Theor Appl Genet (2003) 107:1278–1287
DOI 10.1007/s00122-003-1362-5

J. L. Molina-Cano · J. P. Simiand · A. Sopena · A. M. Pérez-Vendrell · S. Dorsch · D. Rubiales · J. S. Swanston · A. Jahoor

Mildew-resistant mutants induced in North American two- and six-rowed malting barley cultivars

Received: 21 November 2002 / Accepted: 3 March 2003 / Published online: 30 July 2003
© Springer-Verlag 2003

Abstract Mildew-resistant mutants were induced with sodium azide in three North American malting barley cultivars, two in the six-rowed Ursula (URS1 and URS2), one in the six-rowed Gertrud (GER1), and one in the two-rowed Prudentia (PRU1). Two of the mutants, URS1 and PRU1, showed complete resistance and were shown to have two new alleles at the *mlo* locus; these were designated, respectively, *mlo31* and *mlo32*. Mutant URS2, showing partial resistance, was inherited as a dominant gene, but was not an allele at the *Mla* locus. The mean yield of each mutant was higher than that of its parental line, but yield levels varied across environments, although this was independent of the severity of the mildew attack. Other reasons, for example, the severity of the necrotic lesions in the mutants, may account for yield variations. The malting quality of the GER1 mutant proved similar to that of Gertrud, but both URS1 and URS2 showed lower malt extract than Ursula. This lower extract might be due to the smaller grain size of the mutants that could, in turn, result from necrotic lesions in the leaves, as implied by the effects on grain yield.

Keywords Barley · Powdery mildew · Mutants · Malting quality

Communicated by G. Wenzel

J. L. Molina-Cano (✉) · A. Sopena
Centre UdL-IRTA, Av. Rovira Roure 177, 25189 Lleida, Spain
e-mail: joseluis.molina@irta.es

J. P. Simiand · S. Dorsch
Bush Agricultural Resources, Ft Collins, Colo., USA

A. M. Pérez-Vendrell
IRTA, Centre Mas Bové, Reus, Spain

D. Rubiales
Instituto de Agricultura Sostenible, Córdoba, Spain

J. S. Swanston
Scottish Crop Research Institute, Invergowrie, Dundee, UK

A. Jahoor
Risø National Laboratory, Plant Research Department, Roskilde, Denmark

Introduction

Powdery mildew (*Blumeria graminis* f. sp. *hordei*) is one of the most damaging foliar diseases of barley in Europe. Consequently, resistance has been a character selected for since the early days of European barley breeding (Jensen et al. 1992; Friedt et al. 2000). Conversely, mildew is not an important disease in the Midwest USA, the main malting barley-producing region of the USA. Here, other barley pathogens, such as spot blotch, caused by *Cochliobolus sativus*, and stem rust, caused by *Puccinia graminis* f. sp. *tritici* (Steffenson 2000), are the major contributors to losses in both yield and quality over seasons. In addition to differences in disease resistance profile, barley required for the USA brewing industry differs from that used to make beer in Europe with respect to various quality attributes. American beer mostly utilises malt made from six-rowed barleys with very high diastatic power and moderate extract (Burger and LaBerge 1985). In Europe, the prevalent malting barleys are of the two-rowed type, and very high malt extract is required (Schildbach and Burbidge 1992; Friedt et al. 2000). These differences in the barley quality profile result from the addition, to the mashing wort, of a substantially higher quantity of non-malt adjuncts in the USA.

In recent years American-style beers, brewed under license in Europe, have greatly increased in popularity. The higher enzyme levels required for brewing could be met by technical means – for example, by reducing the temperature at which the malt is kilned. However, this could have an influence on flavour while, in addition, there may be commercial reasons why companies wish their beers to be brewed with specific types of barley. For that reason a programme was initiated to develop varieties, including six-rowed types, adapted to European-growing conditions, but with the quality attributes required for American style brewing.

In an attempt, described in this paper, to produce mildew-resistant six-rowed North American lines as a genetic resource for exploitation in brewing or to be used

as parents in subsequent crossing programmes, mutagenesis was used instead of transferring the genes for mildew resistance by backcrossing. This approach was chosen to leave the delicate genetic equilibrium on which the malting quality of US six-rowed barleys is constructed largely unaltered, as it is known to result from a narrowly based breeding scheme carried out at Minnesota over more than 20 years (Rasmusson 2000). Mutants resistant to mildew have been induced on many occasions, most being recessive alleles at the *mlo* locus (Jørgensen 1992). The *mlo* mildew resistance has proved durable, but some *mlo* mutants have demonstrated reduced grain yield. This has been attributed to the necrotic flecking, which can cause a significant loss in green leaf area, and is known to be a pleiotropic effect of the *mlo* mutation (Jørgensen 1992).

Two American six-rowed varieties, Gertrud (B 1614) and Ursula (B 2601), were selected to be mutagenised and a two-rowed US malting barley cultivar, Prudentia (B 1202), was also treated. Previously published work suggested that levels of necrotic specking, associated with the *mlo* gene, are affected differentially by two-row and six-row genetic backgrounds (Bjornstad and Aastveit 1990). Here the results of the mutagenesis experiments are reported and, in addition, effects on yield and some agronomic characters are noted. As there is a lack of published information of any effects of the *mlo* mutation on malting quality, mutants were compared to their parental lines to determine whether the required malting attributes were conserved. The malting performance of the mutants could not be generalised to malting quality of other *mlo* carrier lines.

