Open access • Journal Article • DOI:10.1051/AGRO:2000173

The Barley mlo-gene: an important powdery mildew resistance source

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Published on: 01 Nov 2000 - Agronomie (EDP Sciences)

Topics: Powdery mildew, Hordeum vulgare and Plant disease resistance

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Agronomie 20 (2000) 745–756

Original article

The Barley *mlo*-gene: an important powdery mildew resistance source

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(Received 16 May 2000; revised 12 July 2000; accepted 14 July 2000)

Abstract – This review briefly summarises recently generated knowledge about *mlo* powdery mildew resistance in barley. Barley *mlo* resistance has remained highly effective since commercial spring barley varieties with the resistance were first released in 1979. Currently, this resistance is the most used resistance in spring barley grown throughout Europe. Barley *mlo* resistance confers nearly total resistance against fungal penetration attempts. However, the efficiency of the resistance depends on several factors including epidermal cell type, host genetic background, environmental conditions and fungal genotype. Recently, the barley *Mlo*-gene has been cloned, but the exact function of the gene is not known. The *Mlo*-gene most likely regulates several mechanisms involved in penetration resistance against powdery mildew, and *mlo* mutations cause disfunction of the wild type *Mlo*-protein leading to increased resistance. The resistance mechanisms involved probably include earlier deposition and increased size of the host papilla response, callose deposition, production of phenolic compounds and cell wall strengthening by cross binding.

***mlo* resistance / powdery mildew / barley / resistance / virulence**

Résumé – Le gène *Mlo* de l'orge : une importante source de résistance au mildiou. Cette revue résume les connaissances récemment acquises sur la résistance *mlo* à l'oïdium chez l'orge. Cette résistance est actuellement la plus utilisée dans les orges de printemps en Europe, et son efficacité s'est maintenue depuis son introduction en 1979 dans les variétés commerciales. La résistance *mlo* bloque la pénétration du champignon. Cependant, son expression dépend de plusieurs facteurs dont le type de cellules épidermiques, le fond génétique de l'hôte, les conditions climatiques et le génotype du champignon. Chez l'orge, le gène *Mlo* a récemment été cloné mais sa fonction précise n'est pas connue. Le gène *Mlo* régule vraisemblablement plusieurs mécanismes impliqués dans la résistance à la pénétration de l'oïdium, et les mutations *mlo* provoqueraient des dysfonctionnements de la protéine *Mlo.* Les mécanismes de résistance impliqués incluent probablement une déposition précoce et une taille supérieure de la papille émise par l'hôte, du dépôt de callose, de la production de composés phénoliques et du renforcement des parois cellulaires.

résistance *mlo* / oïdium / orge / résistance / virulence

Communicated by Rients E. Niks (Wageningen, The Netherlands)

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1. Introduction

Powdery mildew is one of the most consistently damaging diseases of barley in Europe. It is caused by the obligate biotrophic fungus *Blumeria graminis* DC Speer f.sp. *hordei* Em. Marchal (Syn. *Erysiphe graminis* DC f.sp. *hordei* Em. Marchal). The fungus can infect all green plant parts causing premature tissue senescence leading to severe reductions in yield. Much effort is devoted to the exploitation of genetic plant resistance for disease control. However, most single genes for resistance in barley have proved ephemeral as virulent pathogen races capable of breaking down the resistance are rapidly selected in the agricultural situation. The recessive *mlo*-alleles of barley have proved to be an exception. Since commercial spring barley varieties with resistance based on *mlo*-alleles were first released in 1979, the resistance has remained highly effective. Currently, this powdery mildew resistance is the most used resistance in spring barley grown throughout Europe. Therefore, if the resistance should break down the consequences would be extremely serious.

