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REVIEW
Powdery Mildew Resistance in Cucumber

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Abstract

Using an improved method for evaluating powdery mildew (*Sphaerotheca cucurbitae*) resistance in cucumber, experiments were conducted for the breeding of varieties resistant to powdery mildew that would be adapted to greenhouse cultivation during the period from winter to spring in Japan. The relationship between the resistance to the pathogen and the ambient temperature was analyzed by spray inoculation of a conidial suspension under controlled conditions. Although the varieties, Asomidori-5-gou and Natsufushinari were resistant at temperatures between 25 to 30ºC, they became susceptible at 15 to 20ºC. The resistance was affected by the duration of the exposure to 30 and 15ºC during the day. To screen resistant varieties and lines independently of the temperature, 295 cucumber accessions were inoculated at 20 and 26ºC. All the accessions tested could be divided into 3 types based on the difference in the resistance response to temperature. Many of the resistant accessions were Chinese varieties and lines. PI197088-5, a progeny of PI197088 which originated in India, displayed the highest level of resistance among all the accessions. Thus PI197088-5, whose resistance is temperature-independent appears to be the most suitable parent for the breeding of powdery mildew-resistant cucumber varieties. The inheritance of powdery mildew resistance in PI197088-5 was analyzed using Natsufushinari (resistant under high temperature conditions) and Sharp 1 (susceptible). As a result it was considered that the powdery mildew resistance in PI197088-5 was due to 2 gene pairs, a recessive gene and an incompletely dominant gene, with the genotype designated as *aaBB*, while the genotype of Natsufushinari was designated as *aabb* and that of Sharp 1 as *AAbb*. The nature of the host-pathogen interaction in PI197088-1 which is the sib-line of PI197088-5 was observed microscopically. The retardation of hyphal growth and development was observed at 2 days after inoculation. Conidia were hardly formed on the epidermis and the number of haustoria in the epidermal cells of PI197088-1 was much lower than that in the susceptible variety Sharp 1. Fluorescence of epidermal cells was observed along the hyphae in PI197088-1 at 6 days after the inoculation, suggesting the existence of a hypersensitive reaction.

Discipline: Plant breeding
Additional key words: temperature, genetic resources, *Sphaerotheca cucurbitae*, inheritance, hypersensitive reaction

Introduction

Powdery mildew, caused by *Sphaerotheca cucurbitae*, limits the production of cucumbers (*Cucumis sativus*) throughout the world. Fungicide application and the use of resistant varieties are the major methods of disease control. Since the resistance to powdery mildew was first

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observed in the variety Puerto Rico 37 in the USA[14], a large number of resistant materials were found in accessions from South and East Asia and utilized to develop new resistant varieties[1,2,5,10,17]. However, it remains to be determined whether these varieties are completely resistant[18]. In Japan, powdery mildew of cucumber has become a serious problem in greenhouse cultivation, especially in grafting cultivation for the production of bloomless fruits. Most of the Japanese commercial varieties for greenhouse cultivation during the period from winter to spring are susceptible to powdery mildew, while most of the present summer cucumber varieties are resistant to it. The reason for the absence of resistant varieties in greenhouse-grown cucumber has not been elucidated.

In the cucumber plants grown in the field, infection with powdery mildew varies with the seasons. Powdery mildew development in the field is dependent to a large extent on the environmental conditions. Excessively high temperatures limit the development of the disease, whereas cool temperatures and shading enhance it. Munger[12] reported that a temperature regime of 15–21ºC was much more favorable for mildew development than a 21–27ºC regime, and the effect of temperature was more pronounced on cucumbers with intermediate levels of powdery mildew resistance than on susceptible ones. Then he suggested that the temperatures at which the studies were conducted should be reported and, if possible, that comparisons be made under different temperature regimes. Afterwards, the relation between resistance and temperature had not been studied in detail.

Generally the evaluation of the resistance is performed under field or greenhouse conditions. However, this method may not be adequate for the evaluation because the inoculation environment can not be controlled precisely under such conditions. Therefore, it was deemed necessary to develop a suitable method for evaluating the resistance level of cucumber varieties to powdery mildew[4,8]. We demonstrated that it was possible to evaluate the resistance level of cucumber varieties to powdery mildew by observing the mildew development on the surface of the cotyledons or the first true leaves in petri dishes[11].

