Studies on the Compatibility of Potato Varieties and Races of *Phytophthora infestans* (Mont.) DeBary, With Special Reference to the DNA Fraction of the Suscepts and the Pathogens

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ジャガイモ品種と疫病菌系統の親和性に関する研究
—特にDNAに関連して

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Introduction

Specificity between potato varieties and races of *Phytophthora infestans* may be derived from an interaction of protoplasm in the suscept and pathogen, and the interaction might have a relation to the DNA both in the suscept and in the pathogen. In order to make clear the co-existence of hypersensitive flecks and susceptible lesions on the suceptible cultivars by inoculating zoospores of *Phytophthora infestans* after placing DNA fraction extracted from the resistant interspecific hybrid 96-56[(9)-(16)], experiments were made on the placement of purified DNA, DNA fraction and crude sap of different potatoes, and investigations were also done on the conidial germination of *P. infestans* treated by DNA fraction extracted from different potato varieties or different races of *P. infestans*.

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Materials and methods

The 3rd-6th position potato leaves (cultivar Norin No. 1— gene r and interspecific hybrid 96-56—gene R_1) were inoculated with zoospores of *Phytophthora infestans*. The conidial suspension kept for 2 hours at 10°C was placed on potato petiole midribs and kept for 24 hours at 20°C and then placed at room temperature for 48 hours. Epidermis of these midribs was peeled with a razor and forceps, fixed with 70% ethanol and observed under microscope (150x). The extraction of DNA from potatoes and *P. infestans* was made by Thomas' method[(7)]. The DNA was fractionized through Sephadex G 200. The measnrement of the absorption spectrum of RNA was made according to the phenol method. The RNA fraction was purified through Sephadex G 200, and Sephadex G 200 was placed in the

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column of 2.2×2.2×39.8 cm. Each of 4 ml aliquot was obtained with SF 160 Toyo Fraction Collector at the speed of 30-45 seconds in one drop. The measurement of DNA was made after Burton method [1] at 600mμ, and RNA was measured after Mejbaum method[5] at 670 mμ colorimetrically with Spectronic 20 Shimadzu. DNase (DNase II extracted from calf pancrease—Nakarai Chemicals Ltd) was used in the concentration of 10 μg/ml in the existence of 0.001M Mg^{++} at pH 5.0 with acetate buffer at 15°C. RNase was a kind of gift from Dr. T. Yamamoto (42,000 U/g) and RNase treatment was given at 37°C for 30 minutes in the concentration of 10 μg/ml. On heat treatment, 4 ml aliquot of DNA solution was kept at different temperatures in water bath for 10 minutes and freezed immediately with dry ice-acetone. Conidial germination of *P. infestans* was investigated under microscope (150x) after keeping the conidial suspension at 10°C for 2 hours. Grafting of potatoes was given by cleft grafting when the plants were at the height of 15 cm. Tubers which were obtained on the plants grown up from the grafted plants were also planted and the flecks and lesions appeared by an inoculation of *P. infestans* were also investigated on these plants. Extraction of DNA from mycelia of *P. infestans* was also given after Thomas method.[7] Two ml of mycelial DNA solution was added to 4 ml of conidial suspension and placed for 2 hours at 16°C. The placement of DNA or other substances was given to the epidermis of the suscept midrib petiole with a brush and an inoculation of zoospores of *P. infestans* was made 1.0 and 1.5 hours after the placement.

Experimental results

1. Placement of DNA and other substances

(1) Crude sap of potato leaves

Leaves of potato variety of Norin No. 1 and interspecific hybrid 96-56 were macerated

Table 1. Susceptible lesions and hypersensitive flecks on potato petiole midribs inoculated with *Phytophthora infestans* after placing the crude sap of potato leaves

Varieties extracted/ Varieties placed	Total number of lesions and flecks observed	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Interspecific hybrid 96-56/ Interspecific hybrid 96-56	158	139	88.1	19	11.8
Norin No. 1/ Interspecific hybrid 96-56	228	127	55.7	101	44.0
Norin No. 1/ Norin No. 1	115	15	13.0	100	87.0
Interspecific hybrid 96-56/ Norin No. 1	92	24	15.1	78	84.9
Interspecific hybrid 96-56 Control	242	228	94.2	74	5.8
Norin No. 1 Control	189	15	7.9	174	92.1

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

with Tris-chlorate buffer solution (pH 7.2) in mortar and centrifuged for 10 minutes at 3,000 rpm and supernatant was used as crude sap of potato leaves. The crude sap was placed on the suscept with a brush, and zoospores of *P. infestans* were inoculated. The results were shown in Table 1.

