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THE USE OF BULK SEGREGANT ANALYSIS TO IDENTIFY A RAPD MARKER LINKED TO THE *MLA* LOCUS OF BARLEY

ABSTRACT

Resistance to powdery mildew, *Blumeria graminis* f.sp. *hordei*, is a major goal of many barley breeding programs. Resistance conferred by genes located at *Mla* locus is commonly used by barley breeders for effective control of powdery mildew. The use of molecular markers may facilitate barley breeding for powdery mildew resistance. In this study, bulked segregant analysis (BSA) was used to determine random amplified polymorphic DNAs (RAPDs) markers linked to *Mla* locus. Thirty one homozygous (17 resistant and 14 susceptible) F_3 families from a cross between variety Pallas and single plant line E 1059-1-1 carrying gene at *Mla* locus were used as plant material. A total of 385 random 10-mer primers were screened to identify polymorphism between the appropriate resistant and susceptible DNA bulks and parents in BSA analysis. Only one PCR marker $OPAA3_{400}$ (primer sequence: 5'-TTAGCGCCCC-3'), amplified in polymerase chain reaction (PCR) proved close linkage and was positioned in distance of 10 cM from *Mla* locus with 5.0 LOD threshold.

Key words: barley, *Blumeria graminis* f. sp. *hordei*, *bulked segregant analysis, DNA marker, Mla* locus, RAPD

INTRODUCTION

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 such as most of Europe, West Asia and North Africa (WANA region), Japan and the eastern barley producing areas of Canada and USA (Kiesling 1985, Czembor 1996).

Chemical control and agronomic practices are not sufficient to efficiently reduce disease incidence. Developing resistant cultivars is the most effective and environmentally safe method to control this disease,

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but powdery mildew still remains an important disease because of great pathogen's variability (Hovmøller *et al.* 2000). More than 100 mildew resistance genes have been identified in barley. The resistance alleles *Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla9*, *Mla10*, *Mla12*, *Mla13*, *Mla14*, *Mla22*, *Mla23* in locus *Mla* and the resistance alleles *Mlk*, *Mlg*, *MlLa*, *Mlh*, *Mlra* and *mlo11* are commonly used in European cultivars of barley (Jřrgensen 1994, Czembor and Czembor 2001a). The most commonly used genes located in *Mla* locus were *Mla1* (Algerian resistance), *Mla9* (Monte Cristo resistance), *Mlas12* (Arabische resistance) and *Mla7* (Lyallpur resistance). Especially important role in barley breeding for powdery mildew resistance played Arabische resistance. Generally, genes at *Mla* locus are characterized by infection type 0 and 1 (Giese 1981, Jørgensen 1994). This type of resistance is very popular among breeders because it provides very good control against barley powdery mildew. The *Mla* locus was originally described in accessions Algerian and S.P.I. 45492 (Briggs and Stanford 1939). Later studies have revealed a large number of allelic genes or closely linked at locus *Mla*. Until now, 32 specificities at the *Mla* locus have been differentiated by their specific reaction to unique isolates of *B. graminis* f.sp. *hordei* (Giese 1981, Jahoor and Fischbeck 1993, Kintzios *et al.* 1995, Wise and Ellingboe 1983).

The use of molecular markers may facilitate barley breeding for powdery mildew resistance (Czembor and Talbert 1997, Gupta *et al.* 1999, Manninen *et al.* 1997). Practical barley breeding has already adopted the new marker-based techniques to tag genes of interest and marker-assisted selection (MAS) becomes a common tool for plant breeder (Erpelding *et al.* 1996, Gupta *et al.* 1999). Such DNA markers are random amplified polymorphic DNAs (RAPDs) invented by Williams *et al.* (1990) based on polymerase chain reaction (PCR) developed by Mullis and Faloona (1987). During last decade RAPD markers had proven to be effective in genetic studies of distinctiveness of barley varieties (Baum *et al.* 1997, 2000, Selbach and Cavalli-Molina 2000), estimation of genetic diversity among barley landraces (Papa *et al.* 1998), barley wild progenitor *Hordeum spontaneum* Koch (Nevo *et al.* 1988, Baum *et al.* 1997, Melchinger 1990), *Hordeum* phylogeny (Russell *et al.* 1997, De Bustos 1998) and genes for resistance (Borovkova *et al.* 1995, Kutcher *et al.* 1996, Molnar *et al.* 2000). From the very beginning of using RAPD markers it was demonstrated in many studies that RAPD analysis provides a rapid method of identifying markers for disease resistance loci (Martin *et al.* 1991, Paran *et al.* 1991, Penner *et al.* 1993). Recently, some studies were reported concerning identification of RAPD markers linked to genes at *mlo* and *Mla* loci conferring barley resistance to powdery mildew (Manninen *et al.*1997, Wei *et al.* 1999). Also in many studies, practical value of molecular markers was proven to be useful for monitoring of changes in pathogen populations (Brändle 1994, McDermott *et al.* 1994, Czembor and Arseniuk 1999, 2000). A good