Then we conducted a series of experiments to obtain powdery mildew-resistant varieties mainly for greenhouse cultivation during the period from winter to spring, by using the evaluation method developed by Morishita et al[11]. In the current paper, the effect of the temperature on the gene expression of powdery mildew resistance and screening of genetic resources as well as the mode of inheritance and the nature of the host-pathogen interaction will be reported.

Material and methods

1. Relation between temperature and powdery mildew resistance

Three cucumber varieties, Sharp 1, Asomidori-5-gou and Natsufushinari which differ in their resistance level to powdery mildew were used to study the relation between the temperature and the expression of powdery mildew resistance in cucumber. Seeds were sown in plastic pots and the seedlings were grown in the greenhouse. Twenty days after germination, the first true leaves removed from plants were placed in petri dishes (9 cm diameter) and inoculated with the pathogen. The petri dishes were put into incubators controlled under the following temperature conditions: (1) 15, 20, 25, 27.5 and 30ºC during a day with a 12 h photoperiod for determining the influence of a constant temperature on the degree of disease susceptibility; (2) 30ºC /24 h, 30ºC /16 h+15ºC /8 h, 30ºC /8 h+15ºC /16 h and 15ºC /24 h with a 12 h photoperiod for evaluating the influence of the number of hours of exposure to 30 and 15ºC during a day on the degree of disease susceptibility.

2. Screening of genetic resources for powdery mildew resistance

In total, 295 accessions of cucumber, stored as genetic resources at the National Institute of Vegetables and Tea Science, were subjected to screening for powdery mildew resistance. Three plants from each accession were grown in a greenhouse in pots and used to evaluate the resistance level at both 26 and 20ºC. The inoculations were performed on the cotyledon and the first true leaves. The cotyledons were cut off from the seedlings at 7 days after germination and the first true leaves at 14 days. They were placed in a petri dish and after inoculation, put into incubators controlled at 26 or 20ºC with a 16 h photoperiod, respectively.

3. Inheritance of powdery mildew resistance

PI197088-5 (P), a resistant genotype at both 26 and 20ºC, which was selected from PI197088 (Indian wild cucumber) introduced from South Korea, Natsufushinari (N), a resistant genotype at 26ºC and susceptible at 20ºC and Sharp 1 (S), a susceptible genotype under both temperature conditions, were selected for genetic analysis. The following crosses were made : P×S, S×N and P×N. Several F_1 plants of each cross were grown subsequently in a greenhouse to produce F_2 and backcross seeds. Seeds of the parental, F_1 and F_2 generations from the crosses P×S, S×N and P×N, and BC_1 (F_1 P×S) ×P, BC_1 (F_1 S×N) ×S, BC_1 (F_1 P×N) ×P and BC_1 (F_1 P×N) ×N were planted in the greenhouse. The mildew test of the parental, F_1, F_2 and BC_1 seedlings was performed in incubators controlled at 26 or 20ºC with a 16 h photoperiod, using the cotyledons or first true leaves cut off from the seedlings, respectively.

4. Observation of fungal development on leaf

For the microscopic observation of the process of conidial infection of *S. cucurbitae* on the leaves of resistant and susceptible variety plants, resistant cucumber PI197088-1 which is the sib-line of PI197088-5 and susceptible Sharp 1 were used as experimental materials. The first true leaves of the plants of each variety were inoculated with *S. cucurbitae* and incubated in a growth chamber controlled at 20ºC. For the observation of fungal development, the sampled leaves were fixed with a mixture of acetic acid and ethyl alcohol (1:3, v/v) and made transparent with chloral hydrate, and mounted on a glass slide with a few drops of Calcoflour white M2R (0.1%). The stained specimens were observed under a fluorescence microscope. Also in order to observe the formation of haustoria in the epidermal cells, infected leaves at 5 days after inoculation were fixed with FAA solution (formalin-acetic acid-alcohol) immediately after collection. The fixed specimens were dehydrated in a graded *t*-butanol series, embedded in paraffin, sliced into 10 µm-thick serial sections. The sections were stained with haematoxylin, safranin and fast green. Coverslips were placed on the stained sections using balsam prior to microscopic observation.

