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DERIVING ISOLATES OF POWDERY MILDEW (*Blumeria graminis* DC. f.sp. *avenae* Em. Marchal.) IN COMMON OAT (*Avena sativa* L.) AND USING THEM TO IDENTIFY SELECTED RESISTANCE GENES

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Abstract

Powdery mildew in common oat is caused by *Blumeria graminis* DC. f.sp. *avenae* Em. Marchal. Host-pathogen tests are commonly used to identify and locate resistance genes to powdery mildew in cereals. The aim of the study was to determine the virulence of powdery mildew isolates obtained from powdery mildew populations harvested in Poland and to identify OMR1, OMR2 and OMR3 resistance genes to powdery mildew in F_2 populations of inter-cultivar hybrids of common oat: Bruno × Fuchs, Jumbo × Fuchs and Mostyn × Fuchs. On the basis of the analysis conducted, isolates enabling division of the studied populations into groups of resistant and susceptible plants were selected. M10 and M14 isolates were chosen for the population which was obtained from crossbreeding of 'Bruno' with 'Fuchs'; these isolates demonstrated avirulence to Bruno cultivar containing OMR1 gene. In order to divide population obtained from crossbreeding of 'Jumbo' with 'Fuchs', M13 and M16 isolates were chosen; they demonstrated avirulence to the cultivar Jumbo containing the OMR2 gene. On the basis of the tests conducted, it was impossible to select isolates characterised by avirulence to the OMR3 gene. In the F_2 population of Bruno × Fuchs and Jumbo × Fuchs hybrids, a division was made into resistant and susceptible plants. The obtained results were verified by the ^{2} test; the proportion in the dispersion matching model was found to be 3 resistant plants: 1 sensitive plant both in the Bruno × Fuchs and Jumbo × Fuchs populations. Such dispersion indicated that the resistance to powdery mildew in the studied cultivars Bruno and Jumbo was conditioned by single dominant genes.

Key words: powdery mildew, host-pathogen tests, OMR genes, *Avena*

INTRODUCTION

Powdery mildew on cereals is a disease caused by a parasitic fungus *Blumeria graminis* (Boreck-ki, 2001). This pathogen is characterised by high parasitic specialization: the specific fungus breed affects only one cereal species. Powdery mildew is a worldwide disease; however, it is predominant in regions which are characterised by the cold and humid climate (Roderick et al. 2000). Weather conditions have a significant effect on the development of the fungus. It has been observed that the disease becomes more intense after mild winters and warm springs (Priestley and Bayles, 1979). The result of the effect of powdery mildew is a reduction of photosynthesis efficiency, a drop in the number of grains and grain weight as well as a decrease in the content of carbohydrates in grain (Roderick and Jones, 1988; Roderick et al. 2000). The ability to infect plants within a wide range of temperatures and humidity constitutes an important epidemiological property of powdery mildew. High genetic changeability and the ability to generate new forms by mutations and DNA recombinations make this pathogen easily adaptable to new conditions (Bennet, 1984; Bayles, 1997).

Powdery mildew in oat is caused by *Blumeria graminis* DC. f.sp. *avenae* Em. Marchal. The pathogen is common in northwestern Europe and South America (Aung et al. 1997; Schwarzbach and Smith, 1988). Losses in oat crops in western Europe caused by powdery mildew range from 5 to 10% (Clifford, 1995; Hsam et al. 1997). It also poses a big threat to plants in eastern Europe, including Poland (Sebesta et al. 1991).

Host-pathogen tests are commonly used to identify and locate resistance genes to powdery mildew in cereals (Czembor and Czembor, 1998; Czembor, 1999; Czembor, 2000; Hsam et al. 1997; Kowalczyk et al. 1998; 2004; Zeller et al. 1998).

