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Survey of Resistance to Scald in World Collections of Cultivated Barley

Toshinori FUKUYAMA and Hiroyuki TAKEDA

Faculty of Agriculture, Niigata University, Niigata, 950-21

A total of 1315 barley cultivars originating from various regions of the world were tested for their resistance to scald (*Rhynchosporium secalis* (OUD.) DAVIS). Screening of the resistant cultivars was conducted by the application of two testing methods consisting of natural inoculation for the mature plants and artificial inoculation for the seedlings. In both tests, the disease reaction on a plant was classified into three types as follows; highly resistant (R), moderately resistant (M) and susceptible (S) reactions. In the field test for mature plants, 79 cultivars were found to be R and most of them were of Ethiopian and Turkish origin. There were only a few resistant cultivars of East Asian origin (Table 2). In the artificial inoculation test for the seedlings, 48 cultivars showed a highly resistant reaction, almost all of them being of Ethiopian and Turkish origin (Table 3). No cultivars with a R reaction were found in the East Asian materials. Finally, 17 cultivars were confirmed to be resistant in both tests for the mature plants and seedlings (Table 4). It was concluded that these 17 cultivars could become useful as genetic resources for resistance to scald.

KEY WORDS: *Hordeum vulgare, Rhynchosporium secalis,* barley, resistance, scald, screening, varietal variation.

Introduction

Barley scald caused by *Rhynchosporium secalis* (OUD.) DAVIS is a very important disease with a world wide distribution. The disease has recently become severe in Japan too, especially in the Hokuriku district, where the area of barley cultivation has been increasing due to the curtailment of rice production (SUZUKI and ARAI 1990). The main cultivar grown in the Hokuriku district is Minorimugi which is highly affected by the scald. The farmers exclusively depend upon the use of fungicides for the control of the disease, which is economically disadvantageous to the farmers and also results in environmental pollution due to fungicide use. Therefore, the promotion of a breeding programme to develop cultivars with resistance to scald is particularly important. To implement this programme, it is necessary to survey genetic resources for resistance to scald and carry out studies on the inheritance of resistance.

Many studies have been carried out on the genetic mechanism of resistance to scald and a total of 11 resistance genes have been identified (BAKER and LARTER 1963, DYCK and SCHALLER 1961, HABGOOD and HAYES 1971, MACKIE 1929, RIDDLE and BRIGGS 1950, WELLS and SKOROPAD 1963). All of them, however, were mainly detected in materials originating from Europe and the U.S.A. Moreover, the existence of physiologic races of *R. secalis* has been reported by many authors in various countries (ALI and BOYD 1973, ALI *et al.* 1976, BROWN 1985, 1990, CEOLONI 1980, JACKSON and WEBSTER 1976 and others).

In Japan, the physiology, epidemiology and pathogenic differentiation of *R. secalis* have been extensively investigated by OZOE (1956) and KAJIWARA and IWATA (1963). However, information on genetic resources for resistance and gene analysis is very limited. In the present

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study, a number of cultivars from various regions of the world were investigated in relation to their resistance to scald by the use of natural and artificial inoculation tests. The following results were obtained.

Materials and Methods

A total of 1315 barley cultivars collected from various regions of the world were tested for their resistance to scald. These cultivars were given by the courtesy of the Barley Germplasm Center, Research Institute for Bioresources, Okayama University. Screening of the resistant cultivars was conducted according to the following two methods; natural inoculation test for mature plants (Exp. 1) and artificial inoculation test for seedlings (Exp. 2).

1) Exp. 1

Fifteen grains of each cultivar harvested from disease-free plants were hill-seeded in the field at intervals of 30 cm, and the rows were 90 cm apart. The experimental plot consisted of 4 rows surrounded by the susceptible cultivar Minorimugi. The test was repeated in 1989 and 1990 under the same experimental design. Since the experimental plot was located in a field consistently infected with scald, the scald epidemic was allowed to develop naturally.

After the emergence of the flag leaf in all of the cultivars, the scald reaction on a plant was investigated on 2 May 1989 and 24 April 1990, and scored according to the following method modified from DYCK and SCHALLER (1961).