5. Pathogen

The isolate of *S. cucurbitae*, originating from natural infection on cucumber leaves in the plastic film greenhouse, was used for these experiments. The inoculum of *S. cucurbitae* derived from a monospore culture was multiplied on the susceptible variety Sagamihanjiro in the greenhouse. A spore suspension was prepared by soaking heavily infected leaves in distilled water and filtering the suspension through 2 layers of cheesecloth. A suspension of 10^5 spores mL^{-1} was used for the inoculation.

6. Estimation of disease severity

Disease severity of each cotyledon or first true leaf was classified into 5 categories based on the following scale: 0, no visual infection; 1, less than 5% of the surface area of the leaf infected; 2, 6–25% of leaf; 3, 26–50% of leaf; 4, more than 50% of leaf. Final disease assessment was conducted at 12 days after inoculation. Accessions with scores 1 and 0 were classified as resistant and those with scores above 1 as susceptible.

Results and discussion

The influence of a constant temperature on the expression of powdery mildew resistance in cucumber varieties is depicted in Fig. 1. The resistant variety Natsufushinari did not show any incidence of the disease at 25, 27.5 and 30ºC, and a moderate incidence was observed at 15 and 20ºC. The resistant variety Asomidori-5-gou did not show any incidence at 27.5 and 30ºC and mildew was more severe than in Natsufushinari at 15, 20 and 25ºC. The susceptible variety Sharp 1 devel-

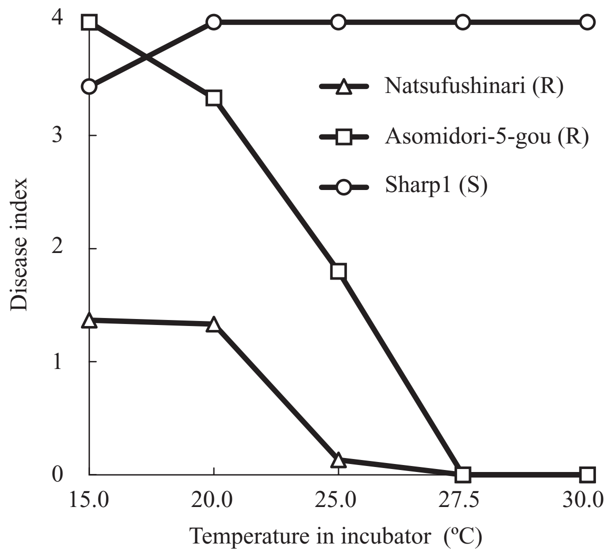

Fig. 1. Influence of temperature on the degree of disease susceptibility in cucumber varieties

Table 1. Influence of number of hours of exposure to 30ºC and 15ºC during the day on the degree of disease susceptibility in cucumber varieties

Variety[a]	30ºC/24 h	30ºC/16 h+15ºC/8 h	30ºC/8 h+15ºC/16 h	15ºC/24 h
Natsufushinari (R)	0.0 a[b]	0.3 a	4.0 c	3.2 b
Asomidori-5-gou (R)	0.0 a	0.3 a	4.0 c	1.7 b
Sharp 1(S)	3.2 a	4.0 b	4.0 b	4.0 b

a): R, Resistant; S, Susceptible.
b): The same letter after the numbers in the same variety indicates the absence of significant difference in Tukey multiple range test at P=0.05 level.

oped extensive lesions of mildew in the temperature range from 15 to 30ºC. The influence of the number of hours of exposure to 15 and 30ºC during a day on powdery mildew resistance in cucumber varieties is shown in Table 1. Sharp 1 was extensively infected regardless of the conditions, while Natsufushinari and Asomidori-5-gou hardly displayed any incidence when exposed to 30ºC for 24 h and 16 h and displayed mildew lesions when exposed for 8 h and less. However, the amount of mildew for less than 8 h exposure was lower than that for 8 h exposure.