The *mlo* powdery mildew resistance effectively prevents the mildew fungus from penetrating host epidermal cells, and thus prevents fungal haustorium formation and infection. This resistance is highly correlated with local papilla deposition in host epidermal cells beneath attempted fungal penetration sites. In susceptible barley lines with the dominant *Mlo*-allele, papillae are also produced, but these are not able to prevent fungal penetration with the same efficiency, so that the fungus is often able to infect, form haustoria and sporulate.

Work relating to the exploitation and characterisation of *mlo* powdery mildew resistance in barley has been reviewed several times, most recently by Jørgensen [30] and Schwarzbach [47]. The present article will summarise briefly knowledge concerning *mlo* powdery mildew resistance generated since then.

2. The barley *mlo*-gene

A major step towards understanding *mlo* powdery mildew resistance was taken when the barley *Mlo*-gene was cloned and sequenced [12]. However, the wild-type *Mlo*-gene encodes a novel, plant-specific, integral membrane protein, the functions of which are still unknown [12, 44]. The gene encodes a 533 amino acid protein that resides in the plasma membrane of leaf cells and is anchored by seven transmembrane helices so that the amino-terminus is extra-cellular and the carboxy-terminal tail is intra-cellular [12, 17, 44]. It has been suggested that the *Mlo*-protein is involved in regulation of one or more early cell defence responses and if this regulation is missing, i.e. in *mlo* mutants, the cell defence responses are activated earlier and/or more strongly than in susceptible barley [30, 51, 57]. Molecular analysis of eleven different induced *mlo* mutants confirmed the hypothesis that *mlo* resistance is caused by lack of function of the wild type *Mlo*-gene [12]. Further, the *Mlo*-protein seems to work in a cell autonomous way as transformation of epidermal cells in *mlo* resistant barley with the wild type *Mlo*-gene restored susceptibility of the transformed epidermal cells while surrounding cells retained their resistance [48].

Genome search in *Arabdopsis thaliana* has revealed the presence of approximately 35 *Mlo*-gene family members sharing a common gene structure [17, 44]. The *Mlo* family members show some similarities in topology and subcellular localisation, but not in sequence, to G-protein-coupled receptors in *Caenorhabditis elegans* and *Homo sapiens* [44]. These receptors are involved in mediating extracellular signals into amplified intracellular responses. The *Mlo*-protein may function in a similar way, but this remains to be confirmed [44].

The tendency of *mlo* containing barley to produce necrotic flecks, even under axenic conditions [57], seems to be a pleiotropic effect of the *mlo*-alleles. This suggests a possible involvement of the wild-type *Mlo*-gene in cell death control that functions independently of the *mlo* mediated penetration resistance response [12, 42, 44]. How this cell death relates to cell death involved in race specific hypersensitive resistance is not clear, but the data indicate the existence of at least two separate pathways leading to cell death in barley [42].

3. Efficiency of *mlo* powdery mildew resistance

The *mlo* resistance does not confer total resistance and the efficacy of the resistance may be modified by many factors including epidermal cell type, host genetic background, environmental conditions and fungal genotypes.

3.1. Cell types

In *mlo* resistant barley, long epidermal cells, located over vascular tissues, are normally not penetrated at all, while short cells are penetrated very rarely. Stomatal subsidiary cells, the very small epidermal cells directly adjacent to guard cells, are penetrated with a slightly greater frequency than short cells e.g. [5, 8, 31]. The phenomenon of enhanced subsidiary cell susceptibility to powdery mildew infection in cereals is well established e.g. [24] although the reasons for this are not fully explained. Skou [50] suggested that subsidiary cells adjacent to stomata were unable to form papillae because of their unique physiology and the flexibility of their walls that relates to stomatal movement. Infections in this type of cell are normally responsible for the characteristic occurrence of occasional powdery mildew colonies seen on *mlo* resistant barley leaves.

