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Maria Prończuk, Lucjan Madej, Irena Kolasińska

Plant Breeding and Acclimatization Institute, Radzików, Poland

RESEARCH FOR RESISTANCE TO *MICRODOCHIUM NIVALE* AMONG INBRED LINES OF RYE

ABSTRACT

Three hundred twelve inbred lines originated from the hybrid breeding program and two open pollinated cultivars: Zduno and Wibro as a standard were screened for resistance to *Micodochium nivale* during five years at Radzików. In each year about thirty to hundred inbred lines were evaluated under controlled condition in the cold chamber and parallel in the field. Selected lines were repeated in series of testing in subsequent years. Twenty three lines were tested during three years, sixteen during four years and seven during five years. Significant variation was found for disease rating among investigated inbred lines. Majority of them were severe damaged by *M. nivale* in all tests. None of tested lines showed an immune reaction to infection. Relative lowest damage were observed in standard cultivars, but a few inbred lines in each test showed the plant loses on the standard cultivars level. Five the best lines selected in 3 year testing confirmed their value in 4 year testing as well. One line which was tested during 5 years showed its high resistance in all series. This results indicate that selection of inbred lines with higher level of resistance to *M. nivale* is possible by multiyear testing. A positive correlation was found between the disease index of genotypes in cold chamber test and in field test. The coefficients of correlation were much higher in two years of testing than in three other.

Key words: Inbred lines, *Microdochium nivale*, resistance, rye, screening, *Secale cereale*, snow mould.

INTRODUCTION

Microdochium nivale (Fr) Samuels and Hallett is a widespread fungal pathogen which causes snow mould disease of cereals and grasseses. Snow mould decreases considerable grain yield of winter rye (*Secale cereale* L.) in Poland in some years, especially when snow covers unfrozen ground in late autumn or early winter (Bojarczuk and Bojarczuk, 1972; Chojnacka, 1980; Jańczak, 1980). Breeding of rye population cultivars for resistance to *M. nivale* were carried out in Poland with remarkable achievements (Bojarczuk *et al.*, 1990; Koczowska, 1993). Recently large number of rye inbred lines were developed for hybrid breeding program in the Plant Breeding and Acclimatization Institute at Radzików. During last winter some lines were severely infected by *M.*

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nivale despite of mild winter, and lack of snow cover. This observation indicated that the snow mould problem can arise during seed production of the hybrid components (multiplication of inbred lines). Preliminary study showed significant genotype variation among the lines in resis–tance to *M. nivale*. High heterosis effects of resistance in F_1 hybrids were observed (Madej and Prończuk, 1995).

The objective of the study was to determine the level of resistance to *M. nivale* of rye inbred lines developed at Radzików.

MATERIAL AND METHODS

Evaluation of the inbred lines resistance to *M. nivale* was conducted in the laboratory according to the modified cold chamber method (Prończuk and Madej, 1998) and parallel in the field test.

Plant material

Three hundred twelve inbred lines from the IHAR hybrid breeding program and two open pollinated standard cultivars: Zduno and Wibro were tested, during winter months in 1998 – 2002 at Radzików. In each year about thirty to hundred inbred lines were evaluated under con–trolled condition in cold chamber and parallel under field conditions. Selected lines were repeated in series of testing in subsequent years. Twenty three lines were tested during three years, sixteen during four years and seven during five years

Cold chamber test

Seeds of inbred lines were surface disinfected (0.001 $HgCl_2$), washed three time with water and then germinated on wet roll–up filter paper strips under darkness at 20˚C for two days. After that they grew at 3–4˚C, 12h light in cold chamber for 18 days. Uniform 20 seedlings were planted in four replications in 16 cm pots filled with peat–sand–soil mixture. Afterward plants were cultivated under natural autumn con–ditions outdoors. In four leaf stages they were hardened in a cold cham–ber at 3–4˚C with a 8h photoperiod and light intensity of 60 $W \times m^{-2}$. After 14 days of hardening, 3 pots were inoculated with 20 grams of *M. nivale* inoculum per pot. One pot without inoculation was remained as a control.