Endo[7] reported that the conidia of powdery mildew of cucumber germinated in the temperature range from 15 to 30ºC, with the optimum temperature being 25ºC. Conidial formation is possible at temperatures ranging from 10 to 35ºC and optimum at 25ºC. Natsufushinari and Asomidori-5-gou expressed both a resistance and susceptibility within the effective temperature range for mildew development. These data indicate that the expression of the powdery mildew resistance in cucumber varieties depends on the temperature. In cabbage, it has been recognized that the resistance to yellows of cabbage (*Fusarium oxysporum*) was affected by the temperature[16]. Type A resistant varieties in cabbage expressed the resistance to *F. oxysporum* at 22ºC or less, and shifted to susceptibility at temperatures above 24ºC. Our data indicate that the powdery mildew resistance of the resistant varieties in cucumber shifts to susceptibility at temperatures below 25ºC, and that the critical temperature for shifting from resistance to susceptibility may vary with the varieties. In addition, the expression of the resistance was affected by the number of hours of exposure to high temperature in a day. In this study, the leaves of the resistant varieties Natsufushinari and Asomidori-5-gou were not infected with powdery mildew when they were exposed for 16 h or more in a day to a high temperature (30ºC) and became infected when exposed for 8 h or less. In fact, Natsufushinari and Asomidori-5-gou are resistant to powdery mildew in the summer in Japan and are infected in late autumn, winter and spring. Munger[12] suggested that a temperature regime of 15-21ºC was much more favorable for mildew development than a 21-27ºC regime. Our results related to the effect of the temperature were slightly different from those of Munger. However, both results are in agreement with the fact that a cool temperature is suitable for the infection with powdery mildew in resistant cucumber varieties. Since the cucumber growers in Japan expect that the resistance to powdery mildew will be maintained through 4 seasons, it is necessary to develop varieties with complete resistance in which the expression of the resistance is not affected by the temperature. Then we attempted to identify

Table 2. Accessions with resistance to powdery mildew under 20 or 26ºC temperature conditions

Variety and line	Origin[a)]	Disease index[b)]		Resistance type[c)]
		26ºC	20ºC	
PI197088-5	I	0.0 ± 0.0[b)]	0.0 ± 0.0	I
Jinza 1 hao	C	0.0 ± 0.0	0.5 ± 0.3	I
808	C	0.0 ± 0.0	0.7 ± 0.3	I
Luchun 32 hao	C	0.0 ± 0.0	0.7 ± 0.3	I
Ji 8 huanggua	C	0.0 ± 0.0	1.0 ± 0.0	I
Ludi 1 hao	C	0.0 ± 0.0	1.0 ± 0.0	I
SC-8	C	0.3 ± 0.3	1.0 ± 0.0	I
2027-90	R	0.0 ± 0.0	1.3 ± 0.3	II
F9-5-2	C	0.0 ± 0.0	1.3 ± 0.3	II
Xiachun 2 hao	C	0.0 ± 0.0	1.3 ± 0.3	II
82-1	C	0.0 ± 0.0	1.7 ± 0.3	II
Jinyan 7 hao	C	0.3 ± 0.3	1.7 ± 0.7	II
Natsufushinari	J	0.7 ± 0.3	1.7 ± 0.3	II
Dihuanggua	C	0.0 ± 0.0	2.0 ± 0.6	II
Jicai 3 hao	C	0.3 ± 0.3	2.0 ± 0.0	II
Ben 1-4-1-7	C	0.7 ± 0.3	2.0 ± 0.6	II
Natsusuzumi	J	0.7 ± 0.3	2.0 ± 0.6	II
Xiafeng 1 hao	C	0.3 ± 0.3	2.3 ± 0.3	II
Lu 103 hao	C	0.0 ± 0.0	2.7 ± 0.3	II
0096	C	0.0 ± 0.0	3.0 ± 0.6	II
Fidelio Imp	N	0.0 ± 0.0	3.0 ± 0.0	II
Jinyan 5 hao	C	0.7 ± 0.3	3.0 ± 0.6	II
Linsuqiufeng	C	1.0 ± 0.0	3.0 ± 0.6	II
Kensui	J	0.0 ± 0.0	3.1 ± 0.6	II
Jinza 4 hao	C	0.0 ± 0.0	3.2 ± 0.6	II
RAR930024	R	0.0 ± 0.0	3.3 ± 0.3	II
Parad	R	0.3 ± 0.3	3.3 ± 0.3	II
Suyo	J	0.0 ± 0.0	3.4 ± 0.7	II
Jinchun 4 hao	C	0.0 ± 0.0	3.5 ± 0.5	II
Xinong 58 hao	C	0.0 ± 0.0	3.7 ± 0.3	II
Caizao 1 hao	C	0.7 ± 0.7	3.7 ± 0.3	II
Status Natsu	J	0.0 ± 0.0	3.9 ± 0.2	II
00100	C	0.0 ± 0.0	4.0 ± 0.0	II
74-18	C	0.0 ± 0.0	4.0 ± 0.0	II
Asomidori-5-gou	J	0.0 ± 0.0	4.0 ± 0.0	II
SKW-690	P	0.0 ± 0.0	4.0 ± 0.0	II
Harvestmore	A	0.3 ± 0.3	4.0 ± 0.0	II
Marketmore 76	A	0.3 ± 0.3	4.0 ± 0.0	II
Alty-Aryk	R	0.7 ± 0.3	4.0 ± 0.0	II
Yomaki Risyu	J	0.7 ± 0.3	4.0 ± 0.0	II
Shchedry	R	1.0 ± 0.0	4.0 ± 0.0	II