R; high resistance without visible lesions or with lesions smaller than 5 mm in diameter, with necrosis (DYCK and SCHALLER's scores 0,1).

M; moderate resistance with somewhat larger lesions from 5 to 10 mm in diameter, with necrosis (score 2).

S; susceptibility with large blue-gray lesions more than 10 mm in diameter, or coalescing lesions (scores 3,4).

2) Exp. 2

A *R. secalis* culture for artificial inoculation on seedlings was obtained from a single spore isolated from susceptible lesions on Minorimugi grown in the field test in 1989. This culture was maintained on a potato-agar medium with 2% sucrose at 18°C.

Five plants of each barley cultivar were grown in a flat (55 × 35 × 15 cm) supplied with 7, 5 and 6.5 g of N, P_2O_5 and K_2O, respectively, in a glasshouse at temperatures ranging from 15 to 25°C. Each flat contained 42 cultivars and susceptible check cultivar Minorimugi.

At the 1~2 leaf stage, a spore suspension of the cultivar described above was sprayed on the leaves of the plants at a density of 30 or 40 spores in a microscope field of view (10 × 15). The inoculated plants were transferred to a tent made of polyvinyl chloride kept at a high humidity with a humidifier, where the temperature ranged from 5 to 20°C. After 48 hr, the plants were retransferred to a glasshouse. Fourteen to 20 days after inoculation, the reaction of each cultivar was examined based on the same scale as that indicated in Exp. 1. Such inoculation experiments were repeated two times for the same cultivar. When there was a discrepancy in the reaction types between two replications, a weaker score was given to the cultivar.

Results

1) Exp. 1 (Test for mature plants)

Since the disease occurred severely in Minorimugi both in 1989 and 1990, the experimental field was considered to be suitable for the screening of the cultivars resistant to scald. Frequencies of the three reaction types in the world barley collections tested in those two years are shown in Table 1. In the 1989 experiment, the frequencies of highly and moderately resistant and susceptible cultivars were 136 (10.3%), 265 (20.2%) and 914 (69.5%), respectively. In the 1990 experiment, 92 cultivars (7.0%) showed a highly resistant reaction, the frequency of which was somewhat lower than that of the 1989 year's test. The frequencies of moderately resistant and susceptible cultivars were 166 (12.6%) and 1057 (80.4%), respectively. Among the 1315 cultivars, 1084 (82.4%) showed the same reaction type in the two years' tests, and the remaining showed a different reaction. Such a discrepancy in the reaction types resulted in cultivars showing a moderate resistance in either of the two years, and the opposite reaction from high resistance to susceptibility was found only in 26 cultivars (2.0%). When a difference in the reactions was found in a cultivar between the two years, a score for a weaker reaction was given to the cultivar. Table 2 shows these two years' data classified by the origin of the cultivars. Total frequencies of highly resistant, moderately resistant and susceptible cultivars were 79 (6.0%), 157 (11.9%) and 1079 (82.1%), respectively. Almost all of the cultivars originating from East Asia (Japan, Korea and China) and Nepal were susceptible to the disease. On the other hand, those originating from South West Asia, Europe and Africa included highly resistant cultivars, especially those from Ethiopia (20.4%).

2) Exp. 2 (Test for seedlings)

The results obtained from the artificial inoculation test for seedlings at the 1~2 leaf stage are shown in Table 3. The frequency of susceptible cultivars amounted to 1223 (93.0%) in this screening test, which was higher than that of the field test (82.1%, Table 2). All the cultivars originating from Japan, Korea, China and Nepal were susceptible. Forty eight cultivars from S.W. Asia, Europe and Africa showed a highly resistant reaction. A large part of them were of Ethiopian and Turkish origin, although the frequency of Ethiopian resistant

Table 1. Frequencies of highly resistant (R), moderately resistant (M) and susceptible (S) reactions in the field test conducted in 1989 and 1990