Materials and methods

Mutagenesis and mutant selection

Dry grain samples, 1 kg each, of the six-row cultivars Gertrud (B 1614) and Ursula (B 2601) and the two-row Prudentia (B 1202) were mutagenised with sodium azide following the protocol of Molina-Cano et al. (1989). The M_1 generations were grown in isolated plots at Lleida (Spain) in 1993 and harvested in bulk. From each cultivar 30,000 M_2 seeds were screened for mildew resistance in the greenhouse in 1993 and 1994. The seeds were sown individually on plastic trays with 10 × 6 holes each, and kept at a daily cycle of 20 °C for 16 h of light and 15 °C for 8 h of darkness. Sufficient pots with spreader plants of the very susceptible cultivar Ursula, previously infected with a mixture of local isolates of powdery mildew, were placed amongst the M_2 trays to permit a heavy mildew infection. This developed when the plantlets had two unfolded leaves, covering almost all their foliar area. The mixture of isolates used was known to be virulent on all known resistance genes except *Ml-a, Ml-*(*1402*) and *mlo* (Molina-Cano et al. 1992). Putative mutants were selected that showed an almost complete absence of mildew colonies, and these plants were reared until maturity. The mutants were subsequently subjected to various cycles of natural mildew infection, both in the greenhouse and the open field, to check their reaction.

Table 1 Field trial locations and locations where the grain for the malting analyses was produced

Environment code	Country	Site	Year
Field trials locations			
97FR1	France (centre)	Levroux	1997
97FR2	France (north)	Valenciennes	1997
98FR1	France (centre)	Levroux	1998
99FR1	France (centre)	Levroux	1999
99FR2	France (north)	Valenciennes	1999
99SP1	Spain (north-east)	Zaragoza	1999
99SP2	Spain (north-east)	Lleida	1999
Locations where the grain for malting analyses was produced			
98FTC	USA	Fort Collins (Colorado)	1998
98FRN	France (north)	Valenciennes	1998
98SPW	Spain (north-east)	Lleida	1998

Powdery mildew test

Powdery mildew tests were conducted at the seedling stage using detached leaves placed on water agar containing 15 mg/l benzimidazol. The seedlings were raised under controlled powdery mildew-free conditions in order to standardise receptivity and to prevent contamination. Inoculations were carried out 9–10 days after sowing using an infection tower (Aslam and Schwarzbach 1980). For incubation, the infected leaves were placed under controlled conditions at 17 °C and 100% relative humidity. Infection types were scored 9 days after inoculation according to the 0-4 scale described by Islam et al. (1992). For the lines containing the *mlo* powdery mildew resistance gene, 0/4 reaction types were recorded (Jahoor and Fischbeck 1987).

The *mlo* testcrosses were carried out by crossing the mutants PRU1 and URS1 to *Alexis*, a cultivar carrying the *mlo9* allele. Both F_1s and F_2s were tested for resistance to powdery mildew by exposure in the greenhouse to a composite powdery mildew population from the Lleida region (Molina-Cano et al. 1992), using the genotype SM4142, which does not carry any resistance gene (G. Fischbeck, personal communication), as a spreader. The URS2 mutant was test-crossed to *Alexis* (*mlo*) and *Rupal* (*Mla13*) (Table 3). In the first case, the mildew population used for inoculation was the composite noted above, while, to test for *Mla13* resistance, a mildew isolate virulent against it, kindly supplied by Prof. G. Fischbeck, was used.

Field testing

The selected mutants were grown in field trials at various European sites over 3 years. The trial sites covered a wide range of environments, from northeastern Spain to northern France, differing in agronomic practice and, therefore, yield potential. The sites and years are listed in Table 1. Each trial consisted of plots of eight rows, each 6 m long, 0.15 m apart, replicated three times and laid out in an alpha-lattice design.

Malting quality analyses

Samples were taken from the sites and years listed in Table 1. The analytical sample was obtained by pooling together the grain from the three replications of each treatment, followed by two analytical replications per sample. These samples were prior screened over a 2.38-mm sieve and a sub-sample of the seed retained was used for micromalting with the following scheme.

1) Steeping:
– Temperature: 10 °C
– Programme: immersion, 5 h 30 min; air rest, 6 h; immersion, 4 h; air rest, 6 h; immersion, 2 h; air rest, 2 h.

2) Germination:
– Temperature: 14.5 °C
– Duration: 4 days.

3) Kilning:
– Total time: 22 h 45 min
– Programme: 1 h 10 min, 32.5 °C; 2 h, 35 °C; 8 h, 44.5 °C; 4 h 30 min, 58 °C; 2 h, 65 °C; 1 h 20 min, 75 °C; 30 min, 85 °C; 3 h 30 min, 90 °C; 40 min, 25 °C.

Malting analyses were carried out according to the methods of the American Society of Brewing Chemists (ASBC 1992), and the following quality parameters were analysed: total malt protein (%), coarse grind malt extract yield (%), Kolbach index (wort protein as a percentage of total malt protein), α-amylase (dextrinising units, DU) and diastatic power (degrees Lintner, °L). The use of coarse grind malt extract instead of the standard fine grind determination is a common practice in some barley breeding programmes to make the selection criteria more stringent, as under-modified portions of the endosperm may not be brought into solution (Swanston et al. 2000).

Statistical analyses

General linear model analyses of variance were carried out with STATGRAPHICS 4.1 (Statistical Graphics Corporation 1999).

Results

Selection of powdery mildew resistant mutants

A number of mutants with superior resistance to that of their parental cultivar were selected. One from Gertrud was apparently partially resistant, showing moderate susceptibility in mildew tests. Two resistant mutants were obtained from Ursula and seven from *Prudentia*, but six of these showed other severe phenotypic defects and were discarded. The other one, designated PRU1, showed extensive necrotic flecking and was considered a possible *mlo* type.

Genetic analysis of powdery mildew resistance

The effect of a particular resistance gene or allele is determined by the specific interactions between the resistance gene(s) in a host plant and different virulence mlgene(s) in the pathogen. Consequently, according to Flor's gene-for gene model (Flor 1955), a resistance gene in the host plant can be identified after infection with specific pathogen isolates. Therefore, a set of single spore isolates with known virulence genes were selected to identify resistance genes in the mutants. In these experiments, the mutants URS2, URS1, GER1 and PRU1 and their mother lines *Prudentia, Ursula* and *Gertrud*, as well as some European varieties possessing known powdery mildew resistance genes, were included. All three mother lines were completely susceptible to all powdery mildew isolates used in this experiment (Table 2). Consequently, these three varieties do not possess any effective powdery mildew resistance gene. The mutants URS1 and PRU1, when exposed to infection with powdery mildew isolates, developed a typical infection 0/4. This particular reaction type is known to be conditioned only by the *mlo* powdery mildew resistance gene. The mutants URS2 and GER1 have improved resistance when compared to their mother varieties, but these two mutants did not exhibit a typical *mlo* reaction type (Table 2). Consequently, URS2 and GER1 do not possess the *mlo* powdery mildew resistance gene.