As shown in Table 1, in the plot where the crude sap of interspecific hybrid on Norin No. 1 on the interspecific hybrid 96-56, and in the plot placed the crude sap of interspecific hybrid 96-56 on Norin No. 1, the co-existence of both hypersensitive flecks and susceptible lesions were found, however, in the case of placing the crude sap of Norin No. 1 on Norin No. 1 and placing that of interspecific hybrid 96-56 on the interspecific hybrid 96-56, there was no difference between the experimental plots and the control, respectively.

(2) Placement of DNA fraction on the suscept

DNA fractions were placed on the suscept and zoospores of *P. infestans* were inoculated. The results were given in Table 2.

As shown in Table 2, co-existence of hypersensitive flecks and susceptible lesions were recognized in both plots, placing the DNA fraction of interspecific hybrid 96-56 on cultivar Norin No. 1 and placing the DNA fraction of cultivar Norin No. 1 on the interspecific hybrid 96-56.

Table 2. Susceptible lesions and hypersensitive flecks on potato petiole midribs after placing DNA fraction of potatoes

Variety extracted/ Variety placed	Total number of lesions and flecks observed	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1/ Norin No. 1	192	16	8.0	176	92.0
Interspecific hybrid 96-56/ Norin No. 1	229	83	30.8	158	69.2
Interspecific hybrid 96-56	218	154	70.5	64	29.5
Norin No. 1/ Interspecific hybrid 96-56	189	55	41.5	115	58.6
Interspecific hybrid 96-56 Control	88	78	88.6	10	11.4
Norin No. 1 Control	67	0	0	67	100.0

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

(3) Placement of purified DNA

DNA fraction was purified through Sephadex G 200 and the elution pattern was shown in Fig. 1.

As shown in Fig. 1, two peaks were recognized in Fraction Nos. 9 and 24. The U. V. absorption spectrum of the aliquot No. 9 was given in Fig. 2.

Purified DNA was obtained by means of RNase treatment. Based on the quantitative analysis (diphenylamine reaction) of the No. 9 aliquot, this substance is found as conside-

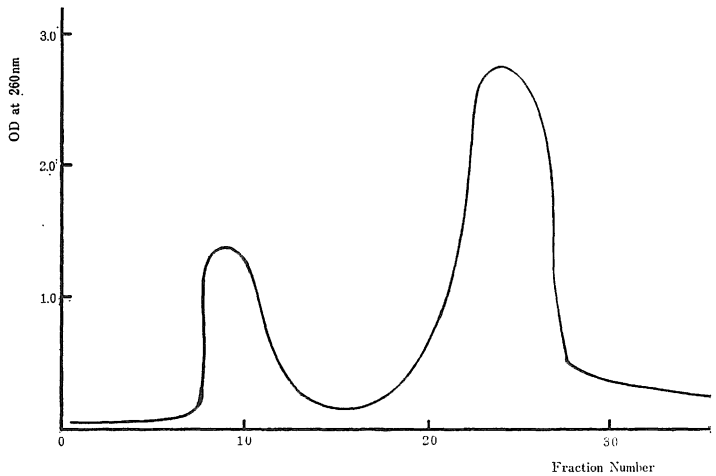

Fig. 1 Elution pattern of the DNA fraction of potatoes by means of G 200

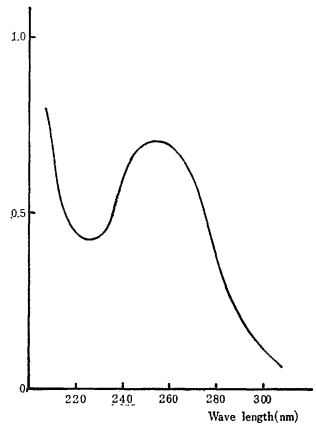

Fig. 2. UV absorption spectrum of Elution No. 9 from Sephadex G 200 column of the DNA fraction of potato leaf (Interspecific hybrid 96-56)

