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Pathogenic Variation of *Pyrenophora teres* Isolates Collected from Japanese and Canadian Spring Barley

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Twenty-two isolates of *Pyrenophora teres* Drechs. collected from Japanese and Canadian spring barleys were inoculated to 38 barley varieties having various genetic backgrounds. The analysis of variance for the disease ratings showed that there were significant differences both in the virulence of isolates and the resistance of varieties. However, the interaction among isolates and varieties was not statistically significant. Both Finlay-Wilkinson regression analysis and principal component analysis by Additive Main effects and Multiplicative Interaction effects (AMMI) model classified the isolates into three groups, which were different in origins and symptom types. A spot type isolate was distinguished from net type isolates by its generally high virulence. A slight pathogenic differentiation was suggested between Japanese and Canadian net type isolates.

Key words: *Hordeum vulgare*, *Pyrenophora teres*, Barley, Net blotch, Race differentiation

INTRODUCTION

Net blotch is a common foliar disease of barley caused by *Pyrenophora teres* Drechs. In Japan, the outbreak of net blotch in barley has been acknowledged in some restricted growing areas and the damage on spring barley has been severe.

In net blotch, two types of symptoms have been reported. One group of isolates gives a typical net-like symptom, whereas another type gives a spot-like circular symptom without netting. In the net type disease, four major genes conferring resistance have been identified in the host (Davis *et*

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al. 1990) and a partial resistance or a quantitative resistance of the host has also been reported (Arabi *et al.* 1990).

For the expression of disease resistance, a gene-for-gene system has been proposed. In this theory, for each gene conditioning resistance in the host, there is a specific gene conditioning pathogenicity in the parasite. After Flor (1956) discovered this system with the flux and flux rust, several gene-for-gene systems have been demonstrated in other host-parasite relationships. Khan and Boyd (1969) reported three physiologic races in net blotch, and Khan (1982) also found changes in the pathogenicity of the fungus population according to changes of barley cultivars. Harrabi (1990) reported a virulence spectrum in the isolates from the Mediterranean region. Pathogenic variation in the spot type isolates was also reported by Karki and Sharp (1986).

Because many major-genic resistances in the host have not been sustainable due to the appearance of new virulent forms of the pathogen, knowledge of pathogenic variation of the parasite is of primary importance in the breeding program for disease resistance. The purpose of this study is to investigate the pathogenic variation among isolates of *P. teres* collected from Japanese and Canadian spring barleys and to acquire the information for race differentiation of the disease.

MATERIALS AND METHODS

1. *Pyrenophora isolates*

Twenty-two *P. teres* isolates, collected from Japanese and Canadian spring barleys were used for this investigation (Table 1). All Japanese isolates and three Canadian isolates formed typical net type symptoms, and one Canadian isolate (No.22) formed a spot type symptom.

For preparing the isolates, the surface of infected barley leaves was sterilized and put onto sterilized wet filter paper in petri dishes, and incubated at 20 °C for about three days to produce conidia. Individual conidia were transferred onto 1 % water agar in petri dishes and incubated at 20 °C for the germination. The tips of hyphae were transferred to the potato dextrose agar medium slanted in test tubes. After hyphae had spread on the surface of media, isolates were stored at 5 °C.

2. *Barley varieties*

Barley varieties and lines were selected to include a wide range of disease resistance observed in a screening test using the isolate K105 (Sato and Takeda 1992). These varieties and lines were supplied from the Barley

Table 1. *P. teres* isolates tested and their characteristics in the pathogenic reaction on the host barley varieties