Tests of resistance to powdery mildew are usually performed on the first leaves of 10-day seedlings. Fragments of leaves are put on Petri dishes filled with agar supplemented with benzimidazole and then inoculated with specific fungal isolates. After incubation the effect of the fungus on the studied forms with unknown resistance genes is determined and compared to the effect on lines or cultivars with known resistance genes. On this basis, resistance genes in the studied forms are determined (Hsam et al. 1997; 1998; Kowalczyk et al. 1998; 2004; Zeller et al. 1998; Wiśniewska and Kowalczyk, 2005). The aim of the study was to determine the virulence of isolates of powdery mildew obtained from powdery mildew populations harvested in Poland and to identify OMR1, OMR2 and OMR3 resistance genes in F_2 populations of inter-cultivar hybrids of oat: Bruno × Fuchs, Jumbo × Fuchs and Mostyn × Fuchs.

MATERIALS AND METHODS

Materials used in the study were the following cultivars of common oat (*Avena sativa* L.): Bruno containing the OMR1 resistance gene to powdery mildew, Jumbo containing the OMR2 gene, Mostyn containing the OMR3 gene, and Fuchs lacking resistance genes to powdery mildew, which were parent components in inter-cultivar crosses and F_2 generation hybrids: Bruno × Fuchs, Jumbo × Fuchs and Mostyn × Fuchs.

Oat leaves affected with powdery mildew were harvested in the regions of Wielkopolska (Choryń), Kujawy (Strzelce), and Lubelszczyzna (Czesławice and the vicinity of Tomaszów Lubelski). In laboratory conditions, a number of isolates were selected from single spores of harvested populations of *Blumeria graminis* f.sp. *avene*. The obtained isolates of powdery mildew were used to determine the effect of powdery mildew in common oat cultivars containing OMR1, OMR2 and OMR3 genes. Fragments of leaves of the control cultivar sensitive to the effect of powdery mildew and of the cultivars containing different OMR genes were places on dishes and inoculated with the obtained isolates.

Host-pathogen tests were carried out on the first leaves of 10-day seedlings in F_2 populations of the inter-cultivar hybrids. Fragments of leaves were placed on 12-well culture plates with benzimidazole agar (6 g of agar per 1 l of water and 35 mg/l of benzimidazole). Control forms were put into the first and last well of every dish in the following order: the cultivar sensitive to the effect of powdery mildew, the cultivar with the OMR1 gene, then with OMR2 and with OMR3. The plates with leaf fragments were inoculated in an inoculation tower by placing about 500-700 spores of powdery mildew on 1 cm^2. Then, the dishes were put in a phytotron at about 17°C and an illuminance of about 4 kLx.

After ten days from inoculation with powdery mildew isolates, the effect on the leaves was determined on a 10-point scale (where 0 means a lack of effect and 9 an effect in which the mycelium takes up more than 50% of leaf surface). Three classes of responses to the isolates used were observed: resistant - when infection of the sensitive cultivar ranged from 0 to 20%, intermediate – when the infection of the susceptible cultivar ranged from 20 to 50%, and susceptible – when the degree of infection exceeded 50%.

The ^{2} test was used in order to confirm the compliance of dispersion in F_2 segregating generations in the studied populations obtained in the resistance tests with the expected values.

RESULTS

From the harvested populations of powdery mildew, 40 isolates were selected which were used to affect common oat cultivars containing different genes of resistance to powdery mildew. 16 of the tested isolates did not show infection differences as compared to the control cultivar Fuchs lacking resistance genes and the cultivars containing OMR1, OMR2 and OMR3 genes. Six derived isolates demonstrated avirulence to the Bruno cultivar containing the OMR1 gene. Five of the tested isolates were avirulent to the Jumbo cultivar containing the OMR2 gene. Three selected isolates were characterised by avirulence to the Mostyn cultivar containing the OMR3 gene as compared to the control cultivar lacking genes of resistance to powdery mildew. However, they were avirulent also with regard to the remaining OMR genes analysed.

In order to verify the results of the first test, 15 powdery mildew isolates showing different reactions to OMR1, OMR2 and OMR3 resistance genes were selected for further analysis. The test was repeated twice.

