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LEAF RUST RESISTANCE IN HYBRID LINES DERIVED FROM CROSSESS BETWEEN *HORDEUM VULGARE* AND *HORDEUM BULBOSUM*

ABSTRACT

Leaf rust caused by fungus *Puccinia hordei* has great economic importance in many barley growing regions in Europe, North America, Australia and West Asia and North Africa. Bulbous barley grass (*Hordeum bulbosum* L.), is the only member of the secondary barley genepool. In presented study 6 recombinant lines obtained from backcrosses of barley cultivars (backcrossing parents) and accessions of *H. bulbosum* were tested with 8 differential isolates of leaf rust. This study showed that resistance to leaf rust is present in 5 from total 6 recombinant lines. Outstanding resistance to leaf rust was identified in line 886Z3/1/10/1/2/1, which showed resistance reaction 0 for inoculation with all isolates used. Another 4 lines were susceptible for inoculation with 2 or 4 leaf rust isolates used. In none of tested lines was possible to postulate presence of known resistance genes for leaf rust resistance. Based on results it may be concluded that leaf rust resistance identified in recombinant lines comes from *H. bulbosum* and may represent new unique type of resistance. Hybrid lines with identified resistance to leaf rust originating from *H. bulbosum* should be used in breeding programmes to provide farmers with cultivars with highly effective resistance to this disease.

Key words: *Hordeum bulbosum*, leaf rust, *Puccinia hordei*, recombinant lines, resistance genes

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world. Barley breeders and geneticists are taking into account three genepools in barley. *H. spontaneum* and *H. vulgare* are in the primary genepool (Bothmer *et al.* 1995). Wild barley especially *H. spontaneum* was used in breeding programmes to transfer of new disease resistances and tolerance to abiotic stress (Fischbeck 2003; Pickering and Johnston 2005). Bulbous barley grass (*H. bulbosum* L.), is the only member of the secondary genepool (Pickering *et al.* 2004a; Bothmer *et al.* 2003; Pickering and Johnston 2005). It is perennial and occurs in the Mediterranean region, West Asia, Caucasus Mountains and part of Central Asia including Iran, Afganistan, Turkmenistan, Uzbekistan, Kazakhstan. It occurs as both dip-

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loid and autotetraploid cytotypes. It normally requires vernalisation to flower and has a strong self-incompatibility system based on two loci (Bothmer *et al.* 1995).

Leaf rust caused by fungus *Puccinia hordei* has great economic importance in many barley growing regions in Europe, North America, Australia and West Asia and North Africa (WANA) (Yahyaoui and Sharp 1987; Park *et al.* 1992; Park 2003; Woldeab *et al.* 2006). In many cases more important than lowering of barley yield is loss of its quality due to infection by leaf rust. This especially concerns plantations of barley for malting purpose. Recently the breeders interest in resistance to barley leaf rust has increased in Europe (Mazaraki and Grabowska 1998; Niks *et al.* 2000; Czembor *et al.* 2006). This interest is caused by observations of increases in fitness of leaf rust populations to many currently grown barley cultivars and to cultivar Vada. Cultivar Vada is well known for possessing high level of partial resistance and it was used wildly as parent in major European barley breeding programs (Czembor *et al.* 2006).

For many years the use of disease-resistant barley cultivars has been an efficient means for controlling major diseases and preventing yield losses (Czembor 1996, 2005; Brooks *et al.* 2000; Finckh *et al.* 2000; Fischbeck 2003; Weibull *et al.* 2003). However, barley breeders, geneticists and plant pathologists are constantly looking for new efficient sources of resistance to major diseases including leaf rust to combine them with already used in modern cultivars in order to increase the resistance durability (Czembor 1996, 2005; Brown and Hovmoller 2002; Bonman *et al.* 2005).

In several studies *H. bulbosum* was described as species with very high level of resistance to barley pathogens including leaf rust (Pickering *et al.* 2004a; Pickering and Johnston 2005). Despite of these observations, there are only few reports on genetic investigations on *H. bulbosum* and on transfer of resistance to major pathogens from *H. bulbosum* to *H. vulgare* (Pickering *et al.* 2004b, 2006a; Zhang *et al.* 2001; Pickering and Johnston 2005). In these reports hybrid lines of *H. bulbosum* x *H. vulgare* expressed resistance to such diseases as leaf rust, powdery mildew, scald, septoria specled leaf blotch, BaYMV/BaMMV, stem rust (Pickering *et al.*, 2004a, 2006b; Shtaya 2007).