No. & name of isolates	Origin	Place of collection	Mean disease rating[2]	Regression[3] b	Regression[3] r	Component[4] z1	Component[4] z2
1 K001	Japan	Kitami	5.2	1.13	0.96	4.92	-1.50
2 K005	Japan	Kitami	4.6	1.05	0.96	-0.44	1.14
3 K006	Japan	Kitami	4.7	1.14	0.96	4.34	2.67
4 K009	Japan	Kitami	4.8	1.21	0.95	7.05	2.39
5 K010	Japan	Kitami	4.3	1.07	0.98	0.90	-0.37
6 K101	Japan	Abashiri	5.1	1.05	0.92	-0.56	5.32
7 K103	Japan	Abashiri	4.1	1.04	0.95	0.56	-0.49
8 K105	Japan	Abashiri	5.3	1.09	0.96	3.38	-2.26
9 K106	Japan	Abashiri	4.5	1.10	0.96	4.17	-0.21
10 K107	Japan	Abashiri	5.1	1.05	0.96	3.39	-2.18
11 K108	Japan	Abashiri	5.1	0.96	0.95	-1.26	1.70
12 K110	Japan	Abashiri	4.8	1.09	0.95	4.17	-3.27
13 K111	Japan	Abashiri	4.5	1.16	0.97	5.64	-0.75
14 K112	Japan	Abashiri	4.6	1.11	0.95	5.09	-1.44
15 K116	Japan	Abashiri	5.2	1.10	0.92	5.22	-0.11
16 K117	Japan	Abashiri	4.7	1.00	0.96	0.29	-0.86
17 K118	Japan	Abashiri	5.0	1.24	0.97	7.49	-1.16
18 K122	Japan	Abashiri	4.2	1.05	0.97	2.71	0.80
19 WRS102	Canada	Saskatchewan	3.7	0.71	0.81	-11.73	3.33
20 WRS1581	Canada	Alberta	5.1	0.60	0.72	-14.80	6.17
21 WRS1607	Canada	Alberta	5.0	0.70	0.77	-13.80	5.00
22 WRS1566[1]	Canada	Alberta	6.4	0.31	0.50	-16.73	-13.93

1): Spot type symptom
2): 1 (Resistant)-10 (Very Susceptible).
3): According to Finlay-Wilkinson model. All correlation coefficients are significant at the 1% level.
4): PCA by AMMI model.

Germplasm Center of Okayama University, Kitami Agricultural Experiment Station and USDA Small Grain Collection. They originated from various areas of the world and included many different types of characters, such as covered or naked and row types (Table 2), which are important key characters for the differentiation of barley varieties.

3. *Inoculation method*

Five seeds each of 38 barley varieties were planted in each seedling box (50 × 35 × 10 cm) which was filled with the soil. The seedlings were grown in a glasshouse at ca. 20 °C for 14 days. At the time of inoculation, seedlings were at the second leaf stage. Inoculation tests were conducted in the spring and the autumn of 1990.

Pyrenophora isolates were cultured on the V-8 medium (V-8 juice 200 ml, $CaCO_3$ 3g, agar 15g, water 800 ml) in 9 cm styrene plastic petri dishes under

Table 2. Origin and characteristics of barley varieties

Variety no. & name	Accession No.	Origin	Covered(+) or naked(n)	Row type	Mean disease rating[1)]
1 Aichi yokozuna	OUJ049	Japan	+	6	2.3
2 Kaikei 39	OUJ068	Japan	+	6	3.1
3 Sazanshu	OUJ097	Taiwan	+	6	3.8
4 Wasemugi	OUJ132	Japan	+	6	2.3
5 Mochimugi	OUJ224	Japan	n	6	5.0
6 Nikaku chevalier	OUJ603	Japan	+	6	7.3
7 Tankawa	OUJ734	Japan	+	6	3.0
8 Kinai 67	OUJ799	Japan	+	6	3.2
9 Konosu 50	OUJ804	Japan	+	6	3.4
10 Hoshimasari	OUJ829	Japan	+	2	6.4
11 Aominori	—	Japan	+	2	9.3
12 Hokuiku 17	—	Japan	+	2	9.6
13 Jeonnam omugi shin 1	OUK016	Korea	+	6	2.2
14 Masan naked 1	OUK043	Korea	n	6	4.9
15 Haman waedong 2	OUK046	Korea	n	6	3.3
16 Sinanju covered 2	OUK114	Korea	+	6	3.7
17 Harumaki rokkakumugi	OUK121	Korea	+	6	4.7
18 Goweon covered 1	OUK717	Korea	+	6	4.9
19 Lissu chiao 1	OUC323	China	+	6	2.9
20 Tibet white 25	OUC357	China	+	6	3.7
21 4887-3	OUC613	China	+	2	5.3
22 Harbin 2-row	OUC649	China	+	2	9.0
23 Katomandu 3	OUN026	Nepal	+	6	7.3
24 Trisui Bazar 8	OUN339	Nepal	+	6	5.8
25 Kagbeni 2	OUN352	Nepal	+	6	2.2
26 Kagbeni 3	OUN358	Nepal	n	6	2.0
27 N113	OUI085	India	n	6	7.2
28 Kabul 10	OUI319	Afghanistan	+	6	4.1
29 C231	OUI424	India	+	6	4.2
30 Yardar 2	OUI755	Afghanistan	+	6	6.0
31 Turkey 45	OUT615	Turkey	+	2	6.0
32 Abusir	OUB061	Egypt	+	6	3.2
33 CSR234a	OUU158	Czechoslovakia	+	2	4.7
34 Kombainiesis	OUU689	United Kingdom	+	2	8.2
35 Canadian lake shore	CI2750	Unknown	+	6	5.3
36 Tifang	CI14373	Unknown	+	6	5.3
37 Ming	CI4797	China	+	6	5.6
38 CI9819	CI9819	Ethiopia	+	2	2.2