knowledge about virulences structure *of B. graminis* f.sp. *hordei* is necessary for use of effective resistance genes by barley breeders (Hovmøller *et al.* 2000, Czembor and Czembor 2001a).

In this paper we report the identification of a RAPD marker linked to the locus *Mla* with genes for resistance to powdery mildew.

MATERIALS AND METHODS

Plant material

Line E 1059-1-1 selected from landrace Ex 1059 of *H. vulgare* was used in this study. Landrace Ex 1059 was provided kindly by Dr. W. Podyma (Polish Gene Bank, IHAR Radzików, Poland) and was collected in Morocco during expedition organized by Warsaw Agricultural University SGGW-AR in 1985 (Podyma 1988). As it was described in the previous study (Czembor and Czembor 2000) during 1996-1998 from this landrace single plant was selected resistant to *B. graminis* f. sp. *hordei*. Line E 1059-1-1 was established as a result of propagation of this plant and testing its progeny for powdery mildew resistance. Then this line was tested with 30 differential isolates of *B. graminis* f.sp. *hordei.* Based on this study it was suggested that powdery mildew resistance gene of this line is located in the locus *Mla*.

In another report the genetic study of inheritance of powdery mildew resistance in this line was described (Czembor and Czembor 2001b). In this study were used Pallas isolines with different genes for powdery mildew (Kølster *et al.* 1986). To determine the number of genes, the types of genes action and the gene loci in this line two types of crosses were made: (1) line was crossed with the susceptible variety Pallas, (2) line was crossed with four Pallas isolines possessing *Mla3* (isoline P2), *Mlat* (isoline P20), *Mlg* (isoline P21) and *mlo5* (isoline P22) genes for resistance. Then seedlings of parents, F_2 generation and F_3 families were evaluated for the powdery mildew resistance infected with isolate R303.1. Chi-square analyses were done to test goodness of fit between observed and expected ratios of resistant and susceptible plants or families. Data from F_3 families originating from cross between line E 1059-1-1 and cultivar Pallas fitted an expected 1:2:1 ratio of resistant, non-segregating: segregating: susceptible, non-segregating progenies. This indicated that resistance to powdery mildew of selection E 1059-1-1 is controlled by a dominant allele of a single gene at locus *Mla* (Czembor and Czembor 2001b).

Pathogen

The isolate R303.1 of *B. graminis* f.sp. *hordei* Em. Marschal was used. The virulence spectrum of this isolate was determined based on observation of its infection types on the Pallas isoline differential set (Kølster *et al.* 1986).

BSA analysis

Conclusion about homogozygosity of F_3 families was based on the infection type showed by seedlings of these families after inoculation with powdery mildew isolate R303.1. Results of this testing showed that among 67 tested F3 families 31 were homozygous (17 resistant and 14 susceptible) and 38 heterozygous (Czembor and Czembor 2001b). Since RAPD markers are dominant, only these 31 homozygous families were further evaluated in BSA analysis. Leaves of two weeks old seedlings were used for DNA extraction with a CTAB method (Poulsen *et al.* 1993) for each sample of the 31 homozygous families. DNA concentration was determined by using TKO 100 Mini Fluorometer (Hoefer Scientific Instruments, 654 Minnesota St, San Francisco, CA 94107, USA) with DNA standards and Hoechst 33258 Dye. In order to conduct BSA analysis two pools of DNA were established. Equal amount of DNA for 6 of the homozygous resistant and 6 of the homozygous susceptible individuals were pooled separately. These two pools together with DNA extracted from two parents were screened with 385 arbitrary sequence 10 base primers selected from kits K-AI of the Operon Technologies (Alameda 1000, Atlantic Ave., CA 94501, USA). If polymorphism between resistant and susceptible components was detected (significant differences in bands illumination between DNA pools was also considered), the marker was selected for further evaluation in PCR using DNA of pooled individuals and the reminder progeny.