These results were confirmed by those from the testcrosses (Table 3). In crosses to cv. *Alexis*, which carries the *mlo* gene, involving both PRU1 and URS1, all F_1 and F_2 progeny were resistant, strongly indicating that they carried a homozygous expression of the *mlo* allele. Testcrosses involving the mutant URS2, however, appeared to give conflicting results. When crossed to *Alexis* (Table 3), the segregation of resistant and susceptible F_2

Table 2 Reaction to powdery mildew of a set of barley mutants, their mother lines and control genotypes

Genotype	Gene	GI-1	VA-3	TR-2	OR-4	VO-2	DK 58-74	RU-3	BO-1	201-60	184-21	WE-3
Ursula		4	3	4	4	4	4	4	4	4	4	4
URS1		2	0	1	0	0/4-	0	0	0	1	0/4	0/4-
URS2		3	3	1	0	3	2	1	2	0	1	3
Gertrud		4	4	4-	4	4	4	4	4	4	4	4
GER1		3-4	3-4-3	2-4	3-4-	3-4-	2-3	4	3-4	3-2	3-4	3-4-
Prudentia		4	4	4	4	4	4	4	3	4	4	4
PRU1		0	0	0	0	0	0/4-	0	1	0/4	0	0
Aramir	*Mla12 + Mlg*	4	4	1	1	2	2	4	4	2	4	1
Ortolan	*Mla7*	2	1-1	4	4	1	4	4	1	4	4	4
Voldagsen	*Mla6*	0	4	4	4	4	0	0	4	4	1	0
Rupal	*Mla13*	1-1-1	2	1	1	0	4	4	1	1	1	1
Vada	*MlLa*	4	3	3	3	4	3	4	3	4	3	4
Welam	*Mla9*	0	3	0	0	0	4	4	0	1	4	4
Gitte	*Mla1*	4	4	0	0	0	0	0	0	4	0	1
Alexis	*mlo*	0	0/4	0/4-	0	0	0	0/4	0	0	0	0
SM4142	None	4-	4-	4	4	4	4	4	3	4	4	4

Table 3 Crosses carried out for studying the inheritance of the mutant genes

Cross PRU1 × Alexis (*mlo*)

F_1: all 15 plants resistant
F_2: all 119 plants resistant
Conclusion: *mlo* gene

Cross URS1 × Alexis (*mlo*)

F_1: all 17 plants resistant
F_2: all 120 plants resistant
Conclusion: *mlo* gene

Cross URS2 × Alexis (*mlo*)

F_1: all 21 plants resistant
F_2: 152 plants resistant: 39 plants susceptible
(non-significantly different from a 13:3 segregation, $P < 0.05$)
Conclusion: a dominant resistance gene

Cross URS2 × Rupal (*Mla13*)

F_1: all 18 plants resistant
F_2: 61 plants resistant: 183 plants susceptible
(non-significantly different from a 3:1 segregation, $P < 0.05$)
Conclusion: a recessive resistance gene

progeny suggested the presence of a dominant gene. The testcross to *Rupal*, which carries the *Mla13* resistance gave a 3:1 ratio of susceptible to resistant progeny, suggesting a recessive resistant gene.

Agronomic performance

The results of analysis of variance of grain yield, powdery mildew and other characters of agronomic interest are summarised in Table 4. There were no statistically significant differences between genotypes for grain yield, but highly significant differences were recorded for powdery mildew susceptibility, and there was also genotype × environment interaction for the latter. There were, however, significant differences for grain yield between environments, indicating that the widely different sites gave, as expected, different agronomic performance. Grain size, days to heading and plant height showed highly significant differences both between genotypes and between environments, and the latter two characters were also subject to significant genotype × environment interaction.

Mean values across environments of these variables (Table 6) indicate a much higher mildew resistance in the mutants URS1 and URS2 (scores 1.17 and 1.24) than in their mother line Ursula (score 6.64). The superiority in resistance shown by mutant GER1 (score 2.20) over its mother cultivar Gertrud (score 4.46) was smaller but statistically significant. The mean grain yields of the mutants across environments were slightly superior to those of the parental genotypes, but the differences proved not to be significant. Both Ursula mutants, URS1 and URS2, had significantly thinner kernels and were later in heading and taller than the parental type. GER1 was not, however, significantly different from Gertrud with regard to these characters.

Genetic and environmental variation in grain yield and powdery mildew susceptibility across the seven environments studied is shown in Fig. 1. For mildew, the mutants were significantly less susceptible than the mother varieties in every environment. Within environments, differences between the mutant and parental genotypes for grain yield were smaller than those for mildew, and they were more variable. For example, the mutant GER1 surpassed Gertrud in three out of the seven environments, but the opposite occurred at the two Spanish sites. Mutant and parental genotypes attained similar yield levels in the remaining two environments. The mutants URS1 and URS2 surpassed Ursula in four environments and were inferior in the remaining three. There was no relationship between the severity of mildew attack and the yield differences observed between mutants and mother lines (Fig. 1), and this was confirmed by analyses of covariance and correlation that showed no significant relationship between these two variables (data not presented).

Other variables of agronomic importance are also recorded in Fig. 1, although in these cases the number of environments was fewer than seven. Grain size of the mutant GER1 was always similar to that of Gertrud, but the mutants URS1 and URS2 showed significantly lower grain sizes than Ursula in all cases. The mutants URS1 and URS2 invariably tended to be later in heading than Ursula, but differences between GER1 and Gertrud were inconsistent across environments. The differences in plant height between the mutants and their respective mother varieties were subject to significant genotype × environment interaction, so it was not possible to determine an overall effect of the mutations on plant height.