3.2. The host

The general host genetic background may strongly affect the efficiency of *mlo* resistance, which may vary in terms of the number of colonies formed from near zero to close to compatibility [6, 29, 31]. Through mutation studies, two genes, *Ror1* and *Ror2,* which may be responsible for this variation, have been identified as required for full expression of *mlo* resistance [19]. If an *mlo*-allele is present together with a mutated *ror1* or *ror2* gene, the *mlo* resistance is compromised and the host becomes susceptible to powdery mildew attack. Different *mlo*-alleles have also been reported to confer differences in the quantitative expression of resistance [22, 45]. However, this could not be confirmed in a microscope examination of 11 different induced *mlo*-alleles back crossed to Cv. Ingrid [6]. Here, no significant differences were found in the overall percentage of successful penetration (Fig. 1), suggesting that the host genetic background in which the *mlo*-gene is placed, and not the actual *mlo*-allele, determines the efficiency of *mlo* resistance in barley.

3.3. Environmental factors

In the field, *mlo* resistant barley cultivars occasionally suffer from outbreaks of powdery mildew. These outbreaks are sporadic and transient, and do not appear to be due to genetic changes in the powdery mildew population (see later), but appear

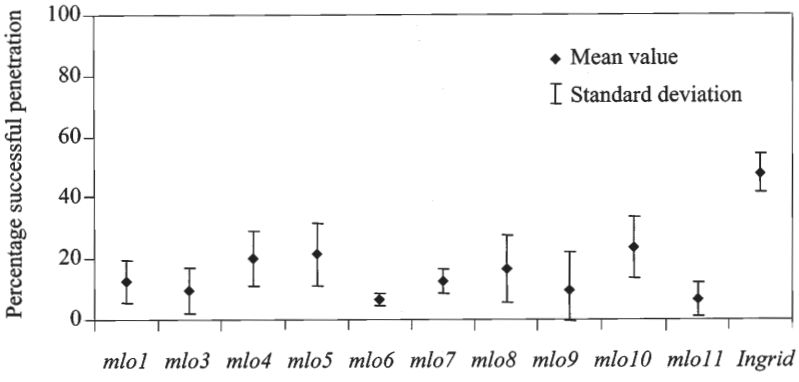

Figure 1. Effects of different resistance alleles on the efficiency of *mlo*-resistance in barley. Percentage successful powdery mildew penetration (haustorium formation) in Ingrid near isogenic barley lines with different *mlo*-alleles, 48 hours after inoculation. From J. Atzema [6].

Table I. Effect of water stress release on *mlo* resistance in the barley cv. Atem. Modified from Newton and Young [40].

	Numbers of powdery mildew colonies per leaf	
Water stress treatment	First formed leaf	Second formed leaf
No stress	0.00	0.00
Mild stress	0.64	0.75
High stress	12.75	0.25
	LSD = 2.44	

related to unusual environmental conditions [30]. Experimentally it was shown that relief from water stress in barley cultivars might result in temporary partial breakdown of *mlo* resistance both under controlled greenhouse conditions and in field experiments [7, 8, 40] (Tab. I). Relief from water stress increased susceptibility of both *Mlo* susceptible and *mlo* resistant barley for a short period of time, with maximum mildew infection frequency being attained when the water stress was relieved approximately seven hours after powdery mildew inoculation [8]. The extent of resistance breakdown also depended on the genetic background of the barley in which the *mlo*-gene was contained, and not the actual *mlo* mutant used [8]. Some barley genotypes do not suffer from breakdown after relief of water stress and QTL mapping has demonstrated that at least one locus independent of the *mlo* gene is responsible for this characteristic (Newton, unpublished data). Similar, partial breakdown of *mlo* resistance has been obtained when barley recovers from cold stress [Lyngkjær and Carver unpublished] (Tab. II).