Primer sequences were synthesised by Ransom Hill Bioscience (P.O. Box 219, Ramona, CA 92065, USA). PCR amplifications were conducted in 12.5 µl volumes containing buffer with $(NH_4)_2SO_4$ (Fermentas AB, Graiciuno 8, Vilnius 2028, Lithuania), 2 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP, 0.5 µM primer, approximately 24 ng of template DNA, and 1 U of *Taq* DNA polymerase (Fermentas) was used. The reaction mixture was overlaid with 9 µl of mineral oil. The DNA Thermal Cycler UNOII 96 (Biometra GmbH, P.O.B. 1544, 37005 Göttingen, Germany) was programmed first for 95°C for 60 s and subsequently for 40 cycles of denaturation at 95°C for 30 s, annealing at 35°C for 30 s, elongation at 72°C for 120 s. The final primer-elongation segment of the run was extended to 12 min. The amplification products were separated on 1.8% agarose gel (1 × TBE buffer, 6V/cm, 4 h) in constant temperature of 15°C controlled by refrigerated and heating circulator FS18-HP (Julabo Labortechnik GmbH, D-77960 Seelbach, Germany). Afterwards, gels were stained with ethidium bromide and photographed on a transilluminator.

Data analysis

Weak and irreproducible bands produced in the PCR assays were not considered in analyses. Linkage between *Mla* locus and marker was estimated using JoinMap version 2.0 software (Stam and Van Ooijen 1995). The recombination fraction was transformed into cM according to

Kosambi function. Molecular weight of bands was counted by FragmwNT Analysis v. 1.1 (Molecular Dynamics, Sunnyvale, CA, USA).

RESULTS

Among 385 ten-base primers screened to identify polymorphism between the appropriate resistant and susceptible DNA bulks and parents in BSA analysis only one marker $OPAA3_{400}$ (primer sequence: 5'-TTAGCGCCCC-3') was identified to be closely linked to *Mla* locus (Fig. 1). Three recombinants were observed among 31 homozygous (17 resistant and 14 susceptible) F_3 families when this marker was used. Marker $OPAA3_{400}$ was positioned in distance of 10 cM from *Mla* locus with 5.0 LOD threshold.

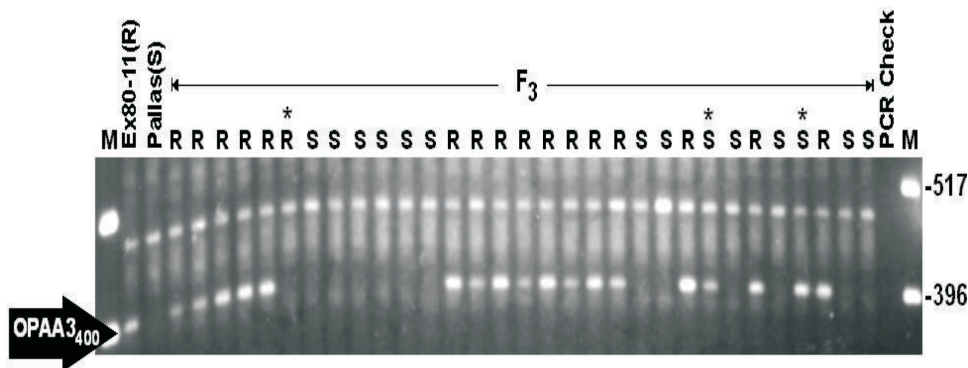

Fig.1. Segregation of marker OPAA3 (black arrow) in population of 31 homozygous F families derived from cross E 1067-1-1 (*Mla*) × Pallas. 'R' and 'S', indicate F families resistant and susceptible, respectively. Recombinants are indicated with '*'. Lanes labeled with 'M' contain size marker (pUC19/Rsa I, Hinf I, Pvu II) in base pairs (bp). Lane labeled with 'PCR check' contain negative control of PCR reaction (HO instead DNA template)

