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Short communication

DNA polymorphism among barley NILs of cv. Pallas, carrying genes for resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*)

Paweł Cz. CZEMBOR, Jerzy H. CZEMBOR

Plant Breeding and Acclimatization Institute, Radzików, Poland

Abstract. Barley powdery mildew, caused by the pathogen *Blumeria graminis* f. sp. *hordei* is an important disease of barley (*Hordeum vulgare* L.). The random amplified polymorphic DNA (RAPD) method was used to detect DNA polymorphism among 7 Pallas near-isogenic lines (NILs) carrying *Mla3*, *Mla12*, *Mlk*, *Mlp*, *Mlat*, *Mlg* and *MlLa* genes for resistance to *B. graminis* f. sp. *hordei*. From among 500 random 10-mer primers tested, 3 were specific for NIL P2 (*Mla3*), 1 for P10 (*Mla12*), 6 for P17 (*Mlk*), 46 for P19 (*Mlp*), 4 for P20 (*Mlat*), 6 for P21 (*Mlg*), and 4 for P23 (*MlLa*). The results of this study demonstrated that the RAPD technique is a useful tool for detecting DNA polymorphism among Pallas NILs.

Key words: *Hordeum vulgare*, Pallas near-isogenic lines, powdery mildew, RAPD markers, resistance genes.

Barley powdery mildew, caused by the pathogen *Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei* Em. Marchal (synamorph *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal.) is an important disease of barley (*Hordeum vulgare* L.) in regions with a maritime climate. In Central Europe it causes annual losses reaching about 10% of barley production (CZEMBOR 1996, DREISEITL, J RGENSEN 2000).

The use of molecular markers may significantly facilitate barley breeding for powdery mildew resistance (CZEMBOR, TALBERT 1997, GUPTA et al. 1999). Such DNA markers include random amplified polymorphic DNAs (RAPDs), in-

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Correspondence: P.Cz. CZEMBOR, Plant Breeding and Acclimatization Institute, Radzików, 05-870 Błonie, Poland; e-mail: p.czembor@ihar.edu.pl

vented by WILLIAMS et al. (1990) on the basis of the polymerase chain reaction (PCR), developed by MULLIS and FALOONA (1987). At the very beginning of the use of RAPD markers, it was demonstrated that RAPD analysis of near-isogenic lines (NILs) is a rapid method identifying markers for disease resistance loci (MARTIN et al. 1991, PENNER et al. 1993). Barley NILs with different specific resistance genes are very useful for genetic studies of host-parasite interactions (HOVM RLLER et al. 2000, CZEMBOR, CZEMBOR 2001). Initially, MOSEMAN (1972) developed 10 barley NILs carrying resistance genes to powdery mildew and next K LSTER et al. (1986) developed 24 NILs based on spring barley cv. Pallas. The latter differential set of lines is commonly used by breeders, geneticists and plant pathologists studying powdery mildew barley interactions (CZEMBOR 1996, HOVM LLER et al. 2000, CZEMBOR, CZEMBOR 2001, 2002).

The purpose of the present study was to examine DNA polymorphism within amplified DNA fragments in Pallas NILs carrying *Mla3*, *Mla12*, *Mlk*, *Mlp*, *Mlat*, *Mlg* and *MlLa* genes for resistance to barley powdery mildew by using the RAPD technique.

DNA was extracted from leaves of two-week-old seedlings with the use of the CTAB method (SAGHAI-MAROOF et al. 1984) from seven Pallas NILs: P2 (*Mla3*), P10 (*Mla12*), P17 (*Mlk*), P19 (*Mlp*), P20 (*Mlat*), P21 (*Mlg*), P23 (*MlLa*) and cv. Pallas as a control (K LSTER et al. 1986). Seven Pallas isolines and cv. Pallas were screened with 500 arbitrary sequences of 10-base primers for their DNA polymorphism.

Primer sequences according to Operon Technologies (Alameda 1000, Atlantic Ave., CA 94501, USA) catalogue's (kits: K-AI) were synthesized by Ransom Hill Bioscience (P.O. Box 219, Ramona, CA 92065, USA). PCR amplifications were conducted according to conditions described by CZEMBOR and CZEMBOR (2002). The amplification products were separated on 1.8% agarose gel (1 × TBE buffer, 6 V/cm, 4 h) at a constant temperature of 15°C, controlled by the refrigerating and heating circulator FS18-HP (Julabo Labortechnik GmbH, D-77960 Seelbach, Germany). Afterwards, gels were stained with ethidium bromide and photographed on a transilluminator. Weak and irreproducible bands produced in the PCR assays were not scored. Molecular weight (bp) of bands was assessed by FragmeNT Analysis v. 1.1 (Molecular Dynamics, Sunnyvale, CA, USA).

