlings have grown to about 5 cm. in height, three of them were transferred to each ERLHENMYER'S flask of 200 c.c. capacity, holding them by the aid of cotton at the mouth. At the end of the experiment, length of the shoots and roots as well as dry weight of the shoots were measured to estimate the effect of the substance under consideration which was melted in the nutrient solution. The experiments were carried out in duplicate or triplicate.

Experiment 1. In this experiment, the filtrate was obtained from 48 days' culture, and 35 c.c. portions of the filtrate were dried up with gentle heat. 35 c.c. of each solvent above mentioned were added to the residue and retained for about 20 hours at room temperature. The residue was all dissolved in distilled water, while some of the residue was left on the bottom of the vessel in the cases of other solvents. The extracted solution was light yellowish in color in alcohol, acetone and chloroform, brown color in distilled water, and hyaline in ether. After the residual substance was omitted by decantation, the solution was heated on a water bath as to drive off the solvent by evaporation. The substance thus obtained was sticky, yellowish in color in the cases of alcohol, acetone, chloroform and distilled water, while hyaline in ether. Additionally, the same evaporation and extraction methods were applied to the RICHARDS' solution for the further use as the control. After the evaporation of each solvent the extracted substance was dissolved in 35 c.c. of distilled water with 500 c.c. of KNOP'S solution, and the effect on the growth of rice seedlings was tested. The experiment.

was carried out between Jan. 9-19, 1931. The results are shown in the following table. In the table, the term "residue" denotes the undissolved substance remaining on the bottom of the vessel when the solvent was applied, and "extract" denotes the substance extracted by the solvent.

Table 1 : - *Effect of the substance extracted from the filtrate on the growth of rice seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
H ₂ O ext. of Cont. M.	6	15.38	17.2	9.8	11.58	0.07
H ₂ O ext. of filt.	6	19.37	29.8	17.6	9.12	0.064
Alcohol ext. of Cont. M.	6	13.95	15.7	10.8	7.73	0.066
Alcohol ext. of filt.	6	21.47	23.7	18.8	9.72	0.06
Alcohol res. of Cont. M.	6	16.03	18.7	14.5	7.17	0.072
Alcohol res. of filt.	6	11.92	15.6	6.8	8.68	0.061
Ether ext. of Cont. M.	6	13.38	16.0	11.5	13.0	0.068
Ether ext. of filt.	6	17.38	20.4	13.4	8.97	0.068
Ether res. of Cont. M.	6	10.88	13.0	9.6	8.43	0.056
Ether res. of filt.	6	14.22	16.2	13.0	8.8	0.07
Aceton ext. of Cont. M.	6	14.38	16.0	13.2	12.18	0.072
Aceton ext. of filt.	6	19.58	24.2	11.5	9.35	0.066
Aceton res. of Cont. M.	3	15.3	15.5	15.2	6.57	0.034
Aceton res. of filt.	5	14.68	19.2	11.8	8.96	0.048
Chlorof. ext. of Cont. M.	6	13.83	17.0	11.5	11.88	0.066
Chlorof. ext. of filt.	6	16.68	18.4	15.3	9.62	0.053
Chlorof. res. of Cont. M.	5	11.36	12.6	9.3	9.4	0.044
Chlorof. res. of filt.	6	17.38	20.5	11.6	10.6	0.073

As shown in the above table, the substance extracted from the fungus filtrate caused the overgrowth of rice seedlings. The residual substance did not cause any overgrowth in any cases except in that from chloroform. Even in the latter case, if the treatment would be repeated several times, the substance in it might be thoroughly extracted.

PRATT (10) called attention in the extraction experiment to the fact that the solvents might induce some disturbance on the real effect of the substance, but in our case we could not recognize it, comparing with the results of

control.

The extraction with glacial acetic acid was also done as in the above experiment. In this case, neither the residue nor the extract caused any overgrowth.