Inoculum of *M. nivale* was prepared by growing the fungus on soil me–dium (Prończuk and Prończuk, 1987) at 18˚C for 7 days in darkness. Af–ter colonisation by mycelium the soil medium was macerated and mixed with 7 isolates of *M. nivale* selected for the highest patogenicity.

Pots after inoculation were covered with moistened cellulose wadding and plastic foil and then incubated for 3 weeks in the cold chamber at 1–2˚C. After incubation the plants were transferred to the greenhouse and tops were cut to 2–3 cm high. The response of plants was deter–mined 10 days later. The infection rate was ranked according to

a 5–grade scale from 0 (undamaged) to 4 (totally dead). The final dam–age of lines was calculated to disease index, using the formula of Townsend and Heuberger (Prończuk and Prończuk, 1987).

Field test

Uniform germinated seeds of inbred lines after surface disinfection and germination on wet filter paper in Petri dishes in darkness at 10˚C for four days were planted in 1.2 m long row in 3 replications with 25 seeds per row. In four leaf stages of seedlings, tinning was done and 20 plants per row were left. Inoculation was done in the last days of October by application of 100 g of soil inoculum of *M. nivale* per row and than plants were covered with cellulose material, to keep the higher humidity for one week. In spring, the damage of plants was estimated using the same scale and calculation formula (index of disease) as in cold chamber test.

The data for inbred lines and standard cultivars which were repeated in 3, 4 and 5 series of testing were statistically evaluated with the use of split–plot variance analysis according to the design of Wójcik and Laudański (1989). The honestly significant differences (HSD) among inbred lines were determined by Tukey's multiple comparison test. Cor–relation coefficients were calculated between the disease index of cold chamber and field test in each year.

RESULTS AND DISCUSSION

Significant variation was found for disease rating among investigated inbred lines (Fig.1). Majority of them were severe damaged by *M. nivale* in all tests. None of tested lines showed an immune reaction to infection. Relative lowest damage were observed in standard cultivars, but a few lines in each test showed the plant loses on the standard cultivars level.

Considerable variability was observed in disease prevalence in both methods of testing in particular year and over the years. Generally the inoculation of inbred lines with *M. nivale* caused more severe damage of plants under cold chamber conditions than in field conditions, except the test in 1999/2000. Low disease rating for inbred lines in that test was dif–ficult to explain. It might be connected with plants development before inoculation. Miedaner *et al.* (1993) found that with increasing age of the plants at inoculation, mean snow mould rating dropped. Differences in disease prevalence within the tests under field conditions could be influ–enced by weather conditions. During last years considerable variability in mean monthly temperatures in autumn and winter, period of snow fall and number of days with snow cover were observed in Radzików (Table 1).

Despite the differences in diseases prevalence, a positive correlation was found between the results from cold chamber and field test, however