a): A, USA; C, China; I, India; J, Japan; N, Netherlands; P, Poland; R, Russia.
b): Values are mean ± SE (n = 3).
c): I, Accessions showing resistance under both 20 and 26ºC temperature conditions; II, Accessions showing resistance under only 26ºC temperature conditions.

genetic resources accessions which are not infected with the powdery mildew fungi under both lower and higher temperature conditions.

The powdery mildew resistance of the accessions was screened under both 26 and 20ºC conditions and the accessions were divided into 3 types based on the difference in the resistance response to temperature (Tables 2,3). Type I accessions showing a resistance at both 26 and 20ºC included PI197088-5, Jinza 1 hao, 808, Luchun 32 hao, Ji 8 huanggua, Ludi 1 hao and SC-8 only. PI197088-5 showed the highest level of resistance among all the accessions tested. These results differed from those of Zijlstra & Groot[18] and Clark[3] who indicated that the PI197088 accession was susceptible or showed a moderate level of resistance. The accession PI197088 introduced from South Korea was heterozygous for powdery mildew resistance. PI197088-5 is one of the resistant lines selected from the progenies of PI197088. The Type II varieties that are resistant at 26ºC and susceptible at 20ºC consisted of 34 accessions in total, including Natsufushinari, Marketmore 76, Luchun 32 hao, Jinza 4 hao, etc. These accessions can become infected with powdery mildew in the greenhouse during cultivation from winter to spring. The Type III varieties (254 accessions) were susceptible under both 26 and 20ºC conditions. Most of the resistant accessions consisted of Chinese varieties and lines. It was thus considered that resistant materials might be abundant in South and East Asia, including China.

Table 3. Classification of accessions with resistance to powdery mildew

Origin	No. of accessions	No. of accessions classified		
		I[a)]	II	III
China	93	6	18	69
Japan	101	0	7	94
Russia	26	0	5	21
USA	8	0	2	6
Netherlands	7	0	1	6
Poland	29	0	1	28
India	4	1	0	3
Malaysia	6	0	0	6
Taiwan	4	0	0	4
Nepal	4	0	0	4
Bangladesh	2	0	0	2
Italy	2	0	0	2
Korea	2	0	0	2
Spain	2	0	0	2
Thailand	2	0	0	2
Papua New Guinea	1	0	0	1
Philippines	1	0	0	1
Turkey	1	0	0	1
Total	295	7	34	254

a): I, Accessions showing resistance under both 20 and 26ºC temperature conditions; II, Accessions showing resistance under only 26ºC temperature conditions; III, Accessions failing to show resistance.