Experiment 2. In the present experiment, the filtrate was obtained from 52 days' culture, and 60 c. c. portions of the filtrate were treated for one day with 1 gram of animal black. After the animal black was collected and dried on the filter paper, it was dipped in 35 c. c. of alcohol for one day together with filter paper. Through a filter paper, the filtrate was collected and evaporated to drive away alcohol. The residue thus obtained was dissolved in 50 c. c. of distilled water with 500 c. c. of KNOP'S solution and the effect on rice seedlings tested. The experiment was carried out between Feb. 13-19, 1931. The results are shown in the following table.

Table 2 : - *Effect of the substance extracted with alcohol from the adsorbed animal black on the growth of rice seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of shoots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP'S solution	9	14.09	16.0	11.5	10.31	0.118
Alcohol ext. of filt.	6	15.38	17.3	12.0	5.18	0.048

The extraction experiment from the adsorbed animal black was also conducted with distilled water, ether and acetone in the place of alcohol. The filtrate used was obtained from 14 days' culture, and the extracts of RICHARD'S solution were also used as the control. The experiment was carried out during the period Feb. 27-Mar. 4, 1931. The results are shown in the following table.

Table 3 : - *Effects of the substance extracted with water, ether and acetone from the adsorbed animal black on the growth of rice seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP'S solution	6	15.18	16.9	12.4	13.18	0.069
H ₂ O ext. of Cont. M.	6	16.95	18.6	15.6	12.78	0.091
H ₂ O ext. of filt.	6	15.62	16.6	14.6	12.65	0.083

Ether ext. of Cont. M.	6	14.8	16.2	12.3	11.3	0.078
Ether ext. of filt.	6	18.05	19.5	15.2	9.92	0.082
Aceton ext. of Cont. M.	6	15.98	16.5	15.5	12.83	0.093
Aceton ext. of filt.	6	18.37	19.7	16.9	9.3	0.059

As shown in the above tables, the seedlings cultured in the solution containing the substances extracted with alcohol, ether and acetone were taller than those cultured in the solution which contained the extract of the control medium. Accordingly, we can conclude that the substance adsorbed to the animal black can be extracted with alcohol, ether and acetone, but not with distilled water.

Experiment 3. In this experiment, the filtrate was obtained from 27 days' culture, and each 94 c.c. portion of the filtrate was dialysed with collodion membrane in 50 c.c. of distilled water containing 1 g. of animal black. After four days, the animal black was collected on filter paper, and washed with distilled water for one day to separate some impurity, then dried up in a drying-oven. The extraction with solvents was repeated three times with 20 c.c. of them for each one day. After evaporation of the applied solvent, the residue was dissolved in 50 c.c. of distilled water with 500 c.c. of KNOP'S solution and the effect on the growth of rice seedlings tested. The experiment was done during the period March 4-11, 1931. The results are shown in the following table.

Table 4 : - *Effects of the substance extracted from the animal black added to dialysed filtrate on the growth of rice seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP'S solution	9	12.73	15.4	11.0	9.61	0.136
Alcohol ext. of filt.	6	16.37	18.0	13.9	5.62	0.067
Ether ext. of filt.	6	18.68	20.7	15.5	9.58	0.076
Aceton ext. of filt.	6	15.5	18.0	12.3	8.12	0.073

As shown in the above table, the results of the experiment were essentially the same as those of tables 2 and 3. The substance in problem was extracted also in this case with alcohol, ether and acetone from the animal black.

From the results of the three experiments above described, we may conclude that the growth promoting substance in the cultured solution of "*Bakanae*" fungus (*Gibberella Fujikuroi*) is present in the solution which was treated successively by dialization, adsorption to animal black and extraction with alcohol, ether and acetone. The chemical nature of the substance in question would be suggested from the fact.

