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Modern varieties of spring barley as a genetic resource for disease resistance breeding

G. Statkevičiūtė and A. Leistrumaitė

Cereal Breeding Department, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto av. 1, Akademija, Kedainiai distr., LT 58344, Lithuania; e-mail: grazinastat@lzi.lt

Abstract. The large variation in net blotch resistance is observed among barley germplasm, but the range of European commercial cultivars of spring barley have various degree of susceptibility. This study was designed to test 150 West European ecotype spring barley varieties and breeding lines in the field for resistance to net blotch (*Pyrenophora teres*) and powdery mildew (*Blumeria graminis* f.sp. *hordei*) and to provide information for a successful resistance–breeding program in spring barley under Lithuanian conditions. The experiment was conducted at the Institute of Agriculture of the Lithuanian Research Centre for Agriculture and Forestry. The effectiveness of different methods of artificial inoculation was also tested. About 50 varieties with net blotch resistance level ranging from resistant/moderately resistant to susceptible were chosen for agro-biological trait evaluation. Increased net blotch infection had a negative impact on ear length, number of spikelets and number of grains in the ear in 2007. No significant effects on these traits were found in 2009, but increased net blotch level caused significantly lower grain weight per ear. Artificial inoculation using chopped straw of susceptible varieties is more likely to give desirable effects on infection level. The varieties 'Luokė', 'Otis', 'Anni', 'Landora', 'Beatrix' possessed the highest resistance to net blotch, and the varieties 'Marnie' and 'Isotta' exhibited good resistance to powdery mildew. The variety 'Acrobat' was resistant to both diseases.

Key words: disease resistance, spring barley, net blotch, powdery mildew

INTRODUCTION

Pyrenophora teres Drechs. is the causal agent of barley net blotch. Its imperfect stage is *Drechslera teres* (Sacc.) Shoem. It comprises two types. The net type *P. teres* f. *teres* produces horizontal and vertical crisscrossed dark brown venation on the barley leaves that can turn chlorotic. The spot type *P. teres* f. *maculata* causes dark brown circular or elliptical spots surrounded by chlorosis (Smedegard-Petersen, 1977). Both types can cause significant financial losses due to yield reductions, ranging from 15 to 35%, and decreased grain quality (Khan, 1987; Steffenson et al., 1991). First symptoms of net blotch usually appear when seedlings reach tillering stage, and host reaction may vary with plant age (Tekauz, 1986; Tekauz, 2000; Gupta et al., 2003). The large variation in net blotch resistance is observed among barley germplasm, but range among European commercial cultivars of spring barley varies in susceptibility (Jonsson, 2001). Many attempts have been made to understand the genetic basis of net blotch resistance. While several studies have located quantitative trait loci (QTL) associated with resistance (Steffenson et al., 1996; Raman et al., 2003), a number of single, major genes controlling resistance have been identified, and several mapped (Chelkowski et al., 2003; Friesen et al., 2006; Manninen et al, 2006). However, none of them can confer durable resistance to net blotch. At present, the use of resistant barley cultivars is the most effective and economical method of controlling net blotch disease and the identification of single dominant resistance (R) genes in barley using molecular markers (Graner et al., 1996; Williams et al., 1999; Manninen et al., 2000) has facilitated the development of resistant cultivars via marker assisted selection. Nevertheless, the mixed (sexual and asexual) reproductive system of *P. teres* (Serenius et al., 2005) constitutes a high evolutionary risk for resistance breeding (McDonald & Linde, 2002). The pathogen overcomes the effectiveness of R genes in cultivars, and the use of alternative strategies is also being explored (Bogacki et al., 2008).