Four out of the 15 isolates of powdery mildew selected for further testing affected the studied cultivars completely. Four isolates demonstrated avirulence to the Bruno cultivar containing the OMR1 gene. Two isolates were characterised by avirulence with regard to the OMR 2 gene. No isolates avirulent to the OMR3 gene were obtained (Table 1).

In the third test which should confirm the stability of the obtained isolates and the reaction of OMR1, OMR2 and OMR3 genes to the selected isolates of powdery mildew, it was shown that four studied isolates of powdery mildew affected all cultivars completely. Two isolates were avirulent to the Bruno cultivar with the OMR1 gene. Two from the studied isolates showed avirulance to the Jumbo cultivar containing the OMR2

gene. In the third test, none of the obtained isolates affected the Mostyn cultivar in a way which would make it possible to show the difference between this cultivar and the control cultivar (Table 1).

On the basis of the analysis conducted, isolates enabling division of the segregating populations studied into groups of resistant and susceptible plants were selected for host-pathogen tests. M10 and M14 isolates which demonstrated avirulence to the Bruno cultivar containing OMR1 were selected for a population obtained from crossbreeding of the Bruno cultivar with Fuchs. M13 and M16 isolates which showed avirulence to the Jumbo cultivar containing the OMR2 gene were selected in order to divide the population obtained from crossbreeding of the Jumbo cultivar with Fuchs. On the basis of the tests conducted, it was impossible to select isolates showing avirulence with respect to the OMR3 gene (Table 1).

Table 1.
The effect of selected powdery mildew isolates on cultivars of common oat containing OMR1, OMR2 and OMR3 genes.

Isolates of poldery mildew	The effect of selected isolates on oat cultivars							
	Fuchs		Bruno OMR1		Jumbo OMR2		Mostyn OMR3	
	Test II	Test III	Test II	Test III	Test II	Test III	Test II	Test III
M1	9	7	2	9	3	6	4	7
M2	9	9	9	8	9	8	8	9
M3	7	7	7	7	5	5	8	9
M5	6	7	1	7	3	6	4	4
M6	9	8	9	8	2	7	9	7
M8	9	6	3	7	2	7	8	6
M10	8	8	1	1	7	7	7	5
M11	9	9	7	9	5	8	8	8
M12	8	6	7	6	8	6	8	7
M13	9	8	9	7	0	0	9	8
M14	9	9	2	1	3	5	9	8
M15	9	9	9	9	9	9	9	9
M16	9	8	8	7	0	0	7	8
M17	9	9	9	8	9	9	9	9
M18	9	8	9	8	7	10	8	8

200 seeds from Bruno × Fuchs and Jumbo × Fuchs hybrid populations were spot-sown to pots and placed in a phytotron. After 10 days host-pathogen tests were carried out on the first leaves of the obtained seedlings.

Two isolates of powdery mildew characterised by avirulence to the OMR1 gene were selected to determine the resistance to powdery mildew of plants belonging to the F_2 generation of hybrids of the crossbred combination Bruno × Fuchs. On the basis of the test results, 40 resistant and susceptible plants were selected for further analyses.

Resistance of the Jumbo × Fuchs population was determined by two powdery mildew isolates avirulent to the OMR2 gene. 35 resistant plants and 40 plants susceptible to the effect of the selected powdery mildew isolates were selected out of the total number of 200 plants sown for the test.

In the F_2 generation of Bruno × Fuchs and Jumbo × Fuchs hybrids, segregation of plants was made into resistant and susceptible (Table 2). After the obtained results were verified by the ^{2} test both in the Bruno × Fuchs and Jumbo × Fuchs population, the proportion in the dispersion matching model was found to be 3 resistant plants: 1 sensitive plant. Such dispersion indicated that the resistance to powdery mildew in the studied cultivars Bruno and Jumbo was conditioned by single dominant genes.