Table 3. Susceptible lesions and hypersensitive flecks on the petiole midribs of potatoes after placing purified DNA of potatoes

Varieties extracted	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1/ Norin No. 1	230	28	12.3	202	87.7
Interspecific hybrid 96-56/ Norin No. 1	329	95	28.8	294	71.2
Interspecific hybrid 96-56/ Interspecific hybrid 96-56	110	226	86.2	36	13.8
Norin No. 1/ Interspecific hybrid 96-56	264	158	60.3	106	39.7
Interspecific hybrid 96-56 Control	93	87	93.5	6	6.5
Norin No. 1 Control	76	4	5.3	72	94.7

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

Table 4. Hypersensitive flecks and susceptible lesions on potato petiole midribs after placing the elution No. 24 by means of Sephadex G 200 of DNA fraction from potato leaves

Varieties placed/ Varieties extracted	Total number of lesions and flecks	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Interspecific hybrid 96-56/ Norin No. 1	203	81	39.9	122	60.1
Norin No. 1/ Interspecific hybrid 96-56	278	162	58.5	116	41.5
Interspecific hybrid 96-56 Control	91	86	94.5	5	5.5
Norin No. 1 Control	87	7	8.0	80	92.0

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

Table 5. Susceptible lesions and hypersensitive flecks on the suscept after placing DNA from potato leaves

Verieties placed/ Varieties extraced	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number cf susceptible lesions	(%)
Interspecific hybrid 96-56/ Norin No. 1	299	114	38.1	185	61.9
Interspecific hybrid 96-56 Control	85	82	96.5	3	3.5
Norin No. 1 Control	83	4	4.8	79	95.2

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

Table 6. Susceptible lesions and hypersensitive flecks on the suscept inoculated with *Phytophthora infestans* after placing 0.5 M NaCl solution, ethanol and buffer solution.

Substance	Varieties placed	Races of P. infestans	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
NaCl solution	Interspecific hybrid 96-56	Race 1	125	13	10.4	110	89.6
		Race 0	72	68	94.4	4	5.6
	Norin No. 1	Race 1	103	8	7.9	95	92.2
		Race 0	69	1	1.4	68	98.6
Ethanol	Interspecific hybrid 96-56	Race 1	112	1	0.8	121	99.2
		Race 0	87	81	93.1	6	6.9
	Norin No. 1	Race 1	92	5	5.4	87	94.6
		Race 0	83	3	2.4	80	97.6
Buffer solution	Interspecific hybrid 96-56	Race 1	74	6	8.1	68	91.9
		Race 0	72	66	91.2	6	8.3
	Norin No. 1	Race 1	81	3	3.7	78	98.3
		Race 0	50	0	0.0	50	100.0

N. B. Figures were given as an average of three experiments
Buffer solution : 0.01 M NaCl−0.01 M Tris-HCl buffer solution at pH 7.2
Concentration of ethanol was 98%

Table 7. Hypersensitive flecks and susceptible lesions on the suscept inoculated with *Phytophthora infestans* after placing the DNA after heat treatment

Varieties extracted/ Varieties placed	Traeted temperatures (C)	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1/ Interspecific hxbrid 96-56/	50	143	80	55.9	63	44.1
	60	151	90	35.6	61	40.4
	70	155	92	59.4	63	40.6
	80	180	114	63.3	66	36.7
	90	186	154	82.8	32	17.2
	100	180	141	78.3	39	21.7
Interspecific hybrid 96-56/ Norin No. 1	50	109	53	48.6	56	51.4
	60	101	49	48.5	52	51.5
	70	104	47	45.2	57	54.8
	80	36	6	41.9	50	58.1
	90	99	29	29.3	70	70.7
	100	77	26	33.8	51	66.2
Interspecific hybrid 96-56 Control		101	97	96.0	4	4.0
Norin No. 1 Control		88	7	7.9	81	92.0

N. B. *Phytophthora infestans* used was Race 0

Table 8. Hypersensitive flecks and susceptible lesions on the potato scion after grafting potatoes having different susceptibility to *Phytophthora infestans*

Scion/ Stock	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1/ Norin No. 1	291	78	26.8	213	73.2
Interspecific hybrid 96-56/ Norin No. 1	318	129	39.8	189	60.2
Norin No. 1/ Interspecific hybrid 96-56	239	215	69.8	24	10.2
Interspecific hybrid 96-56	86	79	91.9	7	8.1
Norin No. 1 Control	72	4	5.6	68	94.4

rably pure DNA. The substance, however, was slight positive in the orcinol reaction, indicating the presence of RNA as a small contaminant.