Availability of nutrients may influence the susceptibility of barley to powdery mildew. High nitrogen levels are known to cause a general increase in susceptibility [27] and nitrogen status was also found to increase the level of infection following relief from water stress in *mlo* resistant barley [7, 40]. However, deprivation of silicon, that normally leads to increased powdery mildew susceptibility [58], did not affect the efficiency of *mlo* resistance, although the size of papillae produced

Table II. Effect of cold stress relief on *mlo* resistance in first formed (seedling leaves) of the Risø 5678R barley line. Plants were grown at 16 or 20 ºC. Stress was applied by lowering the temperature to 4 or 8 ºC for 48 hours. Plants were inoculated at the end of the cold period, incubated at the original, higher temperature, and numbers of colonies were counted 7 days later [Lyngkjær and Carver, unpublished].

	Numbers of powdery mildew colonies per leaf		
Growth temperature	No stress	48 hours at 4 °C	48 hours at 8 °C
16 °C	1.2	3.3	4.4
20 °C	0.8	1.2	4.9

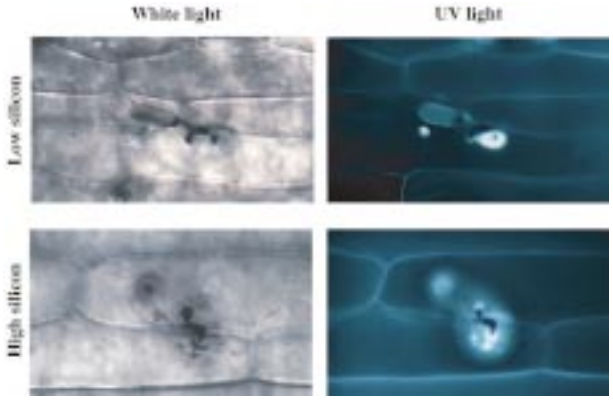

Figure 2. Effects of low and high silicon (Si) levels on papilla formation in the *mlo* resistant barley line Risø 5678R attacked by powdery mildew. Staining with leuco aniline blue and UV light microscopy was used to detect the presence of callose. High Si content is associated with abundant callose while deprivation of Si greatly reduced the abundance of callose ([60], Lyngkjær and Carver, unpublished).

in response to attack was changed dramatically ([60], Lyngkjær and Carver unpublished] (Fig. 2).

3.4. The fungus

Over the past 25 years, barley cultivars carrying *mlo* resistance have become widely used in Europe. By 1988 they occupied about 25 percent of the total area devoted to spring barley [4], and by

1993–94 about 70 percent of the spring barley area in the UK and Germany contained *mlo* [40, 47]. Despite this very intensive use, *mlo* resistance has remained highly effective against powdery mildew populations in the field. In an evaluation of data from the European Barley Disease Nursery from 1976–1988, no indication of increase in disease severity was found on *mlo* resistant cultivars in Europe [4] and this situation remains unchanged.

In Denmark, screening for *mlo*-virulence in isolates collected on *mlo* resistant seedlings from 1992 to 1995 showed no increased virulence [Hovmøller and Lyngkjær unpublished data]. Similar results were obtained from isolates collected in Germany during 1993 and 1994 [6]. However, in the UK Cereal Pathogen Virulence Survey in 1998, the spring barley Riviera, an *mlo* variety, showed noticeable levels of mildew, and pustule transfer experiments in Northern Ireland suggested a shift, albeit small, in virulence for *mlo* [52]. (See also the paper by Hovmøller et al., in this volume).

It would not be surprising if there is an eventual shift towards increased *mlo*-virulence in field populations of powdery mildew. Two powdery mildew isolates with the potential to overcome, at least partially, *mlo* resistance have been described [36, 46]. Both powdery mildew isolates show improved penetration efficiency and are able to infect all epidermal cell types on *mlo* resistant barley. The first, isolate HL3/5 (originally called HL-3 [46]), was selected for the increased number of colonies formed during recurrent propagation on an *mlo* resistant barley line, over 37 successive conidial generations in the laboratory [46]. The other, powdery mildew isolate, Race I, was originally collected from field crops in Japan in the 1950s. This was before any commercial growth of *mlo* resistant cultivars, and only recently its *mlo*-virulence was discovered [36]. In tests on near isogenic barley lines with and without *mlo* resistance, isolate HL3/5 normally develops about 10 to 60 per cent of the colonies it produces on the susceptible barley line. This is between 50 to 2000 times more colonies than its progenitor powdery mildew isolate GE3 e.g. [5, 35, 46] (Fig. 3). Race I was found to be at least as virulent, and possibly more so, than HL3/5 [36].