Based on the above screening test, representative varieties were selected from each type and crossed with each other. Segregation for resistance to powdery mildew in the F_1, F_2 and BC_1 generations under both 26 and 20ºC conditions was studied (Table 4). Consequently there was partial dominance for susceptibility in the F_1 (P×S), complete dominance for susceptibility in the F_1 (S×N) at both 26 and 20ºC, and a slightly partial dominance for resistance in the F_1 (P×N) at 20ºC. In the F_2 populations, the segregation ratio of resistance to susceptible plants in the P×S and S×N crosses at 26ºC satisfactorily fitted to 1:3, supporting the assumption of the presence of a single major gene with dominance for susceptibility, while in the P×S cross at 20ºC, the ratio fitted to 1:15, indicating that 2 major genes controlled the disease reaction. In the BC_1 progenies, a good fit to a 1:1 segregation ratio of resistance to susceptibility was observed in the F_1 (P×S) ×P at 26ºC and 1:3 in the F_1 (P×S) ×P at 20ºC, while all the BC_1 progenies from the F_1 (S×N) ×S cross were estimated to be susceptible at 26 and 20ºC. In the cross of P×N, plants were classified into 3 categories, resistance, intermediate level of resistance and susceptibility, based on the disease severity. In the F_2 progenies, a satisfactory fit to a 1:2:1 ratio of plants with resistance, intermediate level of resistance and susceptibility to powdery mildew was observed, indicating that IP197088-5 harbored an incompletely dominant gene for resistance.

The mode of inheritance of resistance to powdery mildew has been reported in many cucumber varieties and lines. Smith[14] observed that in Puerto Rico 37 the disease was inherited through multiple recessive genes. Barnes & Epps[2] suggested that the resistance in PI197087 from India was due to 1 or 2 major genes and 1 or 2 minor genes. Wilson et al.[17] assumed that the resistance in Yomaki from Japan was inherited through 2 pairs of duplicate recessive genes. Fujieda & Akiya[9] reported that the inheritance of powdery mildew resistance in Natsufushinari from Japan was controlled by a single recessive gene, whereas Kooistra[10] showed that Natsufushinari harbored 2 recessive genes for the resistance to powdery mildew. The results of the genetic analysis of Natsufushinari were in agreement with those of Fujieda & Akiya[9]. In many reports, it is considered that the resistance to powdery mildew in cucumber is controlled by recessive gene(s). Munger et al.[6,13] stated for the first time that cucumber had a dominant gene for powdery mildew resistance in Spartan Salad 77-717 and PI197088, although they did not analyze the data on the resistance of these lines in detail in their reports[6,13]. Our results suggested that the powdery mildew resistance in PI197088-5 selected from PI197088 was due to 2 gene pairs, a recessive gene and an incompletely dominant gene, and the genotype of Natsufushinari was designated as *aabb*, while that of PI197088-5 as *aaBB* and that of the susceptible variety Sharp 1 as *AAbb*. It was considered that *aa++* expresses resistance under high temperature conditions like 26ºC but susceptibility under low temperatures like 20ºC, while the *aaBB* resistance is independent of the temperature conditions.

The development of powdery mildew on the leaf surface of the resistant host PI197088-1 which is the sibline of PI197088-5 was observed at different times after inoculation. Conidia on the leaf surface of PI197088-1 germinated within 12 h after inoculation and the first hyphae began to grow within 24 h. Retardation of hyphal growth and development was observed at 2 days after the

Table 4. Frequency distribution and segregation for resistance to powdery mildew in parental, F_1, F_2 and BC_1 generations under 26 and 20ºC temperature conditions