As shown in Table 3, after the placement of DNA extracted from the interspecific hybrid 96-56 on cultivar Norin No. 1 and the placement of DNA extracted from cultivar Norin No. 1 on the interspecific hybrid 96-56, the co-existence of both hypersensitive flecks and susceptible lesions was recognized. No definite difference was found on the plot of placing DNA extracted from Norin No. 1 on Norin No. 1 and placing DNA extracted from interspecific hybrid 96-56 on the interspecific hybrid 96-56 and the control plots, respectively. In the following experiments, the concentration of DNA was adjusted to be 0.7 of U.V. absorbance at 260 mμ.

(4) Placement of elution No. 24 : Elution No. 24 was obtained through Sephadex G 200 of the DNA fraction.

Table 9. Hypersensitive flecks and susceptible lesions on the stocks after grafting potatoes of different susceptibility to *Phytophthora infestans*

Scion/ Stock	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1/ Norin No. 1	80	4	5.0	76	95.0
Interspecific hybrid 96-56/ Norin No. 1	72	12	16.7	60	83.3
Interspecific hybrid 96-56/ Interspecific hybrid 96-56	88	62	70.1	26	29.9
Norin No. 1/ Interspecific hybrid 96-56	102	83	81.4	19	18.6
Interspecific hybrid 96-56 Control	74	67	90.5	7	9.5
Norin No. 1 Control	83	5	6.0	78	94.0

N. B. *Phytophthora infestans* used was Race 0

Table 10. Hypersensitive flecks and susceptible lesions on the plants grown up from th_e grafted potatoes having different susceptibility to *Phytophthora infestans*

Scion/ Stock	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1/ Norin No. 1	237	77	35.4	135	64.6
Interspecific hybrid 96-56/ Norin No. 1	305	24	8.7	278	91.3
Interspecific hybrid 96-56/ Interspecific hybrid 96-56	324	113	34.7	211	65.3
Norin No. 1/ Interspecific hybrid 96-56	178	124	69.7	54	30.3
Interspecific hybrid 96-56 Control	105	94	89.5	11	10.5
Norin No. 1 Control	96	8	8.3	88	91.7

N. B. *Phytophthora infestans* used was Race 0

Tabll 11. Hypersensitive flecks and susceptible lesions on the suscept inoculated with zoospores of *Phytophthora infestans* germinated indirectly in the DNA solution of different races of *P. infestans*

Race extracted/ Race inoculated	Potato varieties	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Race 1/ Race 0	Interspecific hybrid 96-56	324	292	90.3	32	9.7
Race 0/ Race 1	Interspecific hybrid 96-56	246	17	7.1	229	92.9
Race 1 Control	Interspecific hybrid 96-56	92	6	6.5	86	93.5
Race 0 Control	Interspecific hybrid 96-56	81	77	95.1	4	4.9

N. B. Figures were given as an average of three experiments

Table 12. Hypersensitive flecks and susceptible lesions on the suscept inoculated with zoospores of *Phytophthora infestans* treated with the DNA extracted from a cultivar Norin No. 1

Variety extracted DNA	Inoculated variety	Total number of flecks and lesions	Number of hypersensitive flecks	(%)	Number of susceptible lesions	(%)
Norin No. 1	Interspecific hybrid 96-56	461	441	95.4	20	4.6
	Interspecific hybrid 96-56	128	121	94.5	7	5.5

N. B. Figures were given as an average of three experiments

Table 13. Indirect conidial germination of *Phytophthora infestans* in the potato DNA solution after heat treatment

Potato varieties	Temperature treated (C)	Total number of conidia observed	Number of germinated conidia	(%)
Norin No. 1	50	408	63	15.4
	60	457	92	20.0
	70	444	42	9.5
	80	393	27	7.2
	90	441	65	14.5
	100	529	74	13.8
	Control	449	288	53.1
Interspecific hybrid 96-56	50	382	82	21.3
	60	370	125	24.8
	70	422	56	13.0
	80	395	68	17.2
	90	447	173	11.6
	100	386	36	14.5
	Control	339	238	58.1