DISCUSSION

Marker-assisted selection (MAS) has become recognized as a strategy for increasing selection efficiency (Dudley 1993, Knapp 1994). MAS is the most effective in the early generations of selection among progeny from crosses between inbreed lines because using traditional breeding methods selection in early generation of a pedigree breeding program is often limited by amount of available F_2 and F_3 seed (Lande 1992, Knapp 1994, Stromberg *et al.* 1994). Recent advances in developing molecular techniques have made possible the routine use of MAS to produce gene pyramiding for disease resistance (Witcombe and Hash 2000). The gains from using MAS depend on many factors, including the genetics of the trait of interest, ge-

netic distance between the marker and the gene of interest and the way that MAS is used in the breeding program. In resistance breeding programs, MAS is especially advantageous when the natural inoculum is unreliable, resistance breeding is done against exotic or quarantined pathogens and strong environmental effect on resistance is observed (Melchinger 1990, Witcombe and Hash 2000). Molecular markers can assist backcross breeding for resistance. Use of these markers in selection of desired genes from donor parents reduce linkage drag and amount of undesirable genome of donor parent (Knapp 1998, Gupta *et al.* 1999, Witcombe and Hash 2000).

It was proved in many studies that fungus *B. graminis* f.sp. *hordei* can produce races during few years which overcome a widely used resistance genes (Jřrgensen 1994, Wolfe and McDermott 1994). To avoid this barley breeders developed few strategies of deploing resistance genes. The most common strategy is pyramiding into a single genotype several resistance genes (Wolfe and McDermott 1994). Several resistance genes against barley powdery mildew are known and some of them have been mapped or tagged with DNA-markers. These include the *Mla* locus (Schüller *et al.* 1992, Jahoor *et al.* 1993, Kintzios *et al.* 1995,) the *MlLa* locus (Giese *et al.* 1993) and the *Mlg* locus (Görg *et al.* 1993). DNA markers linked to these specific resistance genes represent valuable tools for barley breeders to enhance backcrossing and pyramiding processes for mildew resistance (Melchinger 1990).

The pyramiding of many different genes for powdery mildew resistance is difficult in breeding practice because many of common resistance genes *Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla9*, *Mla10*, *Mla12*, *Mla13*, *Mla14*, *Mla22*, *Mla23*, *Mlk* and *Mlat* are located on short arm of chromosome 1H (Jřrgensen 1994). Molecular markers may be very useful in this situation for detecting closely linked different genes for resistance in early generations in breeding program.

CONCLUSIONS

In this study, the RAPD technique, in conjunction with BSA, proved to be quick and effective technique for identification of molecular marker linked to *Mla* locus of barley. Marker $OPAA3_{400}$ positioned in distance of 10 cM from *Mla* locus may be used in breeding programs for powdery mildew resistance in barley.

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REFERENCES

Baum B.R., Mechanda S., Soleimani V. 2000. Identification of Canadian six row barley (*Hordeum vulgare* L.) cultivars with primers derived from STSs obtained from RAPD diagnostic bands. Seed Science Technology 28: 45-466.

Baum B.R., Nevo E., Johnson D.A., Beiles A. 1997. Genetic diversity in wild barley (*Hordeum spontaneum* C. Koch) in the Near East: a molecular analysis using Random Amplified Polymorphic DNA (RAPD) markers. Genetic Research Crop Evolution 44: 47-157.

Borovkova I.G., Steffenson B.J., Jin Y., Rasmussen J.B., Kilian A., Kleinhofs A., Rossnagel B.G., Kao K.N. 1995. Identification of molecular markers linked to the stem rust resistance gene *rpg4* in barley. Phytopathology 85: 181-185.

Brändle U.E. 1994. Studies on the genetic structure of local populations of *Erysiphe graminis* f. sp. *hordei* Marchal. Ph. D. thesis, Swiss Federal Institute of Technology, Zürich. 65 pp.

Briggs F.N., Stanford E.H. 1939. Linkage of factors for resistance to mildew in barley. Journal of Genetics 37: 107-117.

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, Bozeman, USA. 144 pp.