II. ON THE EFFECT OF THE SUBSTANCE IN PROBLEM ON THE GROWTH OF SOME PLANTS

It is an interesting problem to determine whether the growth promoting substance excreted by the "*Bakanae*" fungus affects rice seedlings only or also other plants. In this connection, KUROSAWA (8) reported that the toxic substance excreted by the fungus seems to have a growth promoting effect on Panik grass (*Panicum Crus-galli* L. var. *submuticum* MEY.) and Indian corn (*Zea Mays* L.) as on rice-plant.

The writer also experimented with several plants to solve the question. In the first experiment, the filtrate obtained from the old culture of the fungus was directly mixed with KNOP'S solution and its effect on the growth of the seedlings of Indian corn, wheat, barley and rye was examined. These plants thus treated showed somewhat slender appearance compared with those cultured in the KNOP'S solution, but the real length of their shoots was not so long as in the case of rice-plant. Considering that the experimental method was not properly arranged because the substance in question was not purified, the experiment was abandoned.

In the second experiment, the comparatively purified substance that was extracted with alcohol from animal black which was dipped in the dialysed filtrate, was used. The substance, which was derived from the filtrate obtained from cultured solution in three flasks, was dissolved in 50 c.c. of distilled water with 1000 c.c. of KNOP'S solution.

The methods of culture of the seedlings were just the same as those of the preceding experiments. The plants used were wheat, barley, Indian corn,

Azuki bean and soy bean.

1. Wheat ("*Martin's Amber*")

The experiment was made in duplicate, and 3 seedlings were cultured in each flask. The filtrate was obtained from 74 days' culture. The experiment was carried out between June 16 and 27, 1931. The results are shown in the following table.

Table 5 : - *Effects of the substance extracted from the filtrate on the growth of wheat seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP's solution	5	22.48	26.7	19.6	15.44	0.32
Alcohol ext. of filt.	5	29.84	34.6	26.6	12.42	0.321

2. Barley ("*Chevalier*")

Experiment was made at the same time with the same substance as the preceding. The results are shown in the following table.

Table 6 : - *Effects of the substance extracted from the filtrate on the growth of barley seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP's solution	6	22.1	24.7	20.8	13.22	0.274
Alcohol ext. of filt.	6	28.2	34.0	24.3	13.92	0.358

3. Indian corn ("*Long fellow*")

Two seedlings were cultured in each flask. The substance was extracted from the filtrate of 124 day's culture. Experiment was done during the period June 29-July 7, 1931. The results are shown in the following table.

Table 7 :- *Effects of the substance extracted from the filtrate on the growth of the seedlings of Indian corn*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP's solution	6	27.37	32.2	23.4	10.28	0.652
Alcohol ext. of filt.	6	33.45	36.1	27.0	7.65	0.76

4. Azuki bean ("Maruba")

Experiment was carried out at the same time with the substance as the preceding. The results are shown in the following table.

Table 8 :- *Effects of the substance extracted from the filtrate on the growth of Azuki bean seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of roots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP's solution	6	18.98	21.4	15.7	10.8	0.512
Alcohol ext. of filt.	6	29.23	30.5	27.2	14.9	0.406

5. Soy bean ("Akasaya")

The cotyledons of the seedlings was taken off before transference to the culture flask. When the seedlings provided with cotyledon were tested, the growth promoting effect was not observed, while in Azuki bean, the effect was clearly observed upon seedlings either provided with cotyledon or lacking it. The substance was extracted from the filtrate of 101 days' culture. Experiment was done during the period July 14-21, 1931. The results are shown in the following table.

Table 9 :- *Effects of the substance extracted from the filtrate on the growth of soy bean seedlings*

Solution used	Number of plants tested	Length of shoots (in cm.)			Average length of shoots (in cm.)	Total weight of shoots (in g.)
		Aver.	Max.	Min.		
KNOP's solution	4	14.85	16.4	14.0	11.3	0.27
Alcohol ext. of filt.	4	17.88	20.6	15.0	11.98	0.303

As shown in the above tables, all the plants examined grew taller than in the normal case. Accordingly, we can conclude that the substance in question has the growth promoting effect on the seedlings of some plants, not its proper host in nature. Though the mechanism of the growth promoting nature of the substance could not be explained at present, the results obtained may be interesting from the standpoints of pathology, physiology and chemistry of plants.