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

Table 14. Indirect conidial germination of *Phytophthora infestans* in the solution of some substances

Substance	Total number of conidia observed	Germinated conidia	(%)
RNA extracted from cultivar Norin No. 1	317	301	32.5
Elution No. 24 from the DNA fraction extracted from cultivar Norin No. 1	388	134	35.1
Elution No. 24 from the DNA fraction extracted from interspecific hybrid 96-56	277	75	26.9
0.5 M NaCl solution	370	76	20.2
Control	383	129	33.8

N. B. Figures were given as an average of three experiments
Phytophthora infestans used was Race 0

As shown in Table 3, co-existence of hypersensitive flecks and susceptible lesions was recognized on the interspecific hybrid 96-56 by placing on them the substance extracted

from Norin No. 1 and Norin No. 1 by placing the substance extracted from the interspecific hybrid 96-56, respectively. The tendency was similar as shown in Table 2.

(5) Placement of RNA from potato leaves

RNA extractd from potato leaves by the phenol method was placed on the suscept, and *P. infestans* was inoculated. The results obtained were shown in Table 4.

As shown in Table 4, the co-existence of hypersensitive flecks and susceptible lesions was also recognized in the plot treated with RNA. The quantity of RNA used in this experiments was about 45 μg/ml.

(6) Placement of NaCl, ethanol and buffered solution (pH 7.2). They were placed on the suscept, and zoospores of *P. infestans* were inoculated. The resuslts were given in Table 5, 6 and 7. As shown in Table 5, 6 and 7, the existence of other types of flecks or lesions compared with the control was not recognized.

(7) Placement of purified DNA extracted from leaves after heat treatment

DNA extracted from potato leaves was treated as described in "Materials and Method", and placed on the suscept, and *P. infestans* was inoculated on the surface of the leaves. The results were shown in Table 8.

As shown in Table 8, the placement of DNA extracted from Norin No. 1 on the interspecific hybrid 96-56 after heat treatment of Norin No. 1, lesser effect was observed in the plots placed with DNA treated to higher temperatures over 90°C. Hyperchronicity was recognized of the DNA of the interspecific hybrid 96-56 at 60-70°C and 80-90°C, and of the DNA of cultivar Norin No. 1 at 80-90°C.

2. Grafting of potatoes

Cultivar Norin No. 1 and the interspecific hybrid 96-56 were grafted and *P. infestans* was inoculated. The results were given in Table 9 and 10.

As shown in Table 10, the co-existence of hypersensitive flecks and susceptible lesions was recognized on the scions of the grafted plants, both the interspecific hybrid 96-56 on cultivar Norin No. 1 and Norin No. 1 on the interspecific hybrid 96-56.

As shown in Table 11, the co-existence of different types of hypersensitive flecks and susceptible lesions was also recognized on the stocks inoculated with *P. infestans*, but the rate of co-existence was lower than that of the scions with *P. infestans* inoculated on it.

Inoculation of *P. infestans* on the plants grown up from the grafted potatoes showing different types of flecks and lesions was carried out, and the results were given in Table 12.

As shown in Table 12, the co-existence of different types of flecks and lesions was observed on these plants.

3. Inoculation of *P. infestans* germinated in the DNA solution from different races of *P. infestans* on the suscept.

Zoospores germinated indirectly in the DNA solution extracted from different races of *P. infestans* were inoculated to the suscept. The results were shown in Table 13.

DNA extracted from *P. infestans* was applied to the conidia of *P. infestans* (Race 0) and DNA extracted from the conidia of Race 0 was applied to the conidia of Race 1. As shown

in Table 14, the change of pathogenicity was not recognized in both cases, within the limit of these experiments.

4. Other experiments on zoospores of *P. infestans*

(1) Treatment on zoospores of *P. infestans* with DNA extracted from potatoes

In order to obtain an idea whether the placement of potato DNA influences the suscept or the pathogen, DNA extracted from cultivar Norin No. 1 was treated on the zoospores of *P. infestans* (Race 0). The results were given in Table 14

As shown in Table 14, appearance of different types of hypersensifive flecks and susceptible lesions was recognized on the plot treated with DNA extracted from Norin No. 1 on the zoospores of *P. infestans* (Race 0), and inoculated to the interspecific hybrid 96-56.