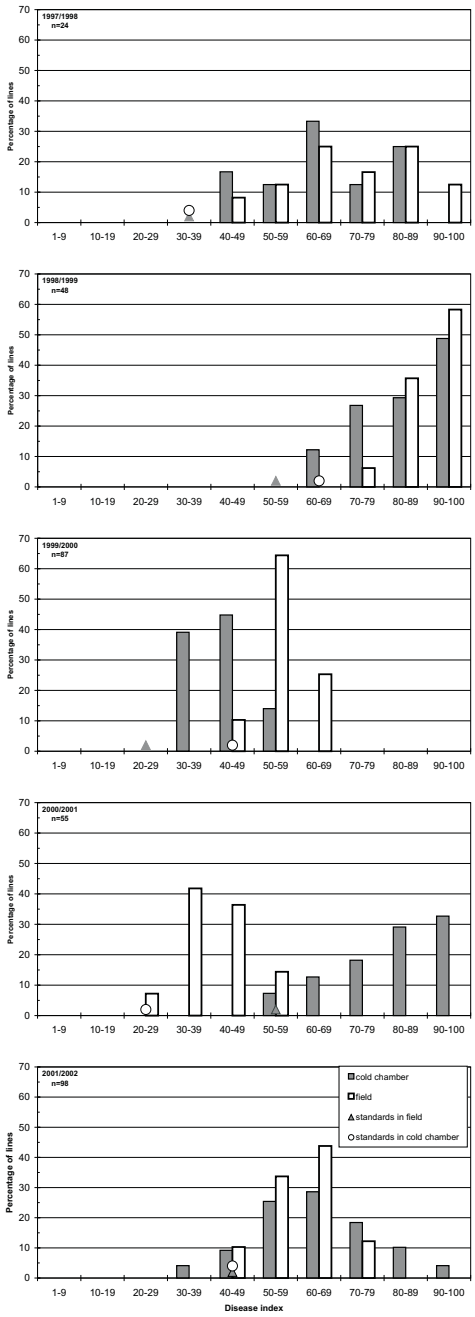

Fig. 1 Distribution of inbred lines damage by *M. nivale* in cold chamber and field tests conducted during winters from 1997/1998 to 2001/2002 at Radzików in comparison to standard cultivars

coefficient of correlation was much higher in 1998 and 1999 than in 2000 and 2001 (Table 2).

Table 1

Monthly mean temperatures and number of days with snow cover during winter 1997/98–2001/02 at Radzików

Months	Mean daily temperatures in˚C					Number of days with snow				
	1997/ 1998	1998/ 1999	1999/ 2000	2000/ 2001	2001/ 2002	1997/ 1998	1998/ 1999	1999/ 2000	2000/ 2001	2001/ 2002
November	3.2	–1.5	2.5	6.4	2.8	1	13	9	0	0
December	0.4	–2.4	1.3	2.5	–3.6	0	14	2	0	16
January	1.1	0.9	–0.7	0.1	–0.1	3	3	0	0	19
February	3.6	–0.5	2.4	0.6	4.5	8	17	0	6	0
March	2.6	5.8	4.0	3.3	5.3	0	0	0	3	0
Mean	2.18	0.46	1.90	2.58	1.78	?=12	47	11	9	35

Table 2

Correlation coefficients between the disease index of inbred lines from cold chamber and field tests

Test in winter	Correlation coefficient
1997/1998	0.74***
1998/1999	0.76***
1999/2000	0.27**
2000/2001	0.20*
2001/2002	0.49***

*, **, ***significant at P ≤ 0.05; 0.01; 0.001, respectively

Table 3

Analysis of variance for susceptibility of rye genotypes to *Microdochium nivale* tested in different number of years in cold chamber.

Number of genotypes tested	Number of years testing	F value for genotypes (A)	F value for years (B)	F value for interaction A × B
25	3	30.87***	1159.89***	8.45***
18	4	18.40***	226.02***	4.33***
9	5	88.13***	183.88***	8.42***

F values are significant at P ≤ 0.001 (***)

This results confirm previous observations of Miedaner *et al.* (1993) and Madej and Prończuk (1995) that it is difficult to obtain high correla–tion coefficient between the damage rating of lines under field and cold chamber conditions. Höxter *et al.* (1991) and Miedaner *et al.* (1993) re–

ported that the correlation could be improved by using the ELISA assay to evaluate disease intensity.

Table 4

Mean disease index for selected inbred lines and open pollinated standard cultivars tested in cold chamber in different number of years

Lines and cultivars	Mean disease index in series:		
	3 years	4 years	5 years
Standard cultivars			
Wibro	42.73	45.07	42.51
Zduno	43.86	47.30	44.83
Inbred lines			
482	44.73	53.24	–
5114	46.04	54.39	56.61
1498	48.59	–	–
343	51.06	54.41	–
399	54.29	64.87	–
463	55.22	60.62	–
854	57.03	67.45	–
2680	57.22	64.24	61.06
447	59.58	72.16	–
95	62.20	–	–
257	64.71	73.33	–
2130	67.62	64.97	69.75
253	67.76	–	–
124	68.54	–	–
5491	68.64	71.10	73.13
53165	69.62	72.08	70.90
130	71.60	76.13	–
99	71.64	–	–
620	72.51	78.46	–
901	72.97	79.28	79.43
51527	75.37	78.76	80.83
458	78.78	–	–
123	79.94	–	–
HSD 0.05	10.62	12.48	6.74