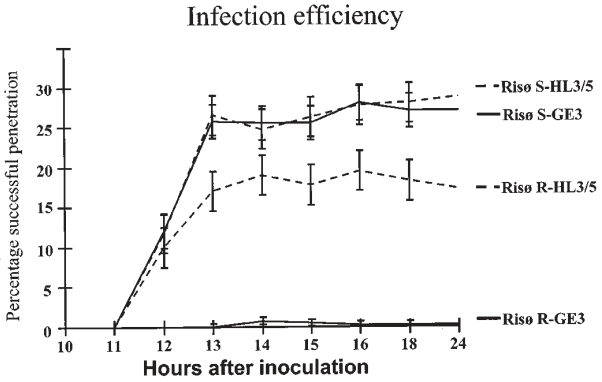

Figure 3. Time-course showing successful penetration (haustorium formation) by the *mlo* virulent HL3/5 and the *mlo* avirulent GE3 powdery mildew isolates on leaves of the two near isogenic barley lines Risø 5678R (with *mlo5* resistance) and Risø 5678S (susceptible), at 10 to 24 hours after inoculation. Error bars represent 95% confidence intervals. From Lyngkjær and Østergård [35].

The genetic basis of *mlo*-virulence in the haploid barley powdery mildew fungus is not well characterised. Schwarzbach [46] noted that the slow increase in fitness during his selection experiment suggested that *mlo*-virulence was controlled by more than one locus. Furthermore, he suggested that at least three genes are involved, because the performance (i.e. the proportion of conidia that formed secondary elongating hyphae) among 6 genotypes sampled at the end of the selection experiment could be grouped into four classes. Based on several crosses between the *mlo* virulent powdery mildew isolate HL3/5 and avirulent field isolates, and later a series of backcrosses, two hypotheses for the genetic basis of *mlo*-virulence were suggested [6]: (1) that *mlo*-virulence depends on the expression of one major virulence gene and two minor additive genes with a fourth gene that can partly inhibit the major gene. (2) that *mlo*-virulence depends on the expression of one major virulence gene and two minor additive genes, and one more additive minor gene with smaller effect.

The indication that at least three genes were involved and the relatively low infection efficiency of these isolates capable of growing on resistant lines, led Jørgensen [28] to suggest that these powdery mildew isolates should be called ‘aggressive’ and not ‘virulent’. However, experimentation using

the selected *mlo*-virulent isolate, HL3/5, and its progenitor isolate, GE3, showed that *mlo*-virulence is not a general increase in pathogen aggressiveness since *mlo* virulent and avirulent isolates have the same fitness on susceptible barley lines [35] (Fig. 3). Because of this and the indication of specificity of the interaction, powdery mildew isolates with increased infection efficiency on *mlo* resistant barley should be termed *mlo* virulent. However, this virulence is quantitative and may not be directly comparable to virulence shown by isolates capable of overcoming other, race specific, major genes for powdery mildew resistance.