Table 4 Probability of *F* after general linear model analysis of variance of grain yield, powdery mildew scores, grain size, days to heading and plant height across different environments of France and Spain during 3 years of the three mutants and their parental genotypes. Significant values are in bold

Source	Grain yield (Dt/ha)	Mildew[a]	Grain size[b]	Days to heading[c]	Plant height (cm)
Genotype	0.7640	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Environment	**<0.001**	0.2537	**0.0004**	**<0.001**	**<0.001**
Genotype × environment	0.4354	**0.0001**	0.2880	**<0.001**	**<0.001**

[a] Scale: 1, no symptoms; 9, maximum incidence
[b] Percentage of grain smaller than 2.38 mm
[c] From January 1

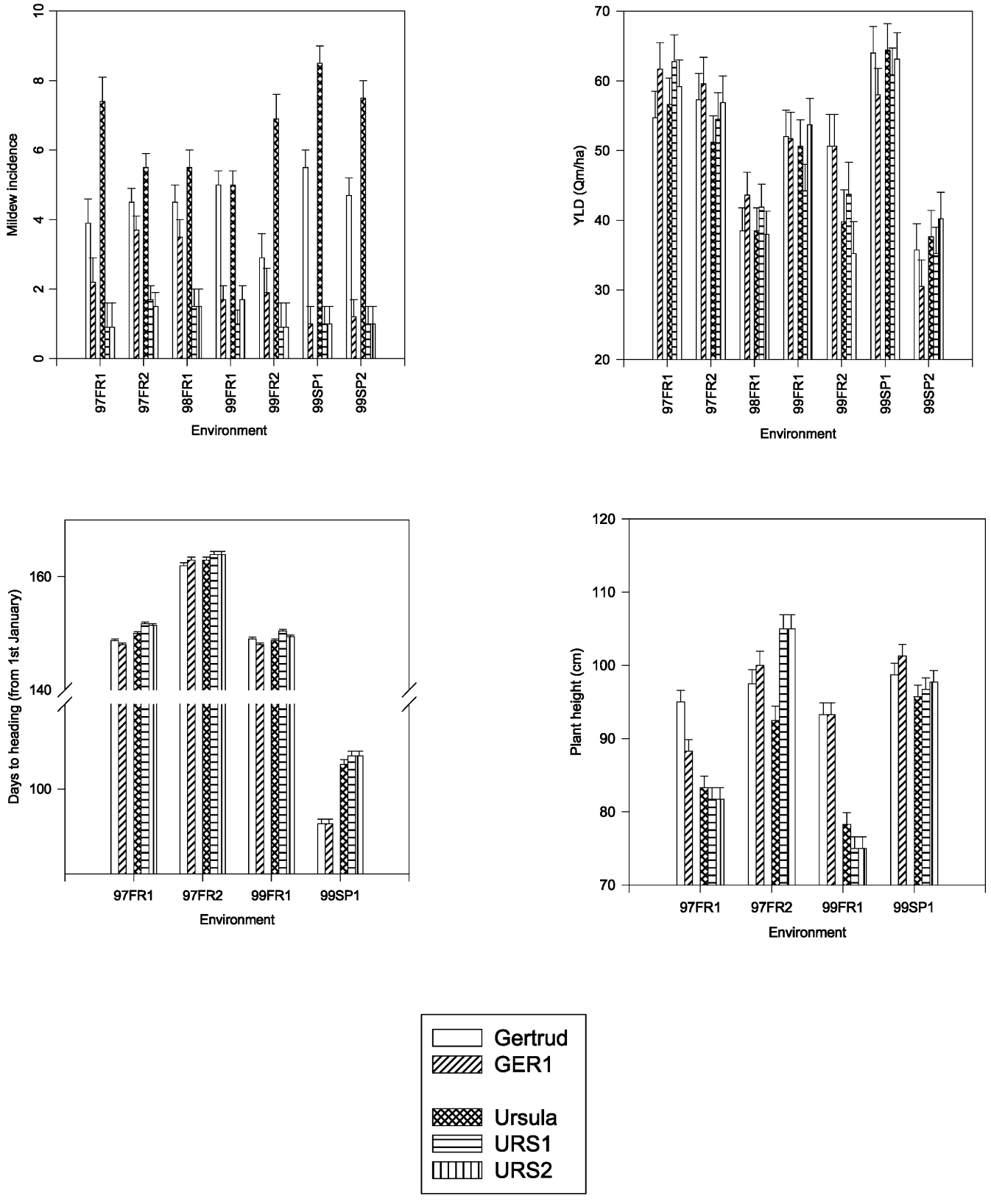


Fig. 1 Genetic and environmental variation in agronomic characters and powdery mildew susceptibility across the seven environments studied (environment codes as in Table 1). Mean values of: mildew incidence, grain yield (YLD), days from sowing to heading and plant height. *Bars*: Standard errors for $P < 0.05$

Table 5 Probability of *F* after general linear model analysis of variance of malting quality parameters of samples of three mutants and their parental genotypes grown at three environments in USA, France and Spain in 1998. Significant values in bold

Source	Total malt protein (%)	Coarse grind malt extract yield (%)	Kolbach index[a]	α-amylase activity (DU)	Diastatic power (°L)
Genotype	**0.0288**	**0.0066**	0.8356	**0.0081**	0.1057
Environment	**<0.001**	**0.0016**	**0.0071**	**0.0138**	**0.0187**
Genotype × environment	0.1582	0.4096	0.2214	0.1324	0.7057

[a] Wort protein as percentage of malt protein

Table 6 Least squares means of grain yield, powdery mildew scores, days to heading and plant height across different environments of France and Spain during 3 years of the three mutants and their parental genotypes. Means followed by a different letter are significantly different after an LSD test ($P < 0.05$)