		Reaction in 1989			
		R	M	S	Total
Reaction	R	79(6.0%)[1)]	13(1.0)	0(0.0)	92(7.0)
in	M	31(2.4)	113(8.6)	22(1.7)	166(12.6)
1990	S	26(2.0)	139(10.6)	892(67.8)	1057(80.4)
Total		136(10.3)	265(20.2)	914(69.5)	1315(100.0)

[1)] Numerals in parentheses indicate the frequency in percentage to the grand total.

cultivars decreased markedly as compared with that of the field test. In contrast, among the 21 highly resistant Turkish cultivars in the test for seedlings, 11 were found to be moderately resistant in the field test and 4 were susceptible. Only 6 Turkish cultivars were resistant in both tests for mature plants and seedlings.

Results of the comparison of the reaction types in the mature plants and in the seedlings are shown in Table 4. A total of 1089 cultivars (82.8%) showed the same reaction in both tests. The remaining cultivars showed different reactions between the tests for mature plants and seedlings, mainly associated with the downgrading of high or moderate resistance in the

Table 2. Geographical difference in the frequency of cultivars with R, M and S reaction types in the field test for mature plants

Origin	Reaction type			No. of cvs tested
	R	M	S	
Japan	1(0.4%)[1)]	6(2.6)	223(97.0)	230
Korea	0(0.0)	0(0.0)	154(100.0)	154
China	0(0.0)	3(4.9)	58(95.1)	61
Nepal	0(0.0)	0(0.0)	85(100.0)	85
S.W.Asia	1(2.4)	1(2.4)	39(95.1)	41
Turkey	15(5.7)	29(10.9)	221(83.4)	265
Europe	3(2.9)	5(4.8)	97(92.4)	105
N.Africa	2(2.9)	8(11.6)	59(85.5)	69
Ethiopia	57(20.4)	103(36.8)	120(42.9)	280
Others	0(0.0)	2(8.0)	23(92.0)	25
Total	79(6.0)	157(11.9)	1079(82.1)	1315

1) Numerals in parentheses indicate the frequency in percentage within each region.

Table 3. Geographical difference in the frequency of cultivars with R, M and S reaction types in the artificial inoculation test for seedlings

Origin	Reaction type			No. of cvs tested
	R	M	S	
Japan	0(0.0%)[1)]	0(0.0)	230(100.0)	230
Korea	0(0.0)	0(0.0)	154(100.0)	154
China	0(0.0)	0(0.0)	61(100.0)	61
Nepal	0(0.0)	0(0.0)	85(100.0)	85
S.W.Asia	1(2.4)	0(0.0)	40(97.6)	41
Turkey	21(7.9)	9(3.4)	235(88.7)	265
Europe	3(2.9)	4(3.8)	98(93.3)	105
N.Africa	4(5.8)	3(4.4)	62(89.9)	69
Ethiopia	19(6.8)	28(10.0)	233(83.2)	280
Others	0(0.0)	0(0.0)	25(100.0)	25
Total	48(3.7)	44(3.4)	1223(93.0)	1315

1) Numerals in parentheses indicate the frequency in percentage within each region.

mature plants to susceptibility in the seedlings (167, 12.7%). On the other hand, a few cultivars with a moderate resistance or susceptibility in the field test showed a high resistance in the test using seedlings. As a result, only 17 cultivars out of 1315 were found to be highly resistant in both tests for the mature plants and seedlings; 9 from Ethiopia, 6 from Turkey, 1 from S.W. Asia, and 1 from N. Africa. All of them exhibited hulled and long-awned spikelets with a lax spike density. Other main morphological characters of these cultivars are shown in Table 5. Two of them showed a 2-rowed head, 12 a 6-rowed head and the remaining 3 belonged to the "irregular" head type. They all showed a spring growth habit except for 2 Turkish cultivars. According to TAKAHASHI *et al.* (1983), these 17 cultivars exhibited a medium or late heading time as shown in the table. It is noteworthy that all of the Ethiopian resistant cultivars belonged to the short-haired rachilla type.