In barley-leaf rust host-pathogen system 19 loci with major genes for resistance were identified (Park and Karakousis 2002, Steffenson 2002, Park *et al.* 2003, Weerasena *et al.* 2004). Most of these resistant loci were identified in old cultivars or landraces of *H. vulgare* (Czembor *et al.* 2006). However, three loci *Rph10*, *Rph11* and *Rph16* were introduced to barley from *H. spontaneum*. In addition, based on reports of Pickering and co-workers two resistance loci *Rph17*, *Rph18* were described which originate from *H. bulbosum* (Pickering *et al.* 1998, Franckowiak 2000).

The objective of this study was to determine leaf rust resistance in recombinant lines obtained in crosses between *H. bulbosum* and *H. vulgare*.

MATERIALS AND METHODS

Plant material

Six recombinant lines obtained from crosses and backcrosses of barley cultivars Vada, Emir, Golden Promise (backcrossing parents) and accessions of *H. bulbosum* (S1, 2920/4, HB2032) were tested (Table 1). In addition 3 cultivars which were backcrossing parents for specific lines were tested. Recombinant lines were obtained at New Zealand Institute for Crop and Food Research, New Zealand (Pickering 1987, 1988; Pickering *et al.* 1987, 1998, 2000a).

Table 1

Recombinant lines, their pedigrees and chromosome location of *H. bulbosum* introgression

No	Recombinant line	Back crossing parent (*H. vulgare*)	*H. bulbosum* parent	Chromosome location of *H. bulbosum* introgression
1	81882/83/3/2/9	Vada	S1	2HS
2	4176/n/3/2/6	Vada	S1	No data
3	886Z3/1/10/1/2/1	Emir	2920/4	2HL
4	38P18/5-13/1-9	Emir	HB2032	2HL
5	38U4/1/3/7	Golden Promise	2920/4	5HL + 6HS
6	102C2/18	Emir	HB2032	2HL

Pathogen

Eight differential isolates of *P. hordei* were used (Table 2). These isolates originated from IHAR Radzikow collection and were chosen according to differences in virulence spectra observed on 12 differential cultivars. None of the isolates used was able to differentiate genes *Rph4* from *Rph8* and *Rph1* from *Rph10* and *Rph11*.

Resistance tests

Five to 10 plants per line were evaluated in a greenhouse with 8 isolates of *P. hordei*. This study was carried out in the IHAR Radzikow growing chambers. Cultivar L94, which does not carry any known genes for resistance to *P. hordei,* was used as a susceptible control.

Testing procedure

The plants were grown with 16 h light and temperature range of 18-22°C. Urediniospores of *P. hordei* were suspended in deionized water with couple

drops of "Tween 20" and inoculated onto one-week old seedling plants (primary leaf fully expanded) using a rate 3 mg urediniospores and 10 ml of water- for 100 plants. Inoculated plants were incubated for 24 hours in a chamber in which the humidity was maintained near saturation by mist from ultrasonic humidifiers n complete darkness and with a temperature range of 12-15°C. Then plants were transferred to a greenhouse bench.

Table 2

Differential isolates and their infection types on differential set.

Accession name	Accession number	Gene	Isolates							
			Ph- 9	*Ph-5*	*Ph-4*	*Ph-6*	*Ph-31*	*Ph-21*	*Ph-17*	*Ph-25*
Sudan	CIho 6489	*Rph1*	4	4	4	4	4	4	4	4
Peruwian	CI 935	*Rph2*	4	4	4	4	4	4	2	4
Estate	CI 3410	*Rph3*	0	4	0	4	4	0	4	4
Gold	CI 1145	*Rph4*	4	4	0	4	4	4	4	4
Magnif	CI 13860	*Rph2+Rph5*	4	1	4	0	0	0	1	4
Bolivia	CI 1257	*Rph2+Rph6*	4	4	4	4	0	4	4	4
Cebada Capa	CI 6193	*Rph7*	0;	0;	0;	0;	0;	0;	0;	0;
Egypt 4	CI 6481	*Rph8*	4	4	0	4	4	4	4	4
HOR 2596	CI 1243	*Rph9*	4	4	4	4	4	1	4	4
Cliper C8	None	*Rph10*	4	4	4	4	4	4	4	4
Cliper C67	None	*Rph11*	4	4	4	4	4	4	4	4
Triumph	PI 290195	*Rph12*	4	4	4	4	4	0;	4	4

Disease assessment

Reactions of each accession were evaluated after an incubation period of 12-14 days in a greenhouse at 20-24°C. Disease symptoms were assessed on the primary leaf of the seedlings according to 0-4 scale adapted from Levine and Cherewick (1952). Infection types 0, 0;, 1 and 2 were considered indicative of incompatibility whereas infection types 3 and 4 of compatibility.