3.5. Interaction with other powdery mildew resistance genes and influence on other diseases

The *mlo* resistance works independently from other powdery mildew resistance genes in barley and can be combined with these to produce powdery mildew 'multi-resistant' barley plants. However, the *mlo* gene seems to confer no resistance to diseases other than barley powdery mildew [30], and it was suggested that *mlo* resistance may affect only pathogens that infect living epidermal cells. Thus, it is not effective against biotrophic fungal pathogens such as leaf rust (*Puccinia hordei*) which enter through open stomata and infect mesophyll cells, or against necrotrophic fungal pathogens, such as net blotch (*Drechslera teres*), which exude toxins and kill the host tissue during infection [30]. However, recently it has been demonstrated that the presence of various *mlo* alleles confer enhanced susceptibility to rice blast (*Magnaporthe grisea*) whereas, by contrast, the wild type *Mlo*-gene confers resistance [26]. This shows that the *mlo* gene does not inevitably confer resistance against fungal pathogens that penetrate epidermal cells, and indicates that the *Mlo*-gene may play a complex role during infection of barley by different pathogens [26]. Increased susceptibility to rice blast of *mlo* barley may cause problems in some areas of the world such as northern Italy, southern France or Asia [26] where *M. grisea* is endemic. It has also been suggested that *mlo* barley is more susceptible to *Rhynchosporium secalis*. However, analysis of crosses between barley cultivars demonstrated that this was not attributable to the *mlo* gene itself but, rather, to the genetic background, and is most likely a consequence of low priority being given to *R. secalis* resistance in breeding programmes ([11], W.T.B. Thomas, Scottish Crap Research Institute, unpublished).

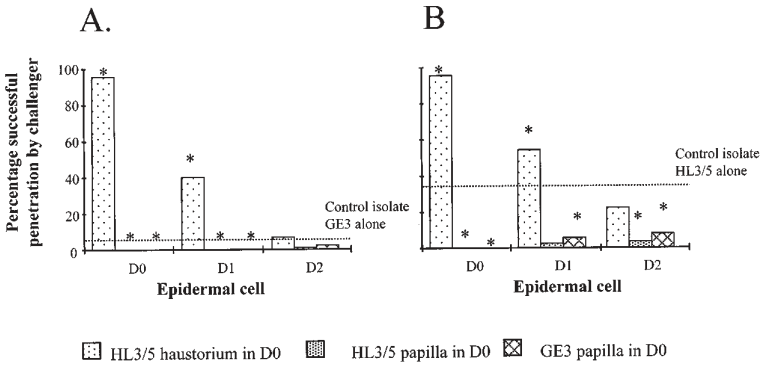

Figure 4. Percentage of challenger attacks by appressoria of the *mlo* avirulent powdery mildew isolate GE3 (A) or the *mlo* virulent isolate HL3/5 (B) associated with successful penetration (haustorium formation) on the *mlo5* resistant Risø 5678R barley line when earlier inducer attack by isolate HL3/5 had successfully penetrated and a haustorium remained in the attacked D0 cell, or had failed to penetrate and a papilla remained in the attacked D0 cell or, similarly, inducer attack by isolate GE3 had failed and a papilla remained in the attacked D0 cell. Data are given for penetration success into D0 cells (those directly attacked by the inducer), D1 cells (immediately adjacent to D0), and D2 cells (separated from the D0 by a D1 cell). * above a column indicates that the 95% confidence interval around the fitted mean differed significantly from the equivalent 95% confidence interval around the control. Modified from Lyngkjær and Carver [34].

3.6. Induced susceptibility and resistance

The powdery mildew fungus may by itself change the efficiency of *mlo* powdery mildew resistance by inducing localised changes in susceptibility of cells to attack by modifying their 'accessibility' (effectively increasing susceptibility) or 'inaccessibility' (effectively increasing resistance) e.g. [34, 41]. The cellular mechanisms behind induced (in)accessibility have been studied in *mlo* resistant barley with both *mlo* virulent and avirulent powdery mildew [34]. This showed that if attack on an *mlo* barley by a virulent isolate is successful and a haustorium is formed, *mlo* resistance is nearly totally suppressed rendering infected cells, and to some extent their immediate neighbours, highly accessible (susceptible) to later attack by both *mlo* virulent and avirulent powdery mildew isolates. By contrast, if the first mildew attack fails, cells are rendered highly inaccessible (resistant) to later attack by both virulent and avirulent powdery mildew isolates [34] (Fig. 4). This means that as long as most individuals in a powdery mildew population are unable to infect *mlo* resistant barley successfully, the plant will express a very high level of induced resistance in the field, including against *mlo* virulent powdery mildew isolates. On the other hand if *mlo* virulence should be common, induced susceptibility may contribute to increased disease severity on *mlo* resistant barley.