Temperature	Material[a)]	Disease index						No. of plants				Expected ratio		x^2	P
		0	1	2	3	4	mean	R[b)]	I[c)]	S[d)]	Total	R:I+S	R:I:S		
26ºC	P	10					0.0	10			10				
	S					10	4.0			10	10				
	F_1 (P×S)				4	6	3.6		4	6	10				
	F_2 (P×S)	23	2	28	44	3	2.0	25	72	3	100	1 : 3		0	0.90<
	BC_1 (F_1×P)	38	10	36	9		1.2	48	45		93	1 : 1		0.096	0.75–0.90
20ºC	P	9	1				0.1	10			10				
	S					10	4			10	10				
	F_1 (P×S)				4	6	3.6		4	6	10				
	F_2 (P×S)	2	6	12	20	60	3.3	8	32	60	100	1 : 15		0.522	0.25–0.50
	BC_1 (F_1×P)	17	11	13	21	31	2.4	28	34	31	93	1 : 3		1.29	0.25–0.50
26ºC	S					10	4.0			10	10				
	N	9					0.0	9			9				
	F_1 (S×N)					10	4.0			10	10				
	F_2 (S×N)	38	5	11	24	122	2.9	43	35	122	200	1 : 3		1.31	0.25–0.50
	BC_1 (F_1×S)			1		99	4.0		1	99	100	0 : 1			
20ºC	S					10	4.0			10	10				
	N				2	8	3.8		2	8	10				
	F_1 (S×N)					10	4.0			10	10				
	F_2 (S×N)	1	3	5	18	170	3.8	4	23	170	197	0 : 1			
	BC_1 (F_1×S)					100	4.0			100	100	0 : 1			
26ºC	P	9	1				0.1	10			10				
	N	3	7				0.7	10			10				
	F_1 (P×N)	10					0.0	10			10				
	F_2 (P×N)	140	43	11	4		0.4	183	15		198		1 : 0 : 0		
	BC_1 (F_1×P)	110	54	28			0.6	164	28		192		1 : 0 : 0		
	BC_1 (F_1×N)	137	53	8			0.4	190	8		198		1 : 0 : 0		
20ºC	P	3	5				0.6	8			8				
	N					9	4.0			9	9				
	F_1 (P×N)			9			2.0		9		9				
	F_2 (P×N)	19	38	39	46	54	2.4	57	85	54	196		1 : 2 : 1	3.54	0.10–0.25
	BC_1 (F_1×P)	35	51	32	35	29	1.9	86	67	29	182		1 : 1 : 0		
	BC_1 (F_1×N)	2	7	49	59	78	3.1	9	108	78	195		0 : 1 : 1		

a): P, PI19708 8-5; S, Sharp 1; N, Natsufushinari. b): Plants with scores 0 and 1 are resistant to powdery mildew.
c): Plants with scores 2 and 3 show an intermediate level of resistance to powdery mildew.
d): Plants with scores 4 are susceptible to powdery mildew.

inoculation (Fig. 2, Table 5). Conidia were hardly formed on the epidermis of PI197088-1 (Table 6) and also the number of haustoria in the epidermal cells of PI197088-1 was lower than that of the susceptible variety Sharp 1 (Table 7). Fluorescence of the epidermal cells was observed along the hyphae in PI197088-1 at 6 days after the inoculation (Fig. 3). This fluorescence was considered to correspond to a hypersensitive reaction, as described by Tosa et al.[15] in wheat leaves infected with powdery mildew. It was suggested that the genes for powdery mildew resistance in PI197088-1 might act at various stages of pathogen development, including hyphal growth, haustorium formation and sporulation or induce a hypersensitive reaction of the epidermal cells.

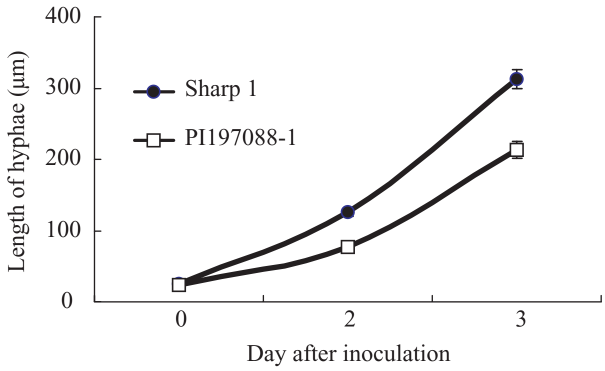

Fig. 2. Growth comparison of first hyphae after inoculation between PI197088-1 and Sharp 1
Vertical bars indicate standard error.