Postulation of leaf-rust resistance genes

Hypotheses about the specific resistance genes present were made by comparing the reaction spectra of the tested lines with those of differential lines.

RESULTS

Line 4176/n/3/2/6 was susceptible for infection with all isolates used. Most probably this line do not possess resistance to leaf rust (Table 3). Another 5 lines tested showed resistance reaction to infection with leaf rust. The most resistant line for inoculation with leaf rust was line 886Z3/1/10/1/2/1. This line showed resistance reaction 0 for inoculation with all isolates used. Another 4 lines were susceptible for inoculation with 2 or 4 leaf rust isolates used. The most common resistance reaction was 0. In none of tested lines was possible to postulate presence of known resistance genes for leaf rust resistance.

Barley cultivars used as back crossing parents showed lack of resistance or very limited one for inoculation with 8 differential isolates.

Table 3

Reaction of 6 recombinant lines and 3 cultivars to infection with 8 isolates of *Puccinia hordei*.

No	Recombinant lines and cultivars	Isolates								Postulated genes	Possible genes
		9	5	4	6	31	21	17	25		
1	81882/83/2/9	4	4	4	1	0;	1	4	2	?*	
2	4176/n/3/2/6	4	4	4	4	4	4	4	4	None	*Rph1* or *Rph10* or *Rph11*
3	886Z3/1/10/1/2/1	0	0	0	0	0	0	0	0	?	
4	38P18/5-13/1-9	0	0	0	0	4	0	4	0	?	
5	38U4/1/3/7	4	4	4	2	4	2	2	2	?	
6	102C2/18	0;	0	0	4	4	0;	0	0	?	
7	Golden Promise	4	4	4	4	4	4	4	4	None	*Rph1* or *Rph10* or *Rph11*
8	Emir	4	4	4	4	4	2	4	4	?	*Rph9*
9	Vada	4	4	4	4	4	4	4	4	None	*Rph1* or *Rph10* or *Rph11*

DISCUSSION

Presented study confirmed findings of other investigators that hybrid lines of *H. bulbosum* x *H. vulgare* possess resistance to major pathogens of barley including leaf rust (Pickering *et al.* 1999, 2004b; 2006b; Pickering and Johnston 2005; Shtaya 2007). Based on screening tests it may be concluded that resistance to leaf rust is present in 5 from total 6 recombinant lines obtained from crosses and backcrosses of barley cultivars and accessions of *H. bulbosum*. Outstanding resistance to leaf rust was identified in line 886Z3/1/10/1/2/1, which showed resistance reaction 0 for inoculation with all isolates used. Another 4 lines were susceptible for inoculation with

2 or 4 leaf rust isolates used. In none of tested lines was possible to postulate presence of known resistance genes for leaf rust resistance. However based on resistance reaction we can conclude that line 81882/83/2/9 may have more than one resistance gene because it expressed 3 different resistance reactions: 0;, 1 and 2. Lines 886Z3/1/10/1/2/1 and 38P18/5-13/1-9 may have one or more resistance genes expressed as resistance reaction 0. In line 38U4/1/3/7 we can postulate presence of one or more resistance genes expressed as resistance reaction 2. In addition, expression of resistance reaction 2 is showing also possibility for the presence of some level of partial resistance in this line. Barley cultivars used as back crossing parents showed lack of resistance or very limited one for inoculation with 8 differential isolates. It confirms that resistance loci present in tested recombinant lines originate from *H. bulbosum* parents.

In presented study seedling resistance tests were used in order to describe infection types expressed by barley plants after inoculation with differential isolates of *P. hordei.* This kind of testing as sufficient for screening for disease resistance and it is used commonly in many breeding programs to postulate the presence of specific genes for resistance in modern cultivars and to screen for new sources of effective resistance (Czembor 1996, 2005; Czembor and Czembor 2001). However, by using this kind of tests it is not possible or at least it is difficult to identify and describe partial resistance. For description of this kind of resistance we need to conduct additional to infection type of measurements of characteristics. In addition, partial resistance is generally better expressed at the adult plant stage (Ochoa and Parlevliet 2007). It will be very interesting if further studies of described hybrid lines will also include parameters describing partial resistance and extension of resistance studies to plants at adult stage. Final determination of the number of resistance genes and the type of their action in tested hybrid lines may be established by crosses and backcrosses among appropriate genotypes (Czembor 1996, 2005).