Genotype	Grain yield (Dt/ha)	Mildew[a]	Days to heading[b]	Plant height (cm)
Ursula	48.35a	6.64d	141.2b	87.46b
URS1	48.98a	1.17a	142.5a	89.58a, b
URS2	49.44a	1.24a	142.2a	89.83a
Gertrud	50.36a	4.46c	138.9c	96.13c
GER1	50.77a	2.20b	138.8c	95.75c

[a] Scale: 1, no symptoms; 9, maximum incidence
[b] From January 1

Table 7 Least squares means of malting quality parameters of samples of three mutants and their parental genotypes grown at three environments in USA, France and Spain in 1998. Means followed by a different letter are significantly different after an LSD test ($P < 0.05$)

Genotype	Sieving fraction	Total malt protein (%)	Coarse grind malt extract yield (%)	Kolbach index[a]	α-Amylase activity (DU)	Diastatic power (°L)
Ursula	47.46b	13.80a	74.31a	40.60a	58.46ab	195.30a
URS1	32.79a	14.75b	72.29b	41.47a	66.35c	219.94ab
URS2	34.86a	14.66b	72.13b	41.27a	62.94bc	213.16ab
Gertrud	68.61c	13.75a	75.17a	41.23a	58.54ab	201.80ab
GER1	67.49c	13.85a	74.99a	39.62a	55.39a	199.62a

[a] Wort protein as percentage of malt protein

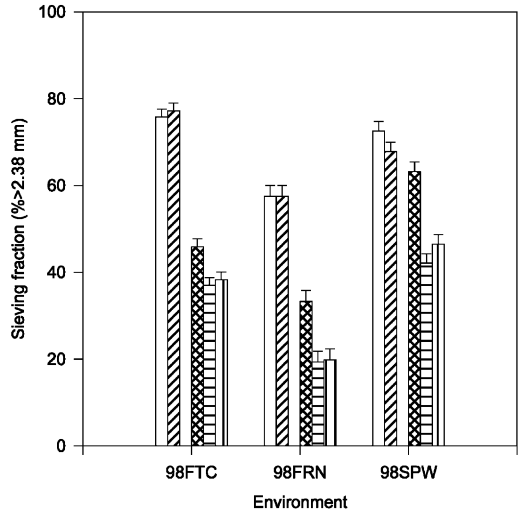
Malting quality analysis

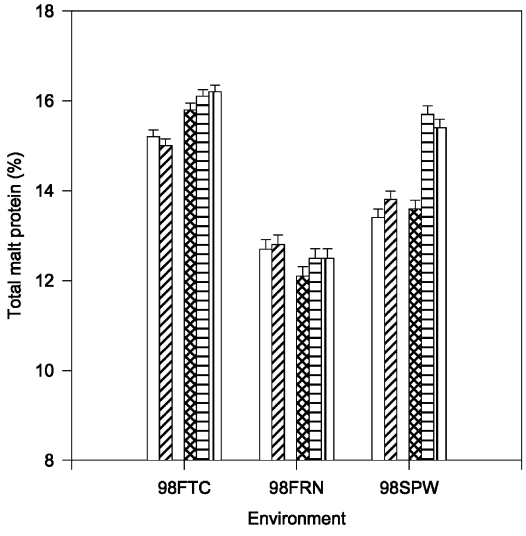
The statistical analyses of the malting quality data are summarised in Tables 5 and 7, and the means of genotypes across environments in Fig. 2. The analyses of variance (Table 5) show statistically significant differences between genotypes for total malt protein, coarse grind malt extract yield and α-amylase. Differences in diastatic power did not reach the level of statistical significance. All quality characters showed significant differences between environments, with environments proving fairly diverse (Fig. 2), but there was no genotype × environment interaction. GER1 was not significantly different from its mother line Gertrud for total malt protein, extract yield and Kolbach index (Table 7) and showed only slightly lower amylolitic activity (α-amylase and diastatic power), so its quality level is similar to that of the original genotype. In contrast, both the Ursula mutants, URS1 and URS2, showed significantly higher protein content and lower malt extract than Ursula. However, their amylolytic activity (α-amylase and diastatic power) was higher than that of the parental genotype.

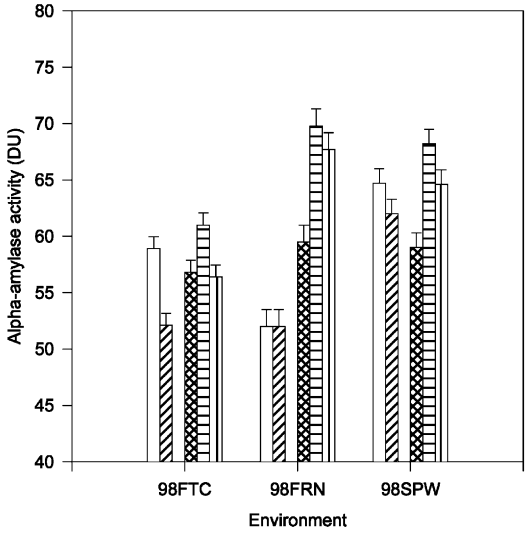
The genetic and environmental variation for the malting quality parameters across three environments is shown in Fig. 2. Differences between GER1 and Gertrud were generally small and variable across environments. In contrast, the relative performance of both the Ursula mutants, URS1 and URS2, compared to their parental genotype was more consistent, except for Kolbach index. Although the magnitude of the difference between parental and mutant types varied across environments, it is possible that variation in grain size might be responsible for the malting quality inferiority of URS1 and URS2 compared to Ursula. Differences in malt protein, malt extract level and diastatic power were all expressed most strongly at the Spanish site (SPW) from the 1998 harvest.