In each year of testing a few lines with high susceptibility and with relative higher resistance were selected. These lines were included in next series of testing. The statistical analysis of results from 3 series of testing revealed significant differences between years and genotypes (Table 3). The analysis also pointed out to interaction between lines and

years, which was highly significant. The tendency was quite similar in respect to 4 and 5 series of testing.

The reaction of inbred lines and standard cultivars was different in each test (Fig. 2). Significant differences in resistance were noted particularly in test conducted in 1997/98, 1998/99, 2000/01 and 2001/02 winter, where the disease severity was relatively high. In the test of 1999/2000 the disease rates were rather low and differences between genotypes were not significant. In each serie of multiyear evaluation, Tukey's multiple range test allowed to separate inbred lines with higher resistance (on the level standard cultivars) and more susceptible to *M. nivale* (Table 4). Five the best lines selected in 3 year testing confirmed their value in the serie of 4 year testing (Table 4). Line 5114, tested during 5 years showed high resistance in all series. This results indicate that selection of inbred lines with higher level of resistance to *M. nivale* is possible by multiyear testing.

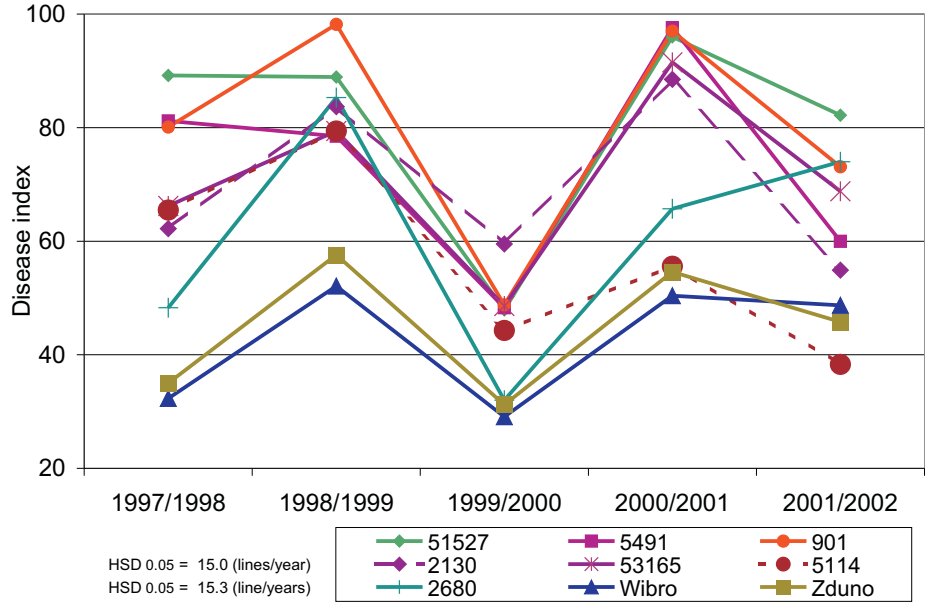

Fig. 2 Response of inbred lines

CONCLUSIONS

1. Inbred lines of rye were damaged much severe by *Microdochium nivale* than open pollinated standard cultivars. Significant variation for damage rating among inbred lines was found. A few lines in

each test showed the resistance on the level of standard cultivars. Five of lines confirmed their value in subsequent years.

2. A positive correlation was found between the index of damage from cold chamber test and from field test, however coefficients of correlation were much higher in 1997/98 and 1998/99 than in 1999/00 and 2000/01.
3. Multiyear testing and more informative laboratory methods are required for selection of inbred lines of rye resistant to *M. nivale*.

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