Description of new sources of resistance are important because future strategies for the control of barley pathogens will have to focus increasingly on more ecologically acceptable methods. Any usage of chemicals (pesticides, fungicides, herbicides, and mineral fertilizers) in agriculture is increasingly criticized in societies of many countries. Breeding for resistance represent such ecologically safe method. In addition to ecological also economical arguments (use of fuel, labour, special machines) are in favour of breeding for resistance versus chemical control (Nierobca *et al.* 2003). Hybrid lines with identified resistance to leaf rust originating from *H. bulbosum* should be used in breeding programmes to provide farmers with cultivars with highly effective resistance to this disease.

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REFERENCES

Bonman J.M., Bockelman H.E., Jackson L.F., Steffenson B.J. 2005. Disease and insect resistance in cultivated barley accessions from the USDA National Small Grains Collection. Crop Sci. 45: 1271-1280.

Bothmer von, R., N. Jacobsen, C.B. Rikke, B. J?rgensen, Linde-Laursen I. 1995. An ecogeographical study of the genus *Hordeum*. IPGRI, Rome, Italy: 1-129.

Brooks W.S., Griffey C.A., Steffenson B.J., Vivar H.E. 2000. Genes governing resistance to *Puccinia hordei* in thirteen spring barley accessions. Phytopathol. 90: 1131-1136.

Brown J.K.M., Hovmřller M.S. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Sci. 297: 537-541.

Czembor J.H. 1996. Presence and expression of resistance genes to powdery mildew of barley in selections from Tunisian barley landraces. Ph. D. thesis, Department of Plant Pathology, Montana State University, USA, 1 - 144.

Czembor J.H. 2005. Powdery mildew [*Blumeria graminis* (DC.) E. O. Speer f. sp. *hordei*] resistance in landraces of barley (*Hordeum vulgare* L.) - habilitation monography. Monographies and Dissertations of IHAR no. 2005(24), p 1-164.

Czembor J.H., Czembor H.J. 2001. Inheritance of resistance to powdery mildew (*Blumeria graminis* f.sp. *hordei*) in selections from Moroccan landraces of barley. Cereal Res. Comm. 29(3-4): 281-288.

Czembor P.C., Pietrusińska A., Czembor H.J. 2006. Mapping new resistance gene to *Puccinia hordei* Otth. in barley. In: Cereal Science and Technology for Feeding Ten Billion People: Genomics Era and Beyond. Proceedings from EUCARPIA - Cereal Section Conference, 13-17 Nov. Lleida, Spain, 54.

Finckh M.R., Gacek E.S., Goyeau H., Lannou C., Merz U., Mundt C.C., Munk L., Nadziak J., Newton A.C., de Vallavielle-Pope C., Wolfe M.S. 2000. Cereal variety and species mixtures in practice, with emphasis on disease resistance. Agronomie 20: 813-837.

Fischbeck G. 2003. Diversification through breeding. 2003. Pp. 29-52. In: R. von Bothmer, T. van Hintum, H. Knüpffer, K. Sato (Eds), Diversity in Barley, Elsevier, Amsterdam.

Franckowiak J.D. 2000. Coordinator's report: Chromosome 2H (2). Barley genetic Newsl. 30: 68 – 71.

Levine M.N., Cherewick W.J. 1952. Studies on dwarf leaf rust of barley. U.S. Department of Agric. Tech. Bull. No. 1056, Washington, DC. 1-17.

Mazaraki M., Grabowska J. 1998. Population structure of barley leaf rust (*Puccinia hordei* Otth.) in Poland. Biul. IHAR 207: 81-86.

Nierobca A., Horoszkiewicz-Janka J., Czembor J.H. 2003. Plant protection as an important element of cereals cultivation technology in the European Union. Pamietnik Pulawski 132: 311-320.

Niks R.E., Walther U., Jaiser H., Martinez F., Rubiales D., Andersen O., Flath K., Gymer P., Heinrichs F., Jonsson R., Kuntze L., Rasmussen M., Richter E. 2000. @Literatura = Resistance against barley leaf rust (*Puccinia hordei*) in West-European spring barley germplasm. Agronomie 20: 769-782.

Ochoa J., Parlevliet J.E. 2007. Effect of partial resistance to barley leaf rust, *Puccinia hordei*, on the yield of three barley cultivars. Euphytica 153(3): 309-312.