Numerous studies have been done on the effectiveness of spring barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) resistance genes in laboratory conditions. The researches on the level of the actual resistance under the field conditions are less frequent (Collins et al., 2002). Resistance breeding based on mono-gene is less time and labour consuming. Nevertheless, such type of resistance is efficient only for a short period (3–5 years) after the cultivars have been grown on a large scale (Collins et al., 2002; Dreiseitl, 2003). In breeding for powdery mildew resistance this situation continued until the wide introduction of the *mlo* gene alleles to practical use, which has been highly effective for nearly three decades (Jørgensen, 1992). But if these genes become inefficient, barley growers will have to use more fungicides. Another type of resistance, partial resistance, is characterized by a compatible interaction in all growth stages, but a lower infection frequency, a longer latent period, or a lower rate or a shorter period of a spore production (Jørgensen, 1994). One of the ways to determine the level of partial resistance is to test the cultivars under field conditions and asses development of disease from its appearance up to the end of the growing season.

Implementation of a successful breeding strategy for disease resistance requires information on the reaction of cultivars and putative resistant lines to local pathogen population. Therefore, the search for new resistance sources and introduction of new germplasm sources into breeding programs as well as pathogen virulence and variability studies remains an important research field. This study was designed to test 150 West European ecotype spring barley varieties and breeding lines under field conditions for resistance to net blotch and powdery mildew, and to provide information for a successful spring barley resistance-breeding program under Lithuanian conditions.

MATERIALS AND METHODS

150 West European ecotype spring barley varieties and breeding lines representing a wide range of reaction to net blotch were selected for field test. The selection of varieties for the experiment was based on their net blotch resistance, recorded in field screenings in previous year. The experiment was conducted at the Institute of Agriculture, of the Lithuanian Research Centre for Agriculture and Forestry during 2007–2009. The pre–crop was fodder galega in 2007. In 2008 and 2009, pre-crop was barley. The field trial was arranged in 3 treatments: natural infection and two treatments with artificial inoculation – plants were inoculated by spreading chopped

barley straw between the rows, and barley was sprayed with net blotch mycelium and conidia suspension. Each treatment consisted 3 replications. The varieties and breeding lines were placed randomly in each replicate. Each variety was sown in two rows, with row length of 1 meter, 2 grams of seeds sown per row. The scorings for the disease reaction were made visually, based on percentage of leaf area covered by necrosis and chlorosis. The first scoring was made before inoculation, when seedlings reached BBCH 20–29 stages. Subsequent scorings were made each 10 days, and there were 5 scorings in total. The severities of diseases were measured in scores, using the scale: 1 (disease severity 0.0%), 2 (0.1%), 3 (1.0%), 4 (5.0%), 5 (10%), 6 (20.0%), 7 (40.0%), 8 (60.0%), 9 (80.0%). 50 varieties with net blotch resistance level ranging from resistant/moderately resistant to susceptible were chosen for agro-biological trait evaluation, and 20 plants were collected of each of them. Shoot number, ear number and length, number of spikelets, number of grains per ear and average weight per ear were assessed.

Spring barley collection resistance to powdery mildew was investigated during 2008–2009. The same varieties and breeding lines were used for powdery mildew resistance trial. It was sown in the same way as in net blotch trial: 3 replications, each variety was sown in two rows, with a row length of 1 meter, 2 grams of seeds per row. The trial was surrounded by winter barley rows, with a row width of 3.4 m, to ensure better spread of powdery mildew. The pre–crop was barley in both years. The scorings were made the same way as in net blotch trial: the first scoring was made when plants reached BBCH 20–29 stage, subsequent scorings were made each 10 days, and there were 5 scorings in total.

$N_{90}P_{60}K_{60}$ fertilization was applied and no fungicide treatment of seeds was made in both net blotch and powdery mildew resistance trials. Statistical analysis were made using SigmaStat 2.03, figures were drawn using Microsoft Office Excel 2003.

RESULTS AND DISCUSSION

Net blotch is one of the most important and widespread diseases of spring barley worldwide and also in Lithuania (Skurdenienė, 1993). Net blotch infection rate and yield damage depends both on host resistance and weather conditions during growing season. In summer 2007 and 2009, weather conditions were favourable for net blotch – it was rather cool and rainy, but in 2008 the growing season was very drought and net blotch infection was low.

