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Title	THE AFTER EFFECT OF THE FUNGUS FILTRATE OF GIBBERELLA FUJIKUROI ON RICE PLANTS
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ascis cylindraceis, apice rotundatis, $50-75 \times 10-13 \mu$, octosporis, brevissime stipitatis, filiformiter paraphysatis; sporidiis distichis, ellipsoideis, continuis, hyalinis.

Hab. in foliis *Coptidis trifolii* SALISB. (Mitsuba-wôren). Peak Kuro-dake, Aug. 11, 1928, G. HAYASHI, Aug. 1931, H. ÔTANI & KIKUCHI; Peak Chûbetsu, Aug. 1931, Y. TOCHINAI.

Remarks: The present fungus attacks the old living leaves of *Coptis trifolia*, and kills them. The pitch-black stromata are formed in the middle of a discolored lesion. Each affected leaf has comparatively few stromata scatteredly.

THE AFTER EFFECT OF THE FUNGUS FILTRATE OF GIBBERELLA FUJIKUROI ON RICE PLANTS

BY

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(With 1 text figure)

Since E. KUROSAWA¹⁾ found, in 1926, that the abnormal elongation of rice seedlings which is the characteristic symptom of the "*Bakanae*"-disease is entirely due to the toxic action of the growth promoting substance excreted by the causal fungus (*Gibberella Fujikuroi*), several investigations of the problem from various standpoints have been reported by many authors. But, in general, the studies hitherto published in connection with the toxic substance in question, have been limited to the early stage of the growth of the affected seedlings.

The present studies have been carried out to learn preliminarily the after effects of the toxin produced by the fungus. The rice seedlings were cultivated under the influence of the fungus filtrate, and the affected seedlings were transplanted at certain intervals in the nutrient solution which contains no fungus excretion. In continuing the sand culture with normal nutrient solution it is examined whether or not the influence of the toxin which worked at the early stage of the development of seedlings affects their further growth.

No. 36 and No. 42, two of the forty seven strains of the "*Bakanae*"-fungus

1) E. KUROSAWA:—Experimental studies on the filtrate of the causal fungus of the "*Bakanae*" of the rice-plants (preliminary report). Trans. Nat. Hist. Soc. Formosa, XVI, pp. 213-227, 1926. (In Japanese).

[Trans. Sapporo Nat. Hist. Soc., Vol. XIII, Pt. 3, 1934]

isolated from the affected plants by the authors in 1932, were used for the purpose of obtaining the fungus filtrate.

Fifty c.c. of Knop's solution containing 0.5 % grape sugar were placed in an Erlenmeyer flask of 200 c.c. capacity. After sterilization in a Koch's steam sterilizer, the medium was inoculated with the fungus and incubated in a thermostat at 25° C. for a month. At the end of the culture period, the cultured solution was filtered through a sheet of filter paper to remove the vigorously developed fungus. Thirty c.c. of the fungus filtrate were diluted by the addition of the original Knop's solution up to 120 c.c. in a flask. It was sterilized for one hour in a Koch's sterilizer. The whole amount of the solution was poured into a Petri-dish, 11.5 cm. in diameter and 3.5 cm. in depth, containing 350 grams of sifted sand which had been sterilized previously. The control nutrient solution for the sand culture of rice seedlings was prepared with the fungus free Knop's solution containing 0.5 % grape sugar diluted by the addition of the original solution to maintain the same proportion of dilution as in the case of the fungus filtrate. The rice grains (*Bôzu No. 5*) were beforehand placed in a thermostat at 25°-28° C. to germinate aseptically, and when they attained a length of about 0.5 cm., ten of them were transplanted to each of the sand-culture beds prepared as above mentioned. They were kept in a greenhouse. Sterilized distilled water was added occasionally to avoid the drying up of the culture bed in Petri-dishes. After 10 (in Experiment 1) or 20 days (in Experiment 2), the seedlings were removed to another culture bed after their length was measured. The preparation of the new culture bed will be explained later in each experiment. The newly started cultivation was continued without adding any nutrient except tap water to keep the soil moistened. After the transplantation the development of the rice plants was observed every day until the harvest time when they were cut off at the base of the culms, and compared with the control plants as to the length of leaves and internodes and as to other characters.