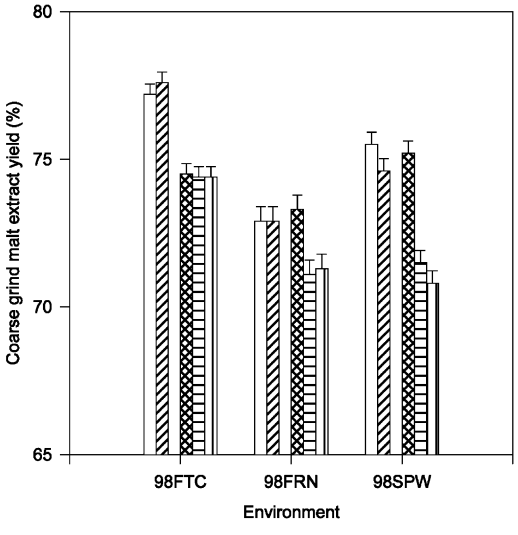
Discussion

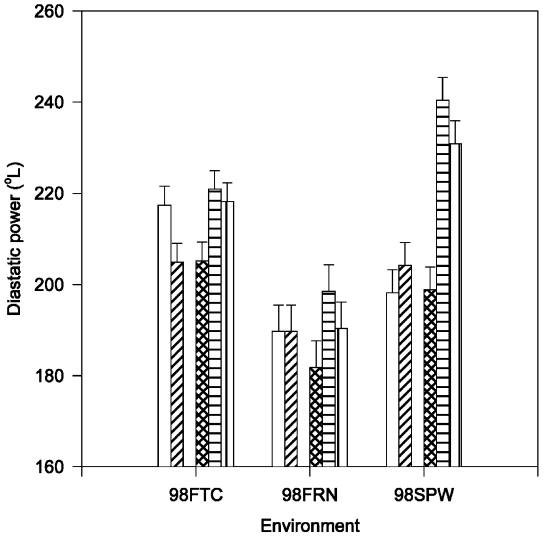
Since Freisleben and Lein (1942), numerous attempts have been made to increase the resistance of barley against powdery mildew by mutagenic seed treatments. In such experiments, mainly *mlo* mutants that are functionally identical have been isolated (Jørgensen 1975). Until


Sieving fraction (%>2.38 mm)
100
80
60
40
20
0
98FTC
98FRN
98SPW
Environment

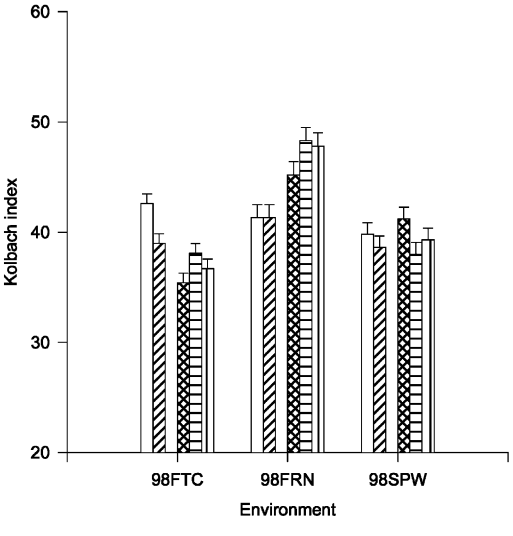

Total malt protein (%)
18
16
14
12
10
8
98FTC
98FRN
98SPW
Environment


Alpha-amylase activity (DU)
80
75
70
65
60
55
50
45
40
98FTC
98FRN
98SPW
Environment


Coarse grind malt extract yield (%)
80
75
70
65
98FTC
98FRN
98SPW
Environment


Diastatic power (°L)
260
240
220
200
180
160
98FTC
98FRN
98SPW
Environment


Gertrud
GER1
Ursula
URS1
URS2


Kolbach index
60
50
40
30
20
98FTC
98FRN
98SPW
Environment

now 26 different alleles have been identified at this locus (Habekuss and Hentrich 1988; Jørgensen 1994; http://www.volny.cz/eschwarzbach). Except for *mlo11*, which has been identified in Ethiopian landraces (Hoffmann and Nover 1959), all alleles of the *mlo* locus have been created by mutation (Jørgensen 1994). The F_1 and F_2 populations of crosses between mutants URS1 and PRU1 with *Alexis*, which carries the *mlo9* resistance gene, support the presence of the *mlo* locus in these mutants (Table 3). In addition, these two mutants give the typical *mlo* reaction type after challenging with the respective powdery mildew isolates (Table 2). On the basis of these facts, it is suggested that the gene in the mutants URS1 and PRU1 should be designated as *mlo31* and *mlo32*, respectively.

The mutant URS2 has a high level of resistance when compared to its mother line (Tables 2, 6). This mutant does not exhibit the typical *mlo* reaction pattern. The segregation analysis of the cross between the URS2 and *Alexis* (Table 3) supports the assumption that the mutated gene is not located at the *mlo* locus. This progeny also provides evidence of the dominant mode of inheritance. Heun and Röbbelen (1984) isolated a resistant mutant from the variety *Bomi* that shows a dominant mode of inheritance. The linkage analysis of crosses involving the mutant and lines possessing different morphological markers from chromosomes 1H and 4H indicated that the mutated gene for resistance is located at or near the *Mlg* locus on chromosome 4H (Heun 1984). Further testcrosses are necessary to localise the gene for resistance in the mutant URS2.

A segregating F_2 progeny was developed from the cross between the mutant URS2 and the variety *Rupal* (carrying the resistance gene *Mla13*). When this progeny was inoculated with an isolate avirulent to the mutant and virulent to the *Rupal* gene, a recessive mode of inheritance was detected (Table 3). Testing two progenies for this mutant with various isolates delivered two different modes of inheritance – recessive and dominant – for the resistance gene present in the mutant URS2 (Table 3). Islam et al. (1992) observed a different mode of inheritance for the *Mla10* powdery mildew resistance gene in barley after challenging with different powdery mildew isolates. The simplest hypothesis to explain different modes of inheritance of the mutated gene could be the involvement of modifier or inhibitor genes (Haggag and Dyck 1973) or minor genes (Khan 1969). Whether these two different modes of inheritance that occurred in the present investigations are due to the interaction of the different genetic backgrounds of the parents used in the crosses (*Alexis* and *Rupal*) or whether the resistance gene of the mutant is acting differently with the various isolates used in these experiments cannot be confirmed. Further powdery mildew tests and the development of new crosses are necessary to verify the mode of inheritance of the mutated gene. The applications of DNA markers will certainly confirm the presence of one or two loci mutated in this experiments that are necessary for resistant reaction against different isolates, and this would help to estimate the number of genes or gene actions involved in the resistant reaction of the mutant. It has already been observed for the several *Mla* alleles (Jørgensen 1996; Freialdenhoven et al. 1994) and for the *mlo* (Freialdenhoven et al. 1996) that *Rar* and *Ror* loci are required for the resistant reaction.