Discussion

In the present study, the screening of resistant cultivars to barley scald was investigated by the application of two methods; natural inoculation test in the field for the mature plants and artificial inoculation test in glasshouse for the seedlings. Our experimental field was covered with a layer of snow 0.5~1 m deep from the end of December to February in the following year. These weather conditions were favorable for the development of scald (SUZUKI and ARAI 1990), and, in fact, the disease occurred severely in the experimental field. Therefore, the reaction to scald of a number of cultivars could be easily evaluated. It should be noted here that since the estimation of the disease reaction in the field test for mature plants was qualitative and not quantitative, the assessment of horizontal resistance was not taken into consideration in the present study.

In some cultivars, there was a discrepancy in the disease reaction between the two tests for mature plants and seedlings, which may be caused by the difference in the growth stage of the materials and/or overlooking of the lesions on plants under the field conditions. However, 1089 (82.8%) out of 1315 cultivars showed the same disease reaction in the two testing methods. ABBOTT *et al.* (1991) reported the presence of a highly positive correlation between the scald rating under field conditions and the seedling infection type in glasshouse conditions. KHAN *et al.* (1984) confirmed the high reproducibility of the scald scores between

Table 4. Comparison of reaction types obtained from the natural inoculation test for mature plants and artificial inoculation test for seedlings

		Reaction in seedlings			
		R	M	S	Total
Reaction in mature plants	R	17(1.3%)[1)]	12(0.9)	50(3.8)	79(6.0)
	M	24(1.8)	16(1.2)	117(8.9)	157(11.9)
	S	7(0.5)	16(1.2)	1056(80.3)	1079(82.1)
Total		48(3.7)	44(3.4)	1223(93.0)	1315

[1)] Numerals in parentheses indicate the frequency in percentage to the grand total.

Table 5. Morphological characters of the cultivars with high resistance to scald through two inoculation tests for mature plants and seedlings

OU No.[1]	Origin	Name	Ear type[2]	Growth type[3]	Heading time[4]	Rachilla hair type
OUI 025	S.W.Asia	Atrantic trailer	6	S	M	Short
OUT 008	Turkey	2022	6	S	L	Short
OUT 030	Turkey	2088	2	W	VL	Long
OUT 031	Turkey	2091	6	W	VL	Long
OUT 070	Turkey	2208	2	S	VL	Long
OUT 086	Turkey	2256	6	S	L	Short
OUT 090	Turkey	2268	6	S	L	Short
OUB 024	N.Africa	Carre 26	6	S	L	Long
OUE 023	Ethiopia	4067	ir	S	VL	Short
OUE 134	Ethiopia	4402	ir	S	VL	Short
OUE 140	Ethiopia	4420	6	S	M	Short
OUE 223	Ethiopia	Debre Zeit 22	6	S	L	Short
OUE 226	Ethiopia	Debre Zeit 31	6	S	M	Short
OUE 233	Ethiopia	Addis Ababa 4	ir	S	L	Short
OUE 244	Ethiopia	Addis Ababa 37	6	S	L	Short
OUE 249	Ethiopia	Addis Ababa 52	6	S	L	Short
OUE 254	Ethiopia	Suluta 1	6	S	L	Short

[1] Accession number of Okayama University.
[2] 2, 6 and ir refer to 2-rowed, 6-rowed and irregular type, respectively.
[3] S and W refer to spring- and winter-growth habit, respectively.
[4] M, L and VL refer to medium, late and very late in heading time, respectively. These data are cited from TAKAHASHI *et al.* (1983).

the replications in the field test. Then, it is considered that the field test method applied in the present study was suitable for the screening of cultivars or lines resistant to scald.