In 2007, net blotch infection level in the treatment inoculated with straw was significantly higher compared to the control. Chopped straw as supplementary source of net blotch inoculum proved to be more effective than spraying with mycelium suspension. Varieties inoculated by spraying did not show any significant increase in net blotch level. In 2008, net blotch infection level was very low in all three treatments due to unfavourable weather conditions. The AUDPC (area under disease progress curve) values for all varieties were similar; therefore it was not possible to make clear distinction between resistant and susceptible varieties. Although infection level in artificial inoculation treatment using straw was still higher, the differences were not significant. The net blotch infection level and its spreading rate increased in 2009, it was higher than in 2008 but lower than in 2007 (Fig. 1).

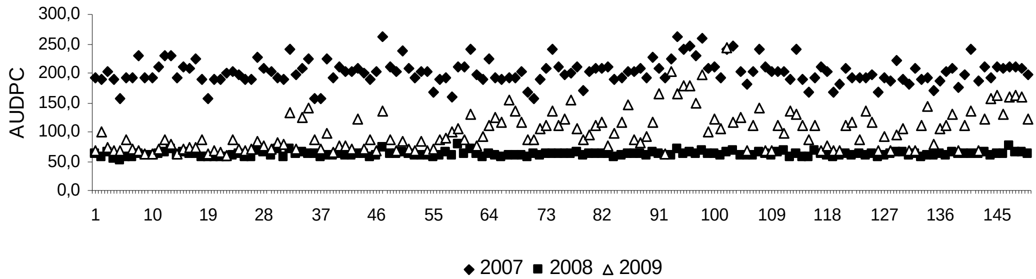

Figure 1. Net blotch AUDPC (area under disease progress curve) values during 2007–2009. The first scoring was made when plants reached BBCH 20–29 stages, subsequent scorings was made each 10 days, 5 scorings in total.

AUDPC values were significantly higher in both artificial inoculation treatments compared to natural infection (Fig. 2). Disease level tended to be lower in treatment sprayed with mycelium/conidia suspension, but the difference was not statistically significant. The net blotch mycelium suspension used for barley inoculation comprised mixture of local net blotch isolates, and each year the mixture was prepared from new isolates. According to Jorgensen et al. (2000), sometimes the same varieties can show substantial differences in resistance level when different pathogen populations are used. This indicates that the local pathogen population may differ in composition and that a successful breeding effort requires the use of a pathogen population composed of several or many local subpopulations. The success to increase net blotch level by spraying mycelium suspension may have been conditioned by using more aggressive pathotypes, but in general chopped straw inoculation proves to be more appropriate to use in net blotch resistance trials because it is simply to prepare and is more likely to give desirable effects on infection level.

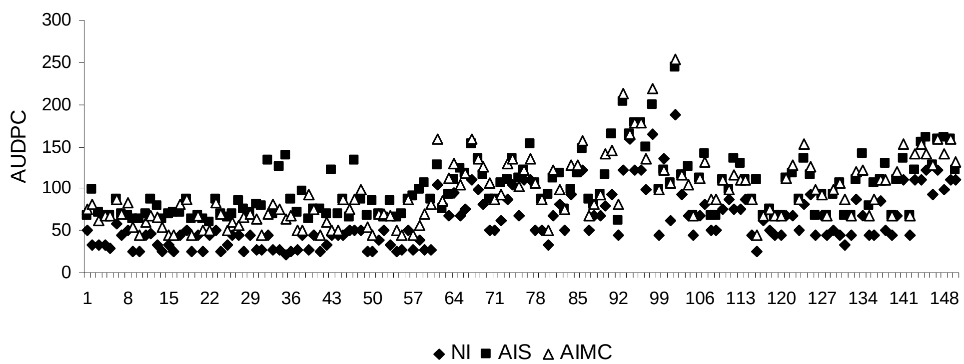

Figure 2. Net blotch AUDPC values in natural infection and artificial inoculation trials in 2009. NI–natural infection, AIS–artificial inoculation using chopped straw, AIMC – artificial inoculation by spraying mycelium and conidia suspension. The first scoring was made when plants reached BBCH 20–29 stages, subsequent scorings was made each 10 days, 5 scorings in total.