The mean yield of the mutants was similar than that of their mother lines (Table 6), but relative yields fluctuated across environments. This occurred independently of the severity of the mildew attack (Fig. 1), so other reasons for these differences are postulated. In the case of the mutant GER1, it seems that it is better adapted to France than to Spain, as it gave inferior yield to its parent at both Spanish sites (Fig. 1). URS1 also gave slightly though not significant lower yield than Ursula in Spain, although it also had, under Spanish conditions, a severe incidence of necrotic lesions in the leaves (data not presented), which may have contributed to the significantly smaller kernel size (Table 7, Fig. 2). The largely unpredictable relative yields of the mutants compared to their mother genotypes could also have resulted from mutations at additional loci. Sodium azide has a high capacity to induce multiple mutations (Olsen et al. 1993). The presence of side effects produced by unwanted additional mutations in mutants selected for breeding purposes has long been documented (Sigurbjörnsson 1977), and backcrossing to the mother genotype is almost always required. Alternatively, any problems associated with the influence of the genetic background of the mother line on these unwanted phenotypic effects, might be solved by transferring the mutation by backcrossing to other, different genetic backgrounds (Borojevic et al. 1977). In this case, crossing with other American cultivars could be a possible solution. In either case, crossing could be facilitated by the use of molecular markers (Barr et al. 2000; Graner et al. 2000).

Adverse agronomic effects associated with the *mlo* mutation have been observed frequently. Wiberg (1973) noted yield reductions in mutants derived from the cultivar *Foma*. Later work attempted to eliminate effects associated with possible differences in genetic background, resulting from additional mutations, by developing populations of inbred lines. These were developed by doubled haploidy (DH) from crosses of the *mlo* mutant to another genotype. Kjaer et al. (1990) noted that DH lines carrying the *mlo* mildew resistance showed, on average, a lower yield that those that did not. This effect was noted for three different resistance alleles. These authors also noted necrotic leaf spotting and reduced thousand-corn weight in the *mlo* lines and concluded that these were pleotropic effects of the *mlo* alleles. Bjornstad and Aastveit (1990) used a similar approach to look at the

Fig. 2 Genetic and environmental variation underlying the malting quality parameters across the three environments explored (environment codes as in Table 1). Mean values of barley sieving fraction, total malt protein, coarse grind malt extract yield, Kolbach index, α-amylase activity and diastatic power. *Bars*: Standard errors for $P < 0.05$

effect of the *mlo*-5 allele in different genetic backgrounds and noted significant modification of the negative pleitropic effects. In particular, the degree of necrosis was found to be much lower in a cross involving six-row lines compared to one with two-row lines. Although they noted that, on average, *mlo* lines yielded slightly less than those without *mlo* resistance, it was possible to identify high-yielding lines carrying the *mlo* gene. Kjaer et al. (1990) reported similar findings and suggested that an initial screen for low levels of necrotic spotting would help in selecting high-yielding *mlo* types.

The malting quality value of the GER1 mutant proved to be similar to that of Gertrud, but both URS1 and URS2 showed lower malt extract than Ursula (Table 7, Fig. 2). This lower extract might have resulted from the smaller grain size of the mutants that, in turn, could have derived from the necrotic lesions in the leaves. The smaller grain size resulted in a higher grain protein level, which was ultimately responsible for the lower extracts. For American-style brewing, however, these lower extract levels may be compensated for by the higher amylolytic capacity of the mutants, both in α-amylase and diastatic power (Table 7, Fig. 2).

The results discussed above show that the main goal of the present research has been achieved: mutants that show a better level of adaptation to the European growing conditions than their mother genotypes have been produced. Advantages result primarily from their much higher resistance to powdery mildew, while the desired malting quality profile of the parental genotypes has been somewhat altered in the mutants. Further work should include backcrossing these mutant lines to their original genotypes and crossing the lines obtained to North American/European barley stocks with high malting quality. With this approach it would be possible to eliminate the small grain size of the mutants and its putative cause, heavy leaf necrosis.

Acknowledgements The present research has been funded by Busch Agricultural Resources, Ft Collins, Colo., USA (one of the Anheuser-Busch companies). The Scottish Crop Research Institute receives grant-in-aid from the Scottish Executive Environment and Rural Affairs Department (SEERAD).

References

American Society of Brewing Chemists (1992) Methods of analysis, 8th edn, St. Paul, Minn.