Judging from this experiment, it might be possible that the DNA placed on the suscept does not give influence on the pathogen but on the suscept.

(2) Indirect germination of *P. infestans* (Race 0) in the solution of DNA after heat treatment

Indirect germination of *P. infestans* (Race 0) in DNA solution extracted from Norin No. 1 and the interspecific hybrid 96-56 was given in Table 15.

As shown in Table 15 and 16, no change was observed of the inhibition of conidial germination of *P. infestans* by heat treatment of DNA from potatoes

Indirect germination of conidia of *P. infestans* (Race 0) was investigated in RNA extracted from Norin No. 1, DNA fraction extracted from Norin No. 1, Elution No. 24 of DNA fraction extracted from the interspecific hybrid 96-56, 0.5 M NaCl solution and Tris-buffer solution, and the results were given in Table 17.

As shown in Table 17, there was no difference in the indirect gemination of conidia of *P. infestans* (Race 0) in the solution of these substances.

Discussions

When the incompatible races of *P. infestans* invade potatoes, the growth of the pathogen is inhibited after hypersensitive death of the suscept cells, but in the case of invading the compatible race, hypersensitive death of the invaded cells of the suscept is not recognized. The formation of phytoalexins, such as rishitin, lyubimin, phytuberin, etc, may be an important factor for the resistance mechanism but the specificity between the races of the pathogen and the varieties of the suscept is not fully explained merely by phytalexines.

The writers anticipated an importance of protoplasm of the suscepts and the pathogens priort to the infection in relation to the specificity, and the writers' attention was focused on the DNA of the suscept and pathogen.

As shown in Table 3, hypersensitive flecks and susceptible lesions were observed on the interspecific hybrid 96-56 after placing the DNA extracted from the cultivar Norin No. 1 and vice versa. These results coincided with the writers' previous report[11]. The same results were observed on potatoes by placing purified DNA through Sephadex G 200. From these experiments, DNA seems to have an important role on the resistance of potatoes against the invasion of *Phytophthora infestans*. However, the same results were also observed on the lower molecular substance (Elution No. 24) and also on total RNA extracted from potatoes (Table 4). From these results, Elution No. 24 substance from the

DNA fraction and the total RNA as well as purified DNA seem to have an ability to influence the resistance of potatoes against the invasion of *P. infestans*. By heat treatment of DNA over 90°C, the decrease of different types of lesions or flecks was observed. From these results, double strand structure of DNA might have a relation to the resistance, but in this point, further detailed studies should be done in the future. Then, what is the basic function of the suscept to make appearance of the different types of flecks or lesions? Salts of nucleic acid, lower molecular nucleotidea, etc might have a chance to do so, but within the scope of the writer' experiments, a definite conclusion is difficult to deduce at present.

Indirect germination of conidia of *P. infestans* (Race 0) in the DNA solution of interspecific hybrid 96-56 after the heat treatment showed no influence of the treatment. The writers[10] previously reported that the appearance of different types of flecks and lesions was strongly inhibited by washing within 10 minutes after placing the DNA on the suscept, but the appearance maintained after 1 hour eeven if it was washed. Ledoux et al[4] studied the fate of exogenous DNA in living organism. From these results, 1-1.5 hours after placing DNA on potatoes may influence the suscept cells, however, in this point, further studies on the behavior of placed DNA in more early stage should be bone. The fact that the co-existence of hypersensitive flecks and susceptible lesions was sometimes observed in one microscopic field leads us to surmise the possible localozation of DNA in the suscept tissues. This is also one of the points to investigate in the next step.

By grafting the scions of interspecific hybrid 96-56 on the stock of Norin No. 1, and vice versa, the co-existence of hypersensitive flecks and susceptible lesions was observed (Table 9, 10, 11). These results may have some relation to the dislocation of DNA or substance in relation to DNA in the stock translocated to the scion or vice versa. This is cioncided with the results of Shinoto's experiments on the grafting of the egg-plant, and some Japanese workers on grafting[2][6]. Their results may have a light on this line.