Table 2.
Segregation of Bruno × Fuchs and Jumbo × Fuchs hybrids of F_2 generation with regard to resistance to powdery mildew

Hybrids	Number of tested plants	Number of resistant plants	Number of susceptible plants	Expected dispersion	^{2}
			M10		
Bruno × Fuchs	174	139	35	3:1	2.210
			M14		
	174	135	39	3:1	0.674
			M13		
Jumbo × Fuchs	171	126	45	3:1	0.149
			M16		
	171	131	40	3:1	0.226

DISCUSSION

Host-pathogen tests are commonly used to identify genes of resistance to powdery mildew in common oat. H s a m et al. (1997) used this method to determine the effect of 12 powdery mildew isolates, characterised by different virulence as compared to genotypes with documented resistance to powdery mildew, on 259 lines and cultivars of common oat. 173 of the studied forms were susceptible to the effect of powdery mildew. The reaction of nine genotypes showed that they had the OMR1 gene. Seven analysed forms showed a similar reaction to that of the line Cc4146 with the OMR2 gene. Eleven of the tested genotypes had the OMR3 gene. The authors did not identify forms which would have the OMR4 gene among the analysed forms. It may result from the fact that this gene is not used in oat cultivation programs (S e b e s t a et al. 1991). Using a resistance test, H s a m et al. (1998) studied the reaction of 207 lines and cultivars of common oat to the effect of 11 selected isolates of powdery mildew. They determined the extent of the effect on the studied forms by comparing their reaction to five genotypes with documented resistance to powdery mildew. 194 tested forms were sensitive to the effect of powdery mildew. Only 5 % of the analysed genotypes showed resistance to selected isolates of powdery mildew. The OMR1 gene was identified in the cultivars Kaap, Tonger Boruta and Dragon and in the line MGH-6374. A Swedish cultivar Galop and the line STH994 from Belarus had the OMR3 gene. OMR2 and OMR4 genes were not identified in the studied lines and cultivars. Host-pathogen tests were also used to identify resistance of oat to powdery mildew by K o w a l c z y k et al. (2004). These authors analysed the reaction of Polish cultivars of common oat to isolates of powdery mildew characterised by avirulence to the OMR2 gene. They used the Jumbo cultivar containing the OMR2 gene and the Kanota cultivar susceptible to the effect of powdery mildew as control forms. The performed experiments showed that only the Skrzat cultivar had the OMR2 gene. The reaction of the remaining cultivars to the powdery mildew isolates used was different from the reaction of the control cultivar Jumbo.

In the experiments presented in this study, F_2 population hybrids obtained as a result of crossbreeding of cultivars containing different OMR genes with a cultivar sensitive to the effect of powdery mildew were tested using host-pathogen tests. Isolates of powdery mildew avirulent to specific OMR genes and derived from *Blumeria graminis* f. sp. *avene* harvested in Poland were used for that purpose. Cultivars with documented resistance to powdery mildew: Bruno (OMR1), Jumbo (OMR2) and Mostyn (OMR3) as well as the Fuchs cultivar lacking genes of resistance to powdery mildew, were used as control forms. The performance of resistance tests on the basis of isolates avirulent to OMR genes provided a possibility for dividing the analysed F_2 populations into groups of plants which were resistant and susceptible to the effect of powdery mildew.

Y u and H e r r m a n n (2006) performed host--pathogen tests on two F_2 populations segregating with regard to resistance to powdery mildew. These authors obtained the populations by crossbreeding of two lines (Am27 and Am28) resistant to powdery mildew with such sensitive cultivars of common oat as Neklan and Flamingsprofi. Resistance of the Am27 and Am28 lines to *Blumeria graminis* f. sp. *avene* was derived from *A. macrostachya.* As a result of the conducted tests the authors obtained the dispersion matching model 3:1 in both F_2 populations, which may indicate that resistance to powdery mildew derived from *A. macrostachya* is controlled by a single dominant gene. Similar results were obtained by H s a m and Z e l l e r (1998) who analysed 18 F_2 populations obtained as a result of crossbreeding of 18 monosomic lines with the cultivar Mostyn containing the OMR3 gene of resistance

to powdery mildew by means of a host-pathogen test. The authors performed resistance tests on the basis of two isolates of powdery mildew: OBB10 and F2/3 avirulent with regard to the OMR3 gene. In 17 crossbred combinations the authors obtained a dispersion matching model 3:1 with regard to resistance to powdery mildew. On the basis of the test results, the authors claimed that resistance to powdery mildew in the cultivar Mostyn was controlled by a single dominant gene.