1): 1 (Resistant) -10 (Very Susceptible).

an alternating irradiation period of 12hr black light (Toshiba Co., range of wave length : 300-400nm) and 12hr darkness at diurnal temperature variation of 25 ± 6 °C for 14 days (Sato and Takeda 1991). After addition of water, mycelia, conidiophores and conidia were collected with brushes and filtered through double layers of gauze to remove mycelia and conidiophores from the conidia suspension. The concentration of the suspension was standardized at 5~10 × 10^4/ml. After addition of a drop of Tween-20, the suspension was sprayed onto seedlings with a glass atomizer driven by an electric air pump. Inoculated plants were maintained in a dew chamber at 20°C for 48hr and grown 10 days in a glasshouse at 20 °C. The second leaf of the inoculated plants was scored by a numerical disease rating, 1 (resistant) to 10 (very susceptible), proposed by Tekauz (1985). The average score obtained from five plants was used to indicate the resistance of the variety.

4 . *Statistical analysis*

The analysis of variance for a randomized block design was applied to the disease ratings regarding two separate inoculation tests, which were conducted in the spring and the autumn, as replications. For further interpretation of isolate-variety interactions, Finlay-Wilkinson regression model (Finlay and Wilkinson 1963) and a principal component analysis by Additive Main effects and Multiplicative Interaction effects (AMMI) model (Crossa 1990) were applied.

RESULTS

1 . *Analysis of variance for disease rating*

The mean disease ratings of the isolates ranged from 3.7 to 6.4 (Table 1). The most and the least virulent isolates were from Canada, while the Japanese isolates showed relatively similar levels of virulence.

As shown in Table 2, the variation of mean disease ratings in barley varieties was very wide compared to those in isolates. The wide range of mean disease ratings from 2.0 to 9.6 suggested that the hosts included both extreme resistant and susceptible varieties. The resistant varieties (with a rating of less than 2.5) were variable in origin and row type, whereas all the susceptible varieties (with a rating of more than 9.0) were two-rowed spring barley of Hokkaido.

The analysis of variance showed that there were significant differences both in the virulence of isolates and in the resistance of varieties, whereas no significant interaction was observed among isolates and varieties (Table 3). The difference between replications (4.72 vs. 4.89) was statistically significant, though quite small.

2. *Finlay-Wilkinson regression analysis*

To characterize the virulent reaction of each isolate, regression analyses were conducted by using an isolate-variety matrix of disease ratings ; for 22

Table 3. Analysis of variance for isolates, variety, and interactions by Finlay-Wilkinson regressions and principal components with AMMI model

Factor	df	SS	MS
Isolate	21	458.79	21.85**
Variety	37	7,121.12	192.46**
Isolate × Variety	777	1,409.74 (100%)	1.81
Regression	21	337.40 (24%)	16.07**
Deviation from regression	756	1,072.34 (76%)	1.42
Component 1	57	564.55 (40%)	9.90**
Component 2	55	171.10 (12%)	3.11**
Residual	665	674.09 (48%)	1.01
Replication	1	11.23	11.23*
Error	835	1,509.27	1.81