Czembor P.Cz., Arseniuk E. 1999. Study of genetic variability among monopycnidial and monopycnidiospore isolates derived from single pycnidia of *Stagonospora* spp. and *Septoria tritici* with use of RAPD-PCR, MP-PCR and rep-PCR techniques. Journal of Phytopathology 147: 539-546.

Czembor P.Cz., Arseniuk E. 2000. Segregation and recombination of PCR based markers in sexual progeny of *Phaeosphaeria* species. Mycological Research 104: 919-926.

Czembor J.H., Czembor H.J. 2000. Powdery mildew resistance in barley landraces from Morocco. Journal of Phytopathology 148(5): 277-288.

Czembor H.J., Czembor J.H. 2001a. Resistance to powdery mildew in barley cultivars and breeding lines included in 1998-2000 Polish registration trials. Plant Breeding and Seed Science 45: 19-28.

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

Czembor H.J., Talbert L.E. 1997. Evaluation of STS-PCR and RFLPs as molecular markers for the *Mla* locus conferring powdery mildew resistance in barley. Plant Breeding and Seed Science 41: 1-14.

De Bustos A., Casanova C., Soler C., Jouve N. 1998. RAPD variation in wild populations of four species of the genus *Hordeum* (*Poaceae*). Theoretical and Applied Genetics 96: 101-111.

Dudley J.W. 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitalive traits. Crop Science 33: 660-668.

Erpelding J.E., Blake N.K., Blake T.K., Talbert L.E. 1996. Transfer of sequence tagged site PCR markers between wheat and barley. Genome 39: 802-810.

Giese H. 1981. Powdery mildew resistance genes in the *Ml-a* and *Ml-k* regions on barley chromosome 5. Hereditas 95: 51-62.

Giese H., Holm – Jensen A.G., Jensen H.P., Jensen J. 1993. Localization of the *Laevigatum* powdery mildew resistance gene to barley chromosome 2 by the use of RFLP markers. Theoretical and Applied Genetics 85: 897-900.

Görg R., Hollricher K., Schulze-Lefert P. 1993. Functional analysis and RFLP-mediated mapping of the *Mlg* resistance locus in barley. The Plant Journal 3: 857-866.

Gupta P.K., Varshney R.K., Sharma P.C., Ramesh B. 1999. Molecular markers and their applications in wheat breeding. Plant Breeding 118: 369-390.

Hovmøller M.S., Caffier V., Jalli M., Andersen O., Besenhofer G., Czembor J.H., Dreiseitl A., Flath K., Fleck A., Heinrics F., Jönsson R., Limpert E., Mercer P., Plesnik S., Rashal I., Skinnes H., Slater S., Vronska O. 2000. The Eurpean barley powdery mildew virulence survey and disease nursery 1993-1999. Agonomie 20: 729-744.

Jahoor A., Fischbeck G. 1993. Identification of new genes for mildew resistance of barley at the *Mla* locus in lines derived from *Hordeum spontaneum*. Plant Breeding 110: 116-122.

Jahoor A., Jacobi A., Schüller C.M.E., Fischbeck G. 1993. Genetical and RFLP studies at the *Mla* locus conferring powdery mildew resistance in barley. Theoretical and Applied Genetics 85: 713-718.

Jørgensen J.H. 1994. Genetics of powdery mildew resistance in barley. Crital Review in Plant Science 13: 97-119.

Kiesling R.L. 1985. The diseases of barley. In: D. C. Rasmusson (ed.). Barley. Madison, Wisconsin: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 269-312.

Kintzios S., Jahoor A., Fischbeck G. 1995. Powdery-mildew-resistance genes *Mla29* and *Mla32* in *H. spontaneum* derived winter-barley lines. Plant Breeding 114: 265-266.

Knapp S.J. 1994. Selection using molecular marker indexes. Proc. Second Plant Breed. Symp, CSSA and ASHS, Corvallis, OR. pp. 1-8.

Knapp S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Science 38: 1164-1174.

Kølster P., Munk L., Střlen O., Lřhde J. 1986. Near-isogenic barley lines with genes for resistance to powdery mildew. Crop Science 26: 903-907.

Kutcher H.R., Bailey K.L., Rossnagel B.G., Legge W.G. 1996. Identification of RAPD markers for common root rot and spot blotch (*Cochliobolus sativus*) resistance in barley. Genome 39:206-215.