Park R.F. 2003. Pathogenic specialization and phenotype distribution of Puccinia hordei Otth. in Australia, 1992-2001. Plant Dis. 87: 1311-1316.

Park R.F., Karakousis A. 2002. Characterisation and mapping of gene *Rph19* confering resistance to *Puccinia hordei* in the cultivar Reka 1 and several Australian barleys. Plant Breed. 121: 232-236.

Park R.F., Cotterill F.J., Rees R.G. 1992. Pathogenic variability in *Puccinia hordei* in Australia. In: Proc. of the Eighth Europ. and Mediter. Cereal Rusts and Mildew Conference Freising-Weihenstephan, 129 - 132.

Park R.F., Poulsen D., Barr A.R., Cakir M., Moody D.B., Raman H., Read B.J. 2003. Mapping genes for resistance to *Puccinia hordei* in barley. Australian J. Agric. Res. 54: 1323-1333.

Pickering R.A. 1987. The influence of *Hordeum bulbose* L. ploidy level on crossability with *H. vulgare* L. cv. Vada and *Triticum aestivum* L. cv. Chinese spring. Barley Genet. Newsl. 17: 43-45.

Pickering R.A. 1988. The production of fertile triploid hybrids between *Hordeum vulgare* L. (2n = 2x = 14) and *H. bulbosum* L. (2n = 4x = 28). Barley Genetics Newsletter 18:25–29.

Pickering R.A., Johnston P.A. 2005. Recent progress in barley improvement using wild species of *Hordeum.* Cytogenet. Genome Res. 109: 344-349.

Pickering R.A., Rennie W.F., Cromey M.G. 1987. Disease resistant material available from the wide hybridization programme at DSIR. Barley Newsletter 31:248–250.

Pickering R.A., Steffenson B.J., Hill A.M., Borovkova I. 1998. Association of leaf rust and powdery mildew resistance in a recombinant derived from a *Hordeum vulgare* x *H. bulbosum* hybrid. Plant Breeding 117: 83-84.

Pickering R. Niks R., Jonhston P.A., Butler R. 2004a. Importance of the secondary gene pool in barley genetics and breeding. II. Disease Resistance, agronomic performance and Quality. Czech J. Genet. Plant Breed. 40: 79 -85.

Pickering R.A., Hudakova S., Houben A., Jonston P.A., Butler R.C. 2004b. Reduced metaphase I associations between the short arms of homologous chromosomes in a *Hordeum vulgare* L. x *H. bulbosum* diploid hybrid influences the frequency of recombinant progeny. TAG 109: 911-916.

Pickering R. Klatte S., Butler R.C. 2006a. Identification of all chromosome arms and their involvement in meiotic homoelogous associations associations at metaphase I in 2 *Hordeum vulgare* L. x *Hordeum bulbosum* L hybrids. Genome 2006, 49: 73-78.

Pickering R., Ruge-Wehling B., Johnson P.A., Schweizer G., Ackermann P., Wehling P. 2006b. The transfer of a gene conferring resistance to scald (*Rynchosporium secalis*) from Hordeum bulbosum into *H. vulgare* chromosome 4HS. 125: 576-579.

Shtaya M.J.Y., Sillero J.C., Flath k., Pickering R., Rubiales D. 2007. The resistance to leaf rust and powdery mildew of recombinant lines of barley (*Hordeum vulgare* L.) derived from *H. vulgare* x *H. bulbosum* crosses. Plant Breeding 126:259-267.

Steffenson B.J. 2002. Coordinator's report: Disease and pest resistance genes. BGN 32: 179-184.

Weerasena J.S., Steffenson B.J., Falk A.B. 2004. Conversion of an amplified fragment length polymorphism marker into a co-dominant marker in the mapping of the Rph15 gene conferring resistance to barley leaf rust, Puccinia hordei Otth. TAG 108(4): 712-719.

Woldeab G., Finisz C., Singh H., Yuen J. 2006. Virulence spectrum of *Puccinia hordei* in barley production systems in Ethiopia. Plant Pathol. 55: 351-357.

Yahyaoui A.H., Sharp E.L. 1987. Virulence spectrum of *Puccinia hordei* in North Africa and the Middle East. Plant Dis. 71: 597-598.

Zhang L., Pickering R.A., Murray B.G. 2001. A *Hordeum vulgare H. bulbosum* tetraploid hybrid provides useful agronomic introgression lines for breeders. NZ J Crop and Hort Science 29:239–246.