**, * : Significant at the 1% and 5% levels, respectively.

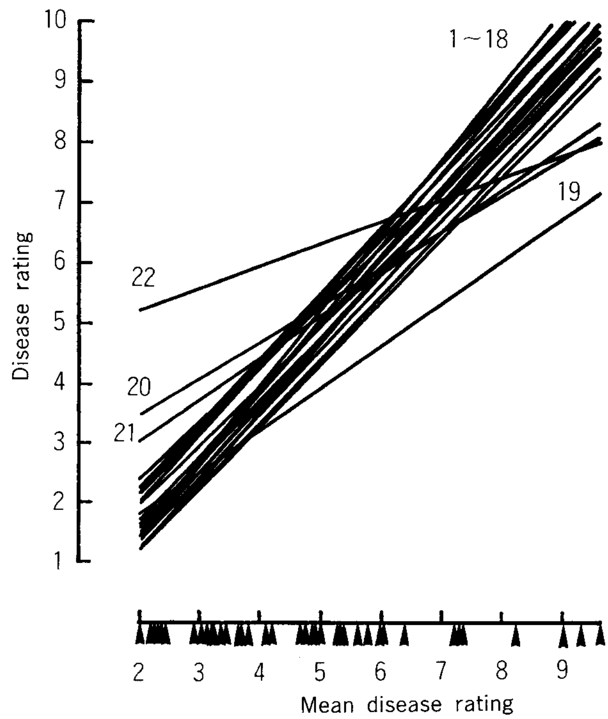

Fig. 1. Regression lines of each rating on mean ratings per variety for 22 individual *P. teres* isolates.
The symbols on the abscissa are mean ratings of varieties.
Names of isolates: cf Table 1

individual isolates, regression of each ratings on mean ratings per variety was calculated (Fig. 1). Table 3 shows the analysis of variance for the regression. The regression was highly significant both against the error and the deviation from regression.

Table 1 shows the regression coefficients (b) and the correlation coefficients (r). In most Japanese isolates, the regression coefficients were above 1.0 and the correlation coefficients were highly significant. On the other hand, the regression coefficients of Canadian isolates were under 1.0, and their correlation coefficients were lower than those of Japanese ones. This findings indicated that Japanese isolates gave a sharper regression line and little deviation from regression in comparison with Canadian isolates. WRS102 showed the lowest mean disease rating and a lower regression coefficient suggesting its virulence was low to all the varieties, while WRS1566 showed a higher mean disease rating and the lowest regression coefficient indicating a universal high virulence to all the varieties tested.

3. *Principal component analysis*

A principal component analysis (PCA) with the AMMI model was applied to the isolate-variety interaction matrix of disease ratings. The contribution of each component was 40 % for component 1 and 12 % for component 2 (Table 3). Table 3 shows that these two components were highly significant against the error, while the third and following components were not statistically significant and thus they were pooled as the sum of square of the residual.

Table 1 shows the component scores for each of the isolates. Differences were clear between Japanese and Canadian isolates in component 1 (z1), and between net type and spot type isolates in component 2 (z2). On the z1-z2 plane isolates were grouped into three, namely Japanese net type, Canadian net type and Canadian spot type (Fig. 2).

Table 4 shows the correlation coefficients among mean disease ratings, Finlay-Wilkinson regression coefficients and principal components; the scores of z1 were closely correlated with the regression coefficients (b), and the scores of z2 were significantly correlated with the mean disease ratings.

The same PCA was applied to the transposed matrix of the isolate-variety interaction. The sum of squares of components and the contribution of each component were the same as in Table 3. Fig. 3 shows the scatter diagram for barley varieties on z1 and z2 axis of PCA. The distribution of varieties was continuous. The varieties that showed the most extreme values in z1 were C231 (No. 27) and N113 (No. 29) from India.

Table 4. Correlation coefficients among mean disease rating, Finlay-Wilkinson regression coefficient (b) and principal components (z1, z2) for the isolates

Item	b	z1	z2
Mean	-.403	-.258	-.498*
b		.963**	-.234

*, ** : Significant at the 5% and 1% levels, respectively.

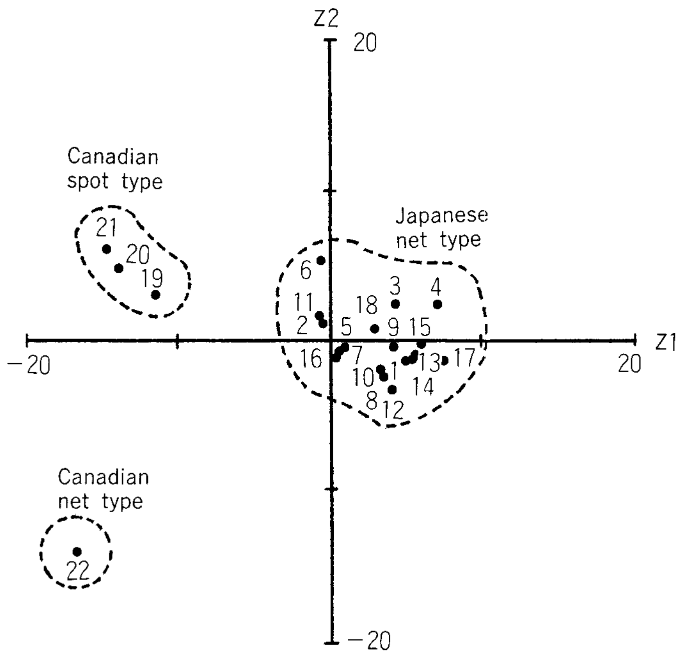

Fig. 2. Scatter diagram of P. teres isolates on z1 and z2 of PCA. Names of isolates: cf Table 1