Aslam M, Schwazbach E (1980) An inoculation technique for quantitative studies of brown rust resistance in barley. Phytopathol Z 99:87–91

Barr AR, Jefferies SP, Warner P, Moody DB, Chalmers KJ, Langridge P (2000) Marker assisted selection in theory and practice. In: Logue S (ed) Barley Genetics 8, Proc 8th Int Barley Genet Symp, vol 1. University of Adelaide, Australia, pp 167–178

Bjornstad A, Aastveit K (1990) Pleiotropic effects of the *mlo*-mildew resistance gene in barley in different genetic backgrounds. Euphytica 46:217–226

Borojevic K, Gottschalk W, Micke A (1977) Factors influencing the mutants spectrum and the quality of mutants. In: Manual on mutation breeding. International Atomic Energy Agency, Vienna, pp 146–149

Burger WC, LaBerge DE (1985) Malting and brewing quality. In: Rasmusson DC (ed) Barley ASA-CSSA-SSSA, Madison, pp 367–402

Flor HH (1955) Host-parasite interaction in flax rust—its genetics and other implication. Phytopathology 45:79–92

Freialdenhoven A, Scherag B, Hollricher K, Collings DB, Thordal-Christiansen H, Schulze-Lefert P (1994) *Nar-1* and *Nar2*, loci required for *Mla12* – specified race-specific resistance to powdery mildew in barley. Plant Cell 6:983–994

Freialdenhoven A, Peterhaensel C, Kurth J, Kreuzaler F, Schulze-Lefert P (1996) Identification of genes required for the function of non-race-specific *mlo* resistance to powdery mildew in barley. Plant Cell 8:5–14

Freisleben R, Lein A (1942) Ueber die Auffindung einer mehltau resistenten Mutante nach Roentgenbestrahlung reinen linie von sommergerste. Naturwissenschaften 30:608

Friedt W, Werner K, Ordon F (2000) Genetic progress as reflected in highly successful and productive modern barley cultivars. In: Logue S (ed) Barley Genetics 8, Proc 8th Int Barley Genet Symp, vol 1. University of Adelaide, Australia, pp 271–279

Graner A, Michalek W, Streng S (2000) Molecular mapping of genes conferring resistance to viral and fungal pathogens. In: Logue S (ed) Barley Genetics 8, Proc 8th Int Barley Genet Symp, vol 1. University of Adelaide, Australia, pp 45–52

Habekuss A, Hentrich W (1988) Charakterisierung funktionell verschiedener *mlo* mutanten durch Primerinfektion, Pustelwachstum, Inkubationszeit und Beffalsverlauf. Tag-Ber Akad Landwirtschaft DDR Berlin, 272:229–237

Haggag MEA, Dyck PL (1973) The inheritance of leaf rust resistance in four common wheat varieties possessing genes at or near the Lr3 locus. Can J Genet Cytol 15:127–134

Heun M (1984) Localization of induced genes in barley for resistance against powdery mildew. Z Pflanzenzuecht 93:158–168

Heun M, Roebbelen G (1984) Heteroallelism in the *mlo* locus of barley. Z Pflanzenzuecht 92:281–288

Hoffmann W, Nover I (1959) Ausgangmaterial fuer die Zuechtung mehltauresistenter Gersten. Z Pflanzenzuecht 42:68–78

Islam MR, Jahoor A, Fischbeck G (1992) Analysis of powdery mildew reaction of barley F_1 plants involving different Mla alleles. Physiol Mol Plant Pathol 40:353–358

Jahoor A, Fischbeck G (1987) Sources of resistance to powdery mildew in barley lines derived from *Hordeum spontaneum* collected from Israel. Plant Breed 99:274–281

Jensen HP, Christensen E, Helms Jørgensen J (1992) Powdery mildew resistance genes in 127 Northwest European spring barley lines. Plant Breed 108:210–228

Jørgensen JH (1975) Identification of powdery mildew resistant barley mutants and their allelic relationship. Barley Genetics 3, Proc Int Barley Genet Symp Garching, pp 446–455

Jørgensen JH (1992) Discovery, characterisation and exploitation of *Mlo* powdery mildew resistance in barley. Euphytica 63:141–152

Jørgensen JH (1994) Genetics of powdery mildew resistance in barley. Critical Rev Plant Sci 13:97–119

Jørgensen JH (1996) Effect of three suppressors on the expression of powdery mildew resistance genes in barley. Genome 39:492–498

Khan TN (1969) Inheritance of resistance to net blotch in barley. I. Factors effecting the penetrance and expresivity of gene(s) conditioning host resistance. Can J Genet Cytol 11:587–591

Kjaer B, Jensen HP, Jensen J, Jørgensen JH (1990) Associations between three *mlo* powdery mildew resistance genes and agronomic traits in barley. Euphytica 46:185–193

Molina Cano JL, Roca de Togores F, Royo C, Pérez A (1989) Fast germinating low beta-blucan mutants induced in barley with improved malting quality and yield. Theor Appl Genet 78:748–754

Molina-Cano JL, Montoya JL, Echarte J, Royo C, Serra J, Marín-Sánchez JP (1992) Effectiveness of twenty-four barley resis-

tance genes against powdery mildew (*Erysiphe graminis* f. sp. *hordei*. Marchal) in Spain. Plant Breed 109:40–45
Olsen O, Wang X, Wettstein D von (1993) Sodium azide mutagenesis: preferential generation of A.T-G.C transitions in the barley *Ant18* gene. Proc Natl Acad Sci USA 90:8043–8047
Rasmusson DC (2000) Breeding barley for forty years. In: Logue S (ed) Barley Genetics 8, Proc 8th Int Barley Genet Symp, vol 1. University of Adelaide, Australia, pp 287–292
Schildbach R, Burbidge M (1992) Barley varieties and their malting and brewing quality. In: Munck L (ed) Barley Genetics 7, Proc 7th Int Barley Genet Symp, Munksgaard International Publ, Copenhagen, pp 953–968
Sigurbjörnsson B (1977) Mutations in plant breeding programmes. In: Manual on mutation breeding. International Atomic Energy Agency, Vienna, pp 1–7
Statistical Graphics Corporation (1999) STATGRAPHICS PLUS 4.1. Manugistics, Rockville, Md.
Steffenson BJ (2000) Durable resistance to spot blotch and stem rust in barley: In: Logue S (ed) Barley Genetics 8, Proc 8th Int Barley Genet Symp, vol 1. University of Adelaide, Australia, pp 39–45
Swanston JS, Thomas WTB, Powell W, Meyer R, Bringhurst TA, Pearson SY, Brosnan JM, Broadhead A (2000) Assessment of spirit yield in barley. J Ins Brewing 106:53–58
Wiberg A (1973) Mutants of barley with induced resistance to powdery mildew. Hereditas 75:83–100