Lande R. 1992. Marker-assisted selection in relation to traditional methods of plant breeding. In: Stalker, H.T., Murphy, J.P. (eds.). Plant Breeding in the 1990s. Proc. Symp. Plant Breeding in the 1990s. C.A.B. International, Wallingford, UK. pp. 437-451.

Manninen O.M., Turpeinen T., Nissilä E. 1997. Identification of RAPD markers closely linked to the *mlo*-locus in barley. Plant Breeding 116: 461-464.

Martin G.B., Williams J.G.K., Tanksley S.D. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proceeding of National Academy of Science USA 88: 2336-2340.

McDermott J.M., Brändle U., Dutly F., Haemmerli U.A., Keller S., Müller K.E., Wolfe M.S. 1994. Genetic variation in powdery mildew of barley: development of RAPD, SCAR, and VNTR markers. Phytopathology 84: 1316-1321.

Melchinger A.E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breeding 104: 1-19.

Molnar S.J., James L.E., Kasha K.J. 2000. Inheritance and RAPD tagging of multiple genes for resistance to net blotch in barley. Genome 43: 224-231.

Mullis K.B., Faloona F.A. 1987. Specific aynthesis of DNA *in vitro* via a polymerase catalyzed reaction. Methods Enzymol. 255, 335-350

Nevo E., Baum B., Beiles A., Johnson D.A. 1988. Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. Genetic Research & Crop Evolution 45: 151-159.

Papa R., Attene G., Barcaccia G., Ohgata A., Konishi T. 1998. Genetic diversity in landrace populations of *Hordeum vulgare* L. from Sardinia, Italy, as revealed by RAPDs, isozymes and morphophenological traits. Plant Breeding 117: 523-530.

Paran I., Kesseli R., Michelmore R. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce using near-isogenic lines. Genome 34: 1021-1027.

Penner G.A., Chong J., Levesque-Lemay M., Molnar S.J., Fedak G. 1993. Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theoretical and Applied Genetics 85: 702-705.

Podyma W. 1988. Report from expedition to Morocco to collect crop landraces in 1985. In: Proceedings of symposium on: Plant Genetic Resources and Initial Material for Plant Breeding. 15-16 Oct. IHAR Radzikow. Zeszyty Problemowe IHAR 61-67 pp.

Poulsen, G.B., Kahl, G., Weising, K., 1993. Abundance and polymorphism of simple repetitive DNA sequences in *Brassica napus* L. Theor. Appl. Genet. 85, 994-1000.

Russell J.R., Fuller J.D., Macaulay M., Hatz B.G., Jahoor A., Powell W., Waugh R. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theoretical and Applied Genetics 95: 714-722.

Schüller C., Backes G., Fischbeck G., Jahoor A. 1992. RFLP markers to identify the alleles on the *Mla* locus conferring powdery mildew resistance in barley. Theoretical and Applied Genetics 84: 330-338.

Selbach A., Cavalli-Molina S. 2000. RAPD characterization of Brazilian barley (*Hordeum vulgare* ssp. *vulgare*) varieties. Euphytica 111:127-135.

Stam P., Van Ooijen J.W. 1995. JoinMap (tm) version 2.0: Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands.

Stromberg L.D., Dudley J.W., Rufener G.K. 1994. Comparing conventional early generation selection with molecular marker assisted selection in maize. Crop Science 34: 1221-1225.

Wei F., Gobelman-Werner K., Morroll S.M., Kurth J., Mao L., Wing R., Leister D., Schulze-Lefert P., Wise R.P. 1999. The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153: 1929-1948.

Wise R.P., Ellingboe A.H. 1983. Infection kinetics of *Erysiphe graminis* f.sp. *hordei* on barley with different alleles at the *Mla* locus. Phytopathology 73: 1220-1222.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Witcombe J.R., Hash C.T. 2000. Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: gene pyramiding, three-way hybrids, and synthetic parent populations. Euphytica 112: 175-186.

Wolfe M.S., McDermott J.M. 1994. Population genetics of plant pathogen interactions: the example of the *Erysiphe graminis-Hordeum vulgare* pathosystem. Annual Reviev of Phytopathology 32: 89-113.