DISCUSSION

As shown in Table 3, the variation of isolates and varieties tested was very wide. Especially, barley varieties tested were from various sources and included known resistant genes (Khan and Boyd 1971, Bokelman *et al.* 1977, Davis *et al.* 1990). However, the interaction between varieties and isolates was not statistically significant.

Although the interaction was not significant, three groups of isolates were detected by the PCA adapted for the interaction matrix, namely Japanese, Canadian net type, and Canadian spot type isolates (Fig. 2). The spot type isolate was clearly distinguished from net type isolates. As the z1 component can separate Japanese and Canadian net type isolate groups, group averages were calculated for varieties with the highest z1 score (No. 29, Japanese : 3.5 Canadian : 7.7) and the lowest score (No. 27, Japanese : 7.8 Canadian : 4.5) in Fig. 3. This indicated the existence of a moderate interaction between Japanese and Canadian net type isolate groups against two varieties with an extreme reaction.

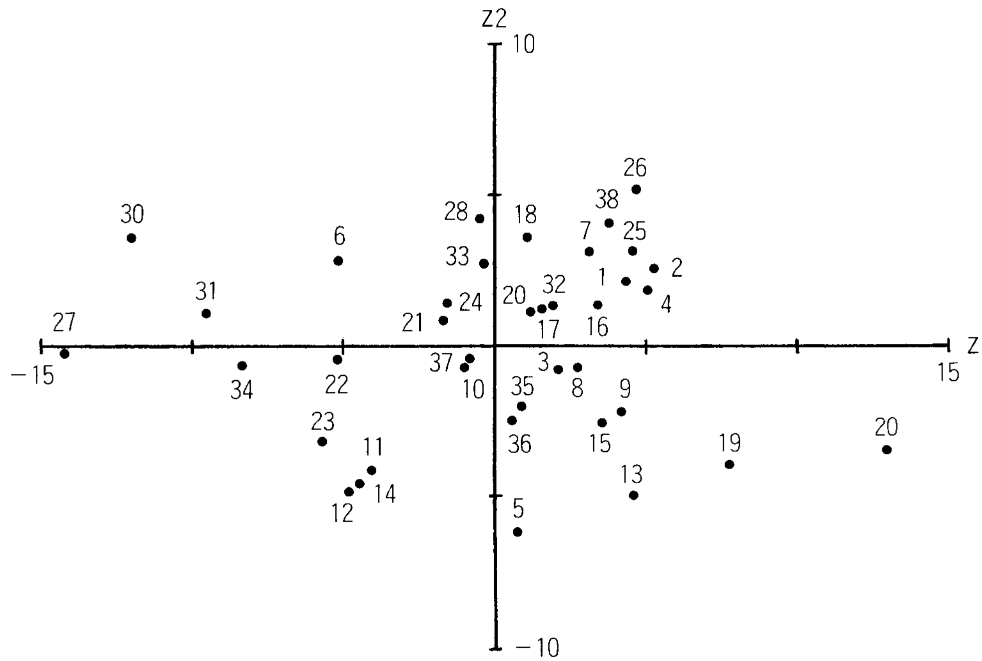

Fig. 3. Scatter diagram of barley varieties on z1 and z2 of PCA.
Names of varieties : cf Table 2

The correlation coefficient among Japanese isolates were high (0.831 ~0.969) indicating that all Japanese isolates infected barley varieties in parallel (Table 5). Among the Canadian net type isolates, the variation was small (r=0.787~0.833). However, the Japanese and Canadian net type isolates infected not always in parallel (r=0.700 in average) suggesting some degree of pathogenic differentiation.

Table 5 Correlation coefficients of disease ratings within and between isolate groups

Isolate group	Japanese net	Canadian net	Canadian spot
Japanese net	.831~.969 (.917)	.601~.814 (.700)	.398~.538 (.455)
Canadian net		.787~.833 (.817)	.302~.441 (.391)

Figures in parentheses indicate the mean of coefficients.