PCR screening of the 500 random 10-mer primers resulted in identification of 61 primers producing desirable polymorphism among the tested Pallas NILs (Table 1). Among these primers, 3 were specific for NIL P2 (*Mla3)*, 1 for NIL P10 (*Mla12)*, 6 for NIL P17 (*Mlk*), 46 for NIL P19 (*Mlp*), 4 for NIL P20 (*Mlat*), 6 for NIL P21 (*Mlg*), and 4 for NIL P23 (*MlLa*). Amplification with eight primers resulted in bands ($OPAC1_{631}$, $OPAC5_{814}$, $OPAC12_{421}$, $OPAF3_{638}$, $OPQ20_{989}$, $OPU9_{766}$, $OPV8_{581}$ and $OPW13_{665}$) differentiating more than one NIL carrying different genes for resistance. In total, 70 potential RAPD markers were identified for seven specific powdery mildew resistance genes (Table 1).

Table 1. RAPD markers detecting DNA polymorphisms among seven near-isogenic lines (NILs) of barley cv. Pallas [P02 (*Mla3*), P10 (*Mla12*), P17 (*Mlk*), P19 (*Mlp*), P20 (*Mlat*), P21 (*Mlg*), and P23 (*MlLa*)]

Pallas NIL	Gene	PCR markers
P2	*Mla3*	$OPR7_{361}$, $OPV7_{499}$, $OPW13_{665}$
P10	*Mla12*	$OPW13_{665}$
P17	*Mlk*	$OPAB9_{759}$, $OPAC1_{631}$, $OPAH3_{913}$, $OPU9_{929}$, $OPV8_{581}$, $OPZ12_{1084}$
P19	*Mlp*	$OPAA1_{499}$, $OPAA17_{887}$, $OPAA18_{675}$, $OPAC4_{425}$, $OPAC5_{814}$, $OPAC12_{421}$, $OPAE18_{853}$, $OPAF3_{638}$, $OPAF16_{244}$, $OPAF18_{1132}$, $OPAG2_{249}$, $OPAG15_{575}$, $OPAH15_{1225}$, $OPK16_{530}$, $OPL12_{513}$, $OPL14_{695}$, $OPM2_{689}$, $OPN9_{572}$, $OPO7_{422}$, $OPO10_{780}$, $OPO19_{1044}$, $OPP2_{754}$, $OPP16_{1122}$, $OPQ16_{1056}$, $OPQ20_{989}$, $OPS19_{359}$, $OPT5_{656}$, $OPT7_{594}$, $OPT12_{1403}$, $OPT16_{443}$, $OPU9_{766}$, $OPU12_{2499}$, $OPU15_{2114}$, $OPV3_{670}$, $OPV8_{776}$, $OPV15_{407}$, $OPW3_{1023}$, $OPW17_{915}$, $OPW18_{707}$, $OPX2_{371}$, $OPX7_{348}$, $OPX18_{1038}$, $OPY3_{2067}$, $OPY11_{512}$, $OPY14_{704}$, $OPZ6_{838}$
P20	*Mlat*	$OPAC5_{565}$, $OPAC12_{345}$, $OPAD11_{726}$, $OPT20_{617}$
P21	*Mlg*	$OPAC1_{836}$, $OPAE10(r)^{1}_{516}$, $OPL5_{689}$, $OPP12_{975}$, $OPV8_{721}$, $OPW12_{681}$
P23	*MlLa*	$OPAF3_{492}$, $OPAI9_{1937}$, $OPN7_{1376}$, $OPQ20_{807}$

[1](r) = marker in repulsion phase

The present study demonstrated that there is a high level of variation within amplified DNA fragments in seven Pallas isolines carrying different genes for powdery mildew resistance. The large number (46 from a total of 70) of potential markers identified for gene *Mlp* present in NIL P19 suggests that during creation of this NIL, a relatively large chromosome fragment was introduced into the genotype of cv. Pallas. No confirmed data are available about the location of this gene on a specific barley chromosome (J RGENSEN 1994). However, on the basis of results of this study we conclude that this gene is probably located on a different chromosome than the other genes present in the tested NILs.