Cultivars which originated from various regions of the world could be classified into two groups according to the frequency of resistance; one group consisted of cultivars originating from East Asia (Japan, Korea and China) and Nepal, almost all of which were susceptible. The other group consisted of cultivars originating from S.W. Asia, Europe and Africa, out of which some were resistant to scald. TAKAHASHI (1955) indicated that cultivated barley could be classified into two groups, Oriental and Occidental types, based on the investigation of several morphological and physiological characters. Such a varietal differentiation approximately corresponds to the grouping of cultivars resistant to scald in the current study. These observations suggest that the Oriental and Occidental types of cultivated barley differ in the gene(s) controlling vertical resistance to the causal fungus, leading to differentiation of the fungus. HIURA (1960) showed that the pathogenicity of *Erysiphe graminis* could be separated into two groups corresponding to the varietal differentiation of barley through the co-evolution between host and parasite. Such a differentiation in pathogenicity was also confirmed in *Pyricularia oryzae* in relation to the phylogeny of *Indica* and *Japonica* rice (MORISHIMA 1969). In addition to the differentiation of the pathogenicity in *R. secalis* described above, many physiological races were reported in various countries, as mentioned previous-

ly. In the Hokuriku district, KAJIWARA and IWATA (1963) recognized the existence of 5 different races.

The present investigation revealed that a total of 17 cultivars was highly resistant to scald in inoculation tests for both mature plants and seedlings when only one isolate of *R. secalis* was used for inoculum. Although further investigations on the genic analysis of these 17 cultivars and allelic tests with known resistance genes should be conducted, it appears that these cultivars are useful breeding materials for resistance to scald. Inoculation tests for the seedlings of these 17 cultivars are underway using various cultures of *R. secalis* collected in the Hokuriku district.

A moderate resistance to scald was detected in a number of cultivars in the present study. However, since the expression of the resistance was unstable between the years tested, it remains to be determined whether the cultivars with this moderate resistance can be used as genetic resources.

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世界各地産の栽培オオムギ品種における雲形病抵抗性の探索

福山利範・竹田宏行

(新潟大学農学部, 新潟市, 〒950-21)

最近, 北陸地方では水田転換作物として栽培面積の増加したオオムギで, 雲形病が多発している. これは, 当地方のほぼ全域で栽培されている品種ミノリムギが, 本病に極弱であることによる. 従って, 本病に抵抗性の品種の育成・導入が強く望まれる.

本研究では, 抵抗性品種育成の基礎知見を得る目的で, 世界各地産の栽培オオムギ1315品種における抵抗性の差異を調べた. 1989, 1990年の2カ年に行った圃場試験では, 試験区に点播された供試品種を罹病性のミノリムギで囲み, これを感染源とした. また, 1990年にはミノリムギの病斑より単胞子分離した菌を, 1～2葉期の幼苗に噴霧接種し, その反応を調査した. 両試験とも, 感染型を高度抵抗性 (R), 中度抵抗性 (M) および罹病性 (S) に3群別した.

2カ年の圃場試験の結果によれば, 両年ともR反応を示した品種は, わずかに79 (6.0%) であり, 大部分の品種 (892品種) は, 2カ年とも罹病性を示した. これらの他に, M反応や年次によって異なる反応を示す品種も認められた (Table 1). 抵抗性の程度を品種の産地別にみると, R反応を示した品種の大部分は, エチオピアおよびトルコ産であった. とくに, エチオピア産の品種は, M反応の頻度も高く (36.8%), 罹病性品種が42.9%と他に比べてかなり少なかった. 日本を含む東アジア地域の品種では, 2カ年を通じてR反応を示たのは, わずかに1品種であり, ほとんどすべての品種が罹病性であった (Table 2).

幼苗試験では, 48品種がR反応を示したが, この場合もエチオピア, トルコ産の品種が大部分で, 東アジア由来の品種には全く見出せなかった. 圃場試験の結果と比べると, M反応の品種は44 (3.4%) と少なく, 逆にS反応の品種が1223 (93.0%) と多かった (Table 3).

圃場および幼苗の両試験で, ともにR反応を示した品種は17で, その産地別内訳は, エチオピア9, トルコ6, 北アフリカおよび西南アジア1つであった (Table 4, Table 5). これら17品種は, 雲形病抵抗性の遺伝資源として有用と思われる. 現在, 北陸各地域の罹病個体から採取した菌の接種試験により, これら17品種の抵抗性の有効性程度を確認している.