There are several experiments showing that net blotch infection suppresses plant height and reduces yield mainly due to 1000-grain weight reduction, but has no effect on ear number (Robinson, 2000). The effect of increased disease level on shoot number, ear number and length, number of spikelets, number of grains per ear and average grain weight per ear were tested in this experiment, and the results differed between years. In 2007, no significant changes were found in ear number either, but the number of spikelets and grains was reduced in many cases, and there were also significant differences in ear length. This was mostly true for susceptible varieties. Since there were no significant differences in net blotch infection level between treatments in 2008, it was not possible to evaluate disease impact on agro-biological traits of barley.

Although net blotch severity was significantly higher in artificial inoculation treatments in 2009, no significant differences in ear length or number of spikelets was found, and only in some cases the number of grains per ear differed significantly (Table 1). However, the mean weight of grains per ear was significantly lower ($P < 0.05$) in

Table 1. Mean values of number of spikelets per ear and number of grains per ear. NI–natural infection, AI–artificial inoculation using chopped straw.

Variety	Number of spikelets per ear				Number of grains per ear			
	2007		2009		2007		2009	
	NI	AI	NI	AI	NI	AI	NI	AI
Class	26.1*	22.8*	23.7	25.3	25.3*	22.2*	23.6	24.6
Barke	26.7	25.3	26.1	26.9	26.3*	24.4*	25.7	25.5
Cicero	26.1*	20.7*	24.3	25.2	25.1*	20.3*	24.1	24.6
Henni	25.8	24.9	24.8	25.6	25.3*	23.7*	23.9	23.0
Henley	26.9*	22.9*	23.7	23.2	26.6*	22.0*	22.9	21.9
Luciana	23.4*	21.1*	22.9*	20.4*	23.2*	20.7*	22.6*	19.5*
Chantal	25.8*	21.6*	24.9	25.4	25.0*	20.5*	24.6	23.8
Quench	26.0*	22.4*	22.2	22.4	24.6*	21.1*	21.7	21.3
Conchita	22.4*	19.3*	25.8	26.0	21.2*	19.0*	25.7	25.8
Luokė	26.3*	23.8*	24.9	25.4	25.4*	22.1*	24.6	23.8

*$P < 0.05$

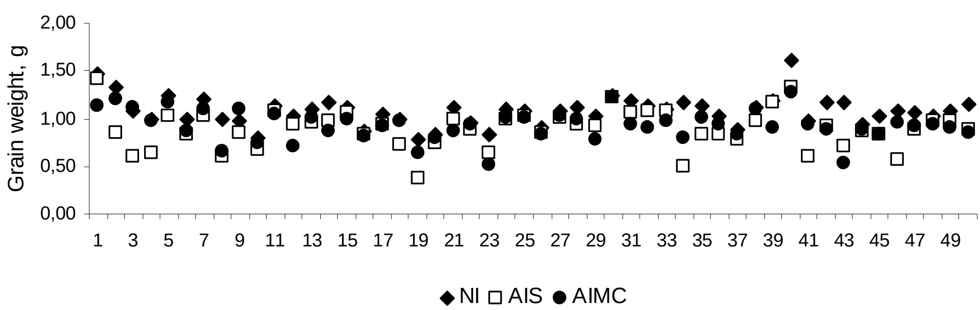

Figure 3. Mean ear grain weight in natural infection and artificial inoculation trials in 2009.

the treatments with artificial inoculation compared to natural infection treatment (Fig. 3). This finding suggests that although there was no negative effect on ear length and grain number, the grain filling was damaged by net blotch infection.

A successful breeding strategy highly depends on correct choice of parent genotypes, therefore it is important to identify the most resistant varieties and exclude the susceptible ones. In this research, varieties ‘Luokė’, ‘Otis’, ‘Anni’, ‘Landora’, ‘Beatrix’, and ‘Acrobat’ possessed the highest resistance to net blotch (Table 2).