There are many reports on the transformation of microorganisms, such as Kern [3], Yamane and Hayashi[18] and others, since the Avery's work on *Pneumococcus*. The writers treated zoospores of *P. infestans* (Race 1) in the stage of indidirect germination with DNA extracted from the mycelia of *P. infestans* (Race 0). However, there was no change of the pathogenicity of *P. infestans* (Race 1), within the scope of the writers' experiments. Similar tendency was observed on the zoospores of Race 0 treated with the DNA from Race 1. It is not clear whether the failure of chnging the pathogenicicity of the pathogen is deduced from the incompatible state of the conidia of *P. infestans* in the writers' experimental condition or not.

Summary

The present paper was designed to elucidate the mechanism of the specificity of suscept-pathogen interrelationships in potato-*Phytophthora*, with special reference to the DNA fraction of potato varieties and the races of *Phytophthora infestaus*.

1. Hypersensitive flecks and susceptible lesions were observed on the resistant interspecific hybrid 96-56 by an inoculation of *P. infestans* (Race 0) after applying the the crude sap

juice, DNA fraction and purified DNA extracted from the susceptible cultivar Norin No. 1.

2. Hypersensitive flecks and susceptible lesions were also observed on the resistant interspecific hvbrid 96-56 by an inoculation of *P. infestans* (Race 0) after applying the DNA fraction (Elution No. 24) through Sephadex G 200 and RNA extracted from the susceptible cultivar Norin No. 1.
3. Ethanol, sodium chloride and Tris-buffer solution have no effect on the appearance of different types of fleck and lesions on potato midribs infected with *P. infestans*.
4. The appearance of different types of flecks and lesions on potatoes infected with *P. infestans* decreased by the treatment of DNA heated over 90°C.
5. Scions of resistant interspecific hybrid 96-56 on the stocks of susceptible cultivar Norin No. 1 and vice versa showed susceptible lesions and hypersensitive flecks by an inoculation of *P. infestans* after grafting. Plants grown up from tubers obtained from grafted plants also showed susceptible lesions and hypersensitive flecks.
6. When the DNA fraction extracted from the mycelium of *P. infestans* (Race 1) was applied to the conidial suspension of *P. infestans* (Race 0) at 12°C (indirect germination) and vice versa, the change of pathogenicity to the suscept was not observed.
7. The appearance of different types of flecks and lesions were not observed on the interspecific hybrid 96-56 by an inoculation of zoospores of *P. infestans* (Race 0) treated with DNA extracted from the susceptible cultivar Norin No. 1 for 1 hour.

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摘　　要

1)　ジャガイモ農林1号の粗汁液，DNAフラクション，精製DNAを種間雑種96-56に塗布し疫病菌 Race 0を接種した場合，逆に種間雑種96-56の粗汁液，DNAフラクション，精製DNAを農林1号に塗布し疫病菌 Race 0を接種した場合，どちらの場合においても羅病型・過敏型両型の病斑の混在が認められた．

2)　農林1号のDNAフラクションのセファデックスG200による溶出番号24の物質や，ジャガイモ葉RNAを種間雑種96-56に塗布し疫病菌 Race 0 を接種した場合，逆に種間雑種96-56のDNAフラクションのセファデックスG200による溶出番号24の物質を農林1号に塗布し疫病菌 Race 0を接種した場合，どちらの場合においても羅病型・過敏型両型の病斑が観察された。

3)　NaCl，エタノール，緩衝液を塗布し疫病菌を接種しても異病斑型の出現は認められなかった．

4)　農林1号から抽出した精製DNAを温度処理したあと種間雑種96-56に塗布した場合，逆に種間雑種96-56から抽出したDNAを温度処理したあと農林1号に塗布した場合，どちらも疫病菌を接種した場合には90°C以上の処理で，異病斑型の出現率が低下した．

5)　農林1号に種間雑種96-56を接木してその接穂あるいは台木に疫病菌 Race 0を接種した場合，逆に種間雑種96-56に農林1号を接木してその接穂あるいは台木に疫病菌 Race 0を接種した場合，どちらの場合にも過敏型・羅病型両型の病斑が観察された．またこの現象は接木後代においても観察された．

6)　Race 1から抽出したDNAを間接発芽時のRace 0の胞子に処理した場合，逆に Race 0から抽出したDNAを間接発芽時の Race 1の胞子に処理した場合，どちらの場合においても本実験の範囲内では病原性の変化は認められなかった．

7)　農林1号から抽出したDNAを疫病菌 Race 0の遊走子に1時間処理し，種間雑種96-56に接種した場合には，異病斑型の出現は認められなかった．