Experiment 1.

The fungus filtrate used in the experiment was obtained from the culture of *Gibberella Fujikuroi* strain No. 36. The rice seedlings were first cultured under the influence of the fungus filtrate from Aug. 19 to 28, 1933. On the 28th, the length of the seedlings was measured. They were then transplanted to another culture bed prepared in a pot, 25 cm. in diameter and 15 cm. in depth and full of farm soil. The conditions of the seedlings grown under influence of the fungus filtrate before transplantation were as follows:

Table 1. Effects of the fungus filtrate on rice seedlings before transplantation.

Solution used	Number of plants tested	Length of shoots (in cm.)			Remarks
		Aver.	Maxim.	Minim.	
Fungus filtrate	5	27.71	32.9	19.3	slender, light green
Control	5	13.27	14.1	12.7	normal, green

On Dec. 14, 1933, the rice plants were harvested. The results of measuring the length are shown in the following table.

Table 2. Conditions of rice plants at harvest time.

Culture solution used before transpl.	plants tested	Whole length (cm.)	Length of internodes (cm.)					Length of leaves (cm.)	Length of ears (cm.)	Number of tillers
			I	II	III	IV	V			
Fungus filtrate	1	96.0	35.0	17.8	11.5	14.0	4.5	32.7	13.5	3
	2	93.0	32.7	17.0	11.0	15.3	4.7	33.0	12.3	2
	3	93.0	31.3	17.2	11.0	15.2	4.8	28.8	13.5	3
	4	89.0	34.0	24.0	16.8	—	—	39.2	13.2	2
	5	89.0	34.0	17.0	11.5	13.0	—	32.0	14.2	2
	Aver.	92.1	33.4	18.6	12.3	11.5	2.8	35.1	13.3	—
Control solution	1	86.0	35.0	18.5	11.5	6.7	—	39.3	14.5	2
	2	79.0	32.0	15.5	10.0	8.0	1.0	31.0	12.5	3
	3	78.0	31.0	18.5	9.0	6.5	—	36.5	13.0	2
	4	76.0	28.5	16.5	9.5	8.0	—	32.5	14.0	2
	5	74.0	31.0	19.6	10.5	—	—	42.4	13.0	2
	Aver.	78.6	31.5	17.7	10.1	5.8	0.2	36.3	13.5	—

In the table, the signs I, II,....., V indicate the order of internodes from the upper to the lower, and the length of leaves means that of the last one.

Experiment 2.

The rice seedlings were cultured with the fungus filtrate obtained from the culture of *Gibberella Fujikuroi* strain No. 42 during the period Oct. 1-20, 1933.

Table 3. Effects of the fungus filtrate on rice seedlings before transplantation.

Solution used	Number of plants tested	Length of shoots (in cm.)			Remarks
		Aver.	Maxim.	Minim.	
Fungus filtrate	4	17.7	18.7	16.4	slender, light green
Control	4	10.5	10.9	10.2	normal, green

On Oct. 20, 1933, these seedlings were transplanted to a porcelain pot, 13 cm. in diameter and depth, each, and full of farm soil. The rice plants were harvested on Jan. 4, 1934. The results are shown in the following table and the text figure.

Table 4. Conditions of rice plants at harvest time.

Culture solution used before transpl.	Plants tested	Whole length (cm.)	Length of internodes (cm.)				Length of leaves (cm.)	Length of ears (cm.)	Number of tillers
			I	II	III	IV			
Fungus filtrate	1	57.0	22.5	12.2	7.0	4.7	36.1	10.6	2
	2	55.5	22.2	12.5	7.2	3.0	48.3	10.6	2
	3	55.0	21.7	12.2	6.7	4.7	40.4	9.7	2
	4	54.5	21.8	12.0	8.0	3.0	41.0	9.7	2
	Aver.	55.5	22.1	12.2	7.2	3.9	41.5	10.2	—
Control solution	1	57.0	22.7	12.4	8.0	1.6	42.0	12.3	3
	2	53.0	23.0	11.8	7.0	2.5	34.7	8.7	2
	3	50.0	21.0	11.3	9.0	—	50.7	8.8	3
	4	44.5	16.0	11.7	8.5	0.5	46.7	7.8	2
	Aver.	51.1	20.7	11.8	8.1	1.2	43.5	9.4	—