Table 2. Net blotch AUDPC values of the most resistant, and the most susceptible varieties in 2007–2009.

Variety	Country of origin	Most resistant			Variety	Country of origin	Most susceptible		
		2007	2008	2009			2007	2008	2009
Luokė	LTU	157.5	57.5	63.5	Antto	SWE	262.5	69.9	164.6
Otis	DEU	157.5	61.0	87.5	Alliot	DNK	244.7	64.0	177.5
Anni	EST	157.5	57.5	71.5	Class	DEU	241.5	65.7	243.5
Landora	DEU	157.5	61.0	86.6	Barke	DEU	241.5	62.5	177.5
Beatrix	DEU	168.0	57.5	68.6	Henni	DEU	241.5	69.9	133.5
Acrobat	GBR	171.2	59.0	79.5	Henley	GBR	241.5	62.5	129.5

Powdery mildew is also economically important fungal disease of barley. Only varieties with *mlo* resistance gene are fully resistant to powdery mildew, although some isolates capable to attack such barley varieties and cause mild infection have been reported. The effectiveness of *mlo* gene also depends on other factors (Lyngkjaer et al., 1995). The highly resistant varieties with *mlo* gene are widely grown in Europe. Yet if new pathotypes capable to infect *mlo* varieties emerged, it could lead to serious economic losses. Therefore powdery mildew resistance screenings and breeding of new varieties with other than *mlo* resistance genes remains important. The same varieties were used in powdery mildew resistance screenings as in net blotch screenings.

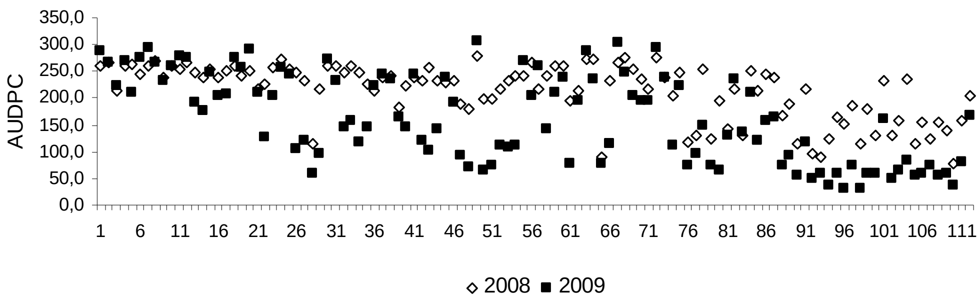

Figure 4. Powdery mildew AUDPC values in 2008–2009. The first scoring was made when plants reached BBCH 20–29 stages, subsequent scorings was made each 10 days, 5 scorings in total.

Contrary to net blotch infection, powdery mildew infection was higher in 2008 than in 2009 (Fig. 4, *mlo* varieties excluded). The varieties ‘Marnie’ and ‘Isotta’ exhibited good resistance to powdery mildew (3–4%). They both have resistance gene

1-B-53. The varieties 'Annabell' (resistance gene We) and 'Orthega' (Ar, We) were moderately susceptible to powdery mildew (15–20%). It may be concluded that these resistance genes are losing their effectiveness. The variety 'Acrobat' (resistance gene unknown) exhibited good resistance to powdery mildew and net blotch.

CONCLUSIONS

In order to enhance net blotch infection level for easier identification of resistant varieties artificial inoculation of spring barley plants using chopped straw of susceptible varieties is more appropriate to use in net blotch resistance trials because it is not only simpler to prepare but it is also more likely to give desirable effects on infection level.

Increased net blotch infection had a negative impact on ear length, number of spikelets and number of grains per ear in 2007. No significant effects on these traits were found in 2009, but increased net blotch level caused significantly lower grain weight per ear.

The varieties 'Luokė', 'Otis', 'Anni', 'Landora', and 'Beatrix' possessed the highest resistance to net blotch. The varieties 'Marnie' and 'Isotta' exhibited good resistance to powdery mildew. They both have resistance gene 1-B-53. The variety 'Acrobat' was resistant to both diseases.

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