In this study, it was found that the proportion of dispersion in both populations studied was 3 resistant plants: 1 plant susceptible to the effect of powdery mildew. It indicated that resistance in the studied cultivars Bruno and Jumbo was controlled by single dominant genes.

CONCLUSIONS

1. Isolates avirulent to OMR1 and OMR2 were selected from *Blumeria graminis* f. sp. *avene* populations harvested in Poland. Selected M10 and M14 isolates may be used to identify the OMR 1 gene by means of host-pathogen tests, whereas M13 and M16 isolates may be used to identify the OMR2 gene.
2. Among the obtained powdery mildew isolates, no isolates avirulent to the OMR3 gene were found. The obtained results show that the population of *Blumeria graminis* f. sp. *avene* completely overcame resistance conditioned by the OMR3 gene.
3. On the basis of dispersion of F_2 populations Bruno × Fuchs and Jumbo × Fuchs with regard to resistance to powdery mildew, it was shown that resistance to this pathogen in the cultivars Bruno and Jumbo is conditioned by single dominant genes.

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Wyprowadzenie izolatów mączniaka prawdziwego owsa (*Blumeria graminis*) i wykorzystanie ich do identyfikacji wybranych genów odporności (*Avena sativa* L.)

Streszczenie

Mączniak prawdziwy owsa powodowany jest przez *Blumeria graminis* DC. f.sp. *avenae* Em. Marchal. Do identyfikacji i lokalizacji genów odporności na mączniaka prawdziwego u zbóż wykorzystywane są powszechnie testy żywiciel-patogen. Celem prezentowanej pracy była ocena wirulencji izolatów mączniaka prawdziwego uzyskanych z populacji mączniaka zebranych na terenach Polski oraz identyfikacja genów odporności n mączniaka prawdziwego OMR1, OMR2 i OMR3 w populacjach F_2 mieszańców międzyodmianowych owsa zwyczajnego: Bruno × Fuchs, Jumbo × Fuchs i Mostyn × Fuchs. Na podstawie przeprowadzonych analiz do testów żywiciel-patogen wybrano izolaty umożliwiające podział badanych populacji segregujących na grupy roślin odpornych i podatnych na porażenie mączniakiem prawdziwym. Dla populacji powstałej z krzyżowania odmiany Bruno z odmianą Fuchs wybrano izolaty M10 i M14, które wykazały awirulencję dla odmiany Bruno zawierającej gen OMR1. W celu podziału populacji powstałej z krzyżowania odmiany Jumbo z odmianą Fuchs wybrano izolaty M13 i M16 odznaczające się awirulencją w stosunku do odmiany Jumbo, zawierającej gen OMR2. Na podstawie przeprowadzonych testów niemożliwa była selekcja izolatów odznaczających się awirulencją w odniesieniu do genu OMR3. W pokoleniu F_2 mieszańców Bruno × Fuchs i Jumbo × Fuchs uzyskano segregację roślin na odporne i podatne na porażenie przez wybrane izolaty mączniaka prawdziwego. Po weryfikacji otrzymanych wyników za pomocą testu ^{2} zarówno w populacji Bruno × Fuchs jak i Jumbo × Fuchs stwierdzono proporcję rozszczepień pasującą do modelu 3 rośliny odporne / 1 roślina wrażliwa. Takie rozszczepienie wskazuje na to, że odporność na mączniaka w badanych odmianach Bruno i Jumbo warunkowana jest przez pojedyncze dominujące geny.