Within the net type isolates, the least correlation (r=0.601) was found between Japanese isolate K116 and Canadian isolate WRS1581. As shown in Fig. 4, two varieties No. 19 and No. 29 seemed to lower the correlation

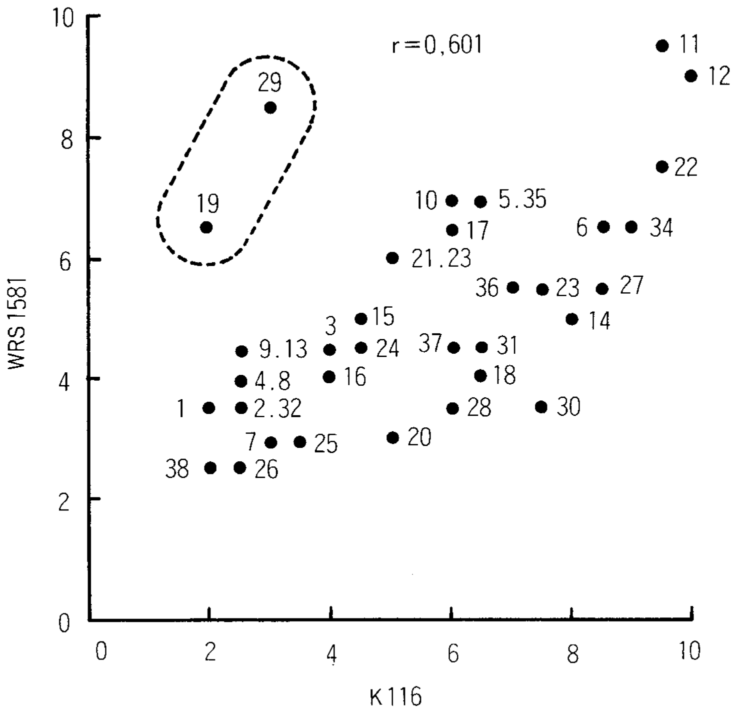

Fig. 4. Scatter diagram for disease ratings of barley varieties for isolates K116 and WRS1581.
Names of varieties: cf Table 2

coefficient. In general, it may be concluded that pathogenic differentiation within net type isolates is small.

However, the Canadian spot type isolate showed a lower correlation (r= 0.302~0.538) with all other isolates suggesting this spot type isolate has different pathogenicity from net type isolates.

As the extent of race differentiation is dependent upon the range of the materials, it is difficult to draw a definite conclusion on the presence or absence of an obvious race differentiation among net type isolates. However, within the range of isolates, an incomplete resistant-susceptible reversal was indicated. One possible explanation for this phenomenon is that the reverse reaction of the major resistance gene was modified by polygenes determining the horizontal resistance (Van der Plank 1968).

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日本とカナダの春播オオムギから採取した大麦網斑病菌株の病原性の変異

佐藤和広・武田和義

北海道およびカナダの春播オオムギから採取した22の大麦網斑病菌株（*Pyrenophora teres* Drechs.）を世界各地のオオムギ38品種に幼苗接種し，病斑指数によって病原性の変異を検討した．

分散分析の結果，菌株の病原力ならびに品種の抵抗性には有意差が認められたが，菌株と品種の交互作用は統計的に有意でなかった．

各菌株の反応を Finlay-Wilkinson (1963) の回帰分析によって解析したところ，北海道とカナダの菌株で病原性反応に差が認められた．この傾向はカナダの菌株のうち，通常の Net type の菌株よりも斑点状病斑を示す Spot type の菌株で顕著であった（Fig. 1）．

さらに，菌株と品種の交互作用を詳細に解析するために相加作用と相乗交互作用モデル（AMMI モデル）を適用して交互作用に関する主成分分析を行った結果，各菌株は日本の Net type，カナダの Net type およびカナダの Spot type の3群に分けられた（Fig. 2）．各菌株について群間ならびに群内の相関係数を算出したところ（Table 5），Net type の菌株相互の相関係数は0.601～0.969と相対的に高かったが，一部の菌株と品種の組合せでは抵抗性反応の逆転がみられた（Fig. 4）．一方，Spot type の菌株と Net type の菌株の相関係数は0.302～0.538と低く，両者の病原性は多少異なることが示された．

このような一部の菌株と品種の間に認められる弱い交互作用は，抵抗性を支配する主働遺伝子の特異的な反応が，いわゆる圃場抵抗性を支配する微働遺伝子の作用によって修飾された結果と考えられる．

キーワード：*Hordeum vulgare*, *Pyrenophora teres*，オオムギ，網斑病，レース分化