Table 5. Number of first and second hyphae generated from a conidium on the epidermis of PI197088-1

Material	Days after inoculation					
	1	2	3		4	
	First hypha	First hypha	First hypha	Second hypha	First hypha	Second hypha
Sharp 1	0.9 ± 0.08	2.2 ± 0.06	3.6 ± 0.12	1.6 ± 0.29	4.8 ± 0.18	7.1 ± 0.46
PI197088-1	0.9 ± 0.06	1.9 ± 0.06	2.8 ± 0.12	0.1 ± 0.06	2.8 ± 0.12	1.7 ± 0.24

Mean ± SE.

Table 6. Formation of conidia on the epidermis in PI197088-1

Material	Days after inoculation				
	3	4	5	6	7
Sharp 1	–	–	+	++	+++
PI197088-1	–	–	–	–	±

–, No formation; ±, Very mild; +, Mild; ++, Medium; +++, Severe.

Table 7. Formation of haustoria in epidermal cells of the resistant line PI197088-1

Material	No. of epidermal cells observed	No. of haustoria	No. of haustoria per 1,000 cells
Sharp 1	4,316	847	196
PI197088-1	3,438	30	9

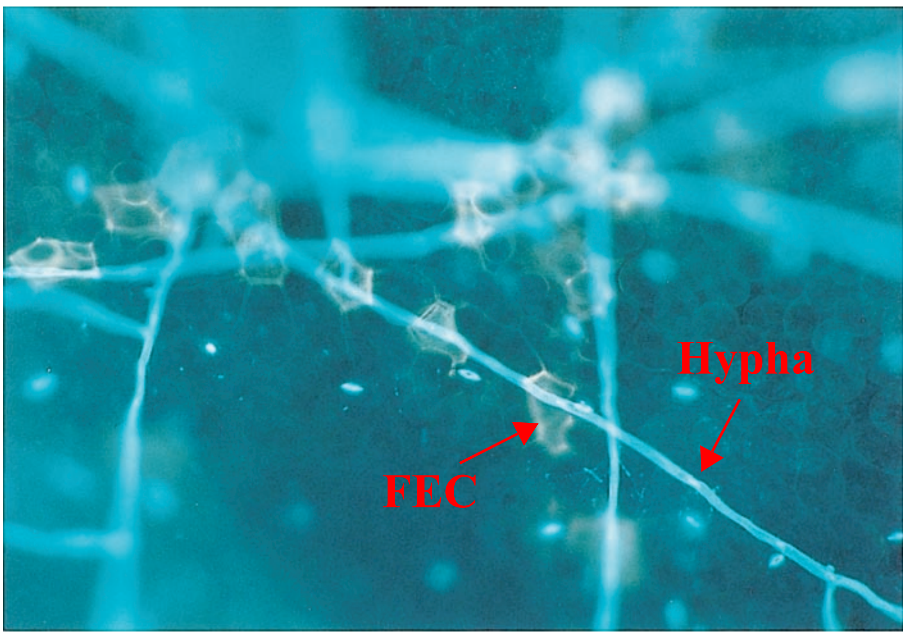

Fig. 3. Fluorescent epidermal cells (FEC) along the hyphae in the resistant line PI197088-1 at 6 days after inoculation with *S. cucurbitae*
The fluorescence was ascribed to a hypersensitive reaction.

It was eventually concluded that PI197088-5 or PI197088-1 is a useful gene source for providing a true powdery mildew resistance expressed independently of the temperature. A backcross breeding procedure should be effective in transferring genes for resistance to commercially valuable varieties. The recurrent parent should be a pure line with homozygous recessive genes (*aa*) such as Natsufushinari which is resistant to powdery mildew under high temperature conditions. It may become possible to maintain the resistance through successive backcrosses without selfing because PI197088-5 harbors recessive (*aa*) and incompletely dominant (*BB*) genes for resistance to powdery mildew.

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