SUMMARY

1. Further studies on the growth promoting substance excreted by "*Bakanae*" fungus were carried out in its extractive nature and its effect on some other plants, not rice-plant.

2. The extraction-experiments were made on the following materials; (1) the filtrate which was dried up by gentle heat, (2) the animal black which adsorbed the substance, and (3) the animal black which adsorbed the substance passed through a semipermeable membrane. From the material (1), the substance was extracted by distilled water, alcohol, ether, acetone and chloroform, and from the materials (2) and (3), it can not be extracted by distilled water. As a whole, the growth promoting substance is extractable by some solvents after various treatments, approaching to the relatively pure state.

3. By the action of the alcohol extracted substance from the filtrate, the seedlings of wheat, barley, Indian corn, Azuki bean and soy bean also grew taller than normal.

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EXPLANATION OF PLATE

- A. 1. Wheat seedlings cultured in KNOP's solution containing alcohol extract of the fungus filtrate.
- A. 2. Wheat seedlings cultured in KNOP's solution.
- B. 1. Barley seedlings cultured in KNOP's solution containing alcohol extract of the fungus filtrate.
- B. 2. Barley seedlings cultured in KNOP's solution.
- C. 1. Indian corn seedlings cultured in KNOP's solution containing alcohol extract of the fungus filtrate.
- C. 2. Indian corn seedlings cultured in KNOP's solution.
- D. 1. Azuki bean seedlings cultured in KNOP's solution containing alcohol extract of the fungus filtrate.
- D. 2. Azuki bean seedlings cultured in KNOP's solution.
- E. 1. Soy bean seedlings cultured in KNOP's solution containing alcohol extract of the fungus filtrate.
- E. 2. Soy bean seedlings cultured in KNOP's solution.

摘 要

稻馬鹿苗菌の分泌する特異なる物質、生長促進物の性質に就きて、曩に伊藤教授と共著にて日本植物病理學會々報第2卷第4號に於て發表せり。本報告は夫れに引續き行ひたる實驗結果なり。

該生長促進物の性質を知らんとするには此物質の純粹分離をなすを第一歩とす。依りて種々なる溶劑を用ひ前實驗に於て證明せる性質に基きて、浸出を試みたり。即(1)菌の培養濾液を蒸發乾固せしめたるもの、(2)菌の培養濾液に作用せしめて、該物質を吸着せしめたる獸炭、(3)半透膜を通したる後該物質を吸着せしめたる獸炭、の三種の物質より溶劑としてアルコール、エーテル、アセトン、クロフォルム(1の場合のみ)及び蒸溜水を用ひて浸出せり。その結果(1)の物質よりは此等5種の溶劑により該生長促進物は完全に浸出せられ、(2)の物質よりは蒸溜水により浸出し得ざるも、他の溶劑により浸出せられ、(3)の物質よりはアルコール、エーテル及びアセトンにより浸出し得る事を証し得たり。

次に此物質の生長促進作用が稻のみに止まるや或は他の植物にも影響を及すや否やを調査せり。1926年黒澤氏はノビエ及び玉蜀黍が此の菌の分泌液により伸長を促がされたる事を報告せり。著者も亦菌の培養濾液を用ひ種々の植物に作用せしめたるに、其の生長の度、對照區に比して大差なかりき。然れども此場合培養液中に含まるる種々なる老廢物質の影響をも亦度外視するを得ずして實驗法の不備なるを認め、更に前記の分離法を利用し半透膜を透したる後獸炭に吸着せしめたる物質をアルコールを以つて浸出し、種々なる植物、小麥、大麥、玉蜀黍、小豆及び大豆の苗に作用せしめたるに、何れも生長促進の現象を呈し此の物質の生長促進作用は稻苗のみに限らざることを証し得たり。