The pathogen is able to overcome quickly (i.e. after 47 years) a widely-distributed resistance gene and the breakdown of resistance can have severe consequences (J RGENSEN 1994). To be able to compete effectively with the evolving pathogen, resistance breeders should have in hand several resistance genes that can be incorporated or pyramided rapidly into advanced breeding lines. During the last decade, marker-assisted selection (MAS) emerged as a strategy for increasing selection gains in the early generations during the barley breeding process (WITCOMBE, HASH 2000). In resistance breeding programs, MAS is especially advantageous when the natural inoculum is unreliable and a strong environmental effect on resistance exists (WITCOMBE, HASH 2000). RAPD markers identified in this study potentially could facilitate molecular-based breeding strategies, such as marker-assisted backcrossing by reducing linkage drag of donor parent (LEE 1995) and selection of individuals with highest amounts of the recurrent parent genome (WITCOMBE, HASH 2000).

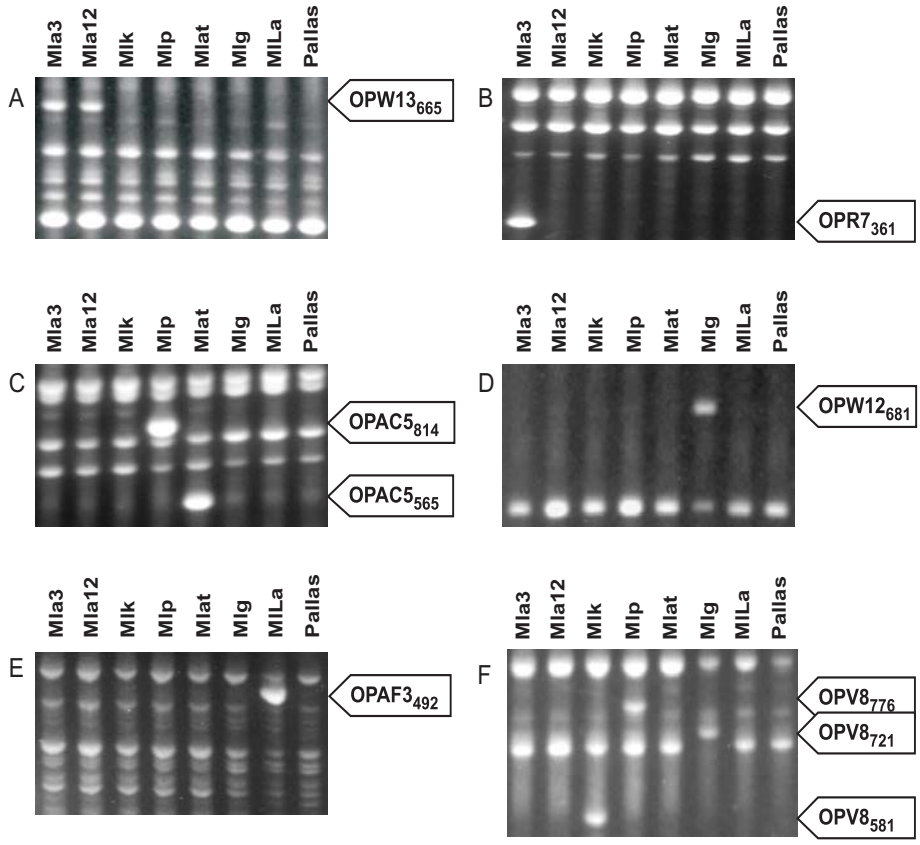

Figure 1. Polymorphic DNA bands (arrows): $OPW13_{665}$ [P2 (*Mla3*) and P10 (*Mla12*)], $OPR7_{361}$ [P2 (*Mla3*)], $OPAC5_{814}$ [P19 (*Mlp*)], $OPAC5_{565}$ [P20 (*Mlat*)], $OPW12_{681}$ [P21 (*Mlg*)], $OPAF3_{492}$ [P23 (*MlLa*)], $OPV8_{776}$ [P19 (*Mlp*)], $OPV8_{721}$ [P21 (*Mlg*)], and $OPV8_{581}$ [P17 (*Mlk*)].

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