As shown in Tables 1 and 3, the rice seedlings grown under the toxic influence of the fungus filtrate in both experiments presented remarkable elongation, slender appearance, and lighter color, corresponding with the typical "*Bakanae*"-symptoms. With in a few days after transplantation into normal culture bed, however, they began to put forth newly developed leaves which were hardly distinguished in morphological appearance from those of the control plants, and in respect to other features of growth produced by them in successive days there was no distinct difference from the control plants. Though they were apparently taller in whole length than the control plants in the first experiment, comparing the corresponding plant-parts, they were almost equal in respect to

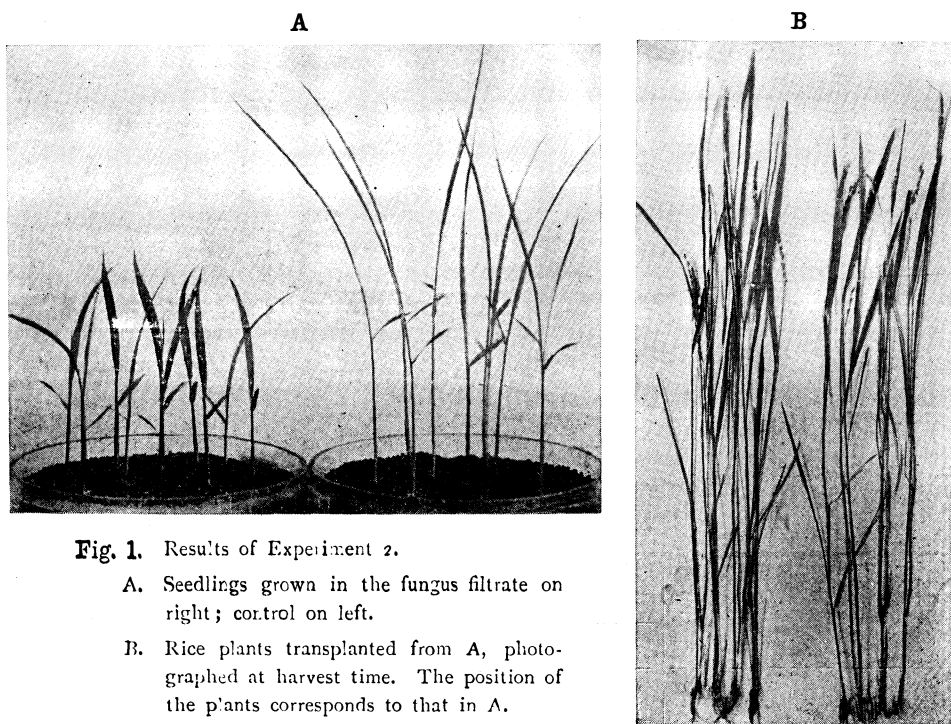


Fig. 1. Results of Experiment 2.

- A. Seedlings grown in the fungus filtrate on right; control on left.
- B. Rice plants transplanted from A, photographed at harvest time. The position of the plants corresponds to that in A.

length of the last leaves, ears, upper internodes of the culms, and number of tillers, but rather conspicuously longer in the lower internodes. This latter point was also clearly recognized in the second experiment. Therefore, the difference in the whole length between affected plants and the control ones seems to be attributable chiefly to the elongation of the lower internodes which occurred before transplantation as a result of the toxic influence of the fungus filtrate.

Considering the results obtained in the above experiments, the following conclusions may be induced:

- 1). The reactions of the rice seedlings to the fungus filtrate of the "Bakanae"-fungus, *Gibberella Fujikuroi*, are limited only to the period during which they are cultured with the nutrient solution containing the growth promoting substance excreted by the fungus.
- 2). When the rice-seedlings abnormally elongated under the toxic influence of the fungus filtrate of the "Bakanae"-fungus are transplanted in an early stage of growth to the toxin-free culture bed, they soon recover from the "Bakanae"-symptoms and continue normal or healthy growing and bear as large a harvest of grains as the control plants.

Finally the authors wish to express their heartiest thanks to Prof. S. ITO for his valuable suggestions given in the course of the present investigation.