4. Resistance mechanisms

It has been suggested that many disease resistance mechanisms may be affected/regulated by the wild type *Mlo*-gene and thereby involved in the penetration resistance conferred by *mlo*-alleles in barley. These mechanisms are all related to apposition/papilla formation and cell wall modifications, and include effects on the timing and size of the host papilla response, callose deposition, production of phenolic compounds and cell wall strengthening by cross linking.

4.1. Timing and size of host response

Rapid papilla formation in *mlo* resistant barley has been suggested as one mechanism involved in resistance [10, 21, 51]. Skou et al. [51] showed a significant difference in initiation of appositions (haloes and papillae) between a resistant and a near isogenic susceptible barley line, and concluded that the early formation of papilla was responsible for *mlo* resistance. Alterations in papilla deposition have also been shown in *mlo* resistant barley coleoptiles where papillae were initiated 60 minutes before attempted penetration by the fungus, in contrast to susceptible coleoptiles where they were initiated 40 minutes after attempted penetration [21]. The importance of timing of papilla deposition in relation to attempted penetration has also been supported by inhibitor studies, in which a delay in papillae formation in treated coleoptiles of an *mlo* resistant barley line was observed coincidentally with an increase in fungal infection [10, 21].

Increased papilla size and frequency at attempted penetration sites also correlates with *mlo* resistance [21, 49, 51, 53, 58]. The diameters of papillae have been measured as almost two times larger in resistant lines compared to susceptible lines at the time of fungal penetration e.g. [35]. However, some susceptible barley lines, i.e. Kobinkatagi, also produced large papillae implying that it is not only the final papilla size that determines efficacy of resistance [51]. Papillae are also formed more frequently during the early stages of attempted penetration in *mlo* resistant barley than in susceptible barley lines although eventual papilla frequency is equal between the two lines [35, 53, 58]. Despite these findings, some evidence suggests that papilla formation is not necessary for *mlo* resistance. Thus, low speed-centrifugation inhibited papilla formation without causing any increase in successful penetration [54].

4.2. Callose

The deposition rate of callose and the final amount of callose in papillae have also been regarded as important to the effectiveness of papillae in *mlo* resistant barley [2, 10, 21, 35, 49, 54]. Callose is synthesised by the plasma membrane bound 1,3-β-glucan synthase [32, 39]. No differences were observed in specific activity of callose synthase between *mlo* resistant barley and susceptible

barley [43], suggesting that regulation of the enzyme must be important in controlling the formation of the large callose containing papillae associated with *mlo* resistance. Calcium (Ca^{2+}) strongly increases the activity of callose synthase [33], and fungal penetration attempts may induce an influx of Ca^{2+} to the cytosol activating the enzyme. When treated with Ca^{2+} chelators *mlo* resistant barley became susceptible [9], and lack of the wild-type *Mlo*-protein may thus allow for an early and rapid elevation of the Ca^{2+} level in the host cell [1, 9].

Bayles et al. [10] provided further experimental evidence supporting the importance of callose for papilla formation. They showed that treatment with the glucose analogue, 2-deoxy-D-glucose (DDG), increased the susceptibility of *mlo* resistant barley. The glucose analogue DDG is described as a powerful inhibitor of callose formation in vivo [25] but it does not inhibit activity of the callose synthase enzyme in vitro [37, 39]. However, the effects of DDG, and another glucose analogue mannose [37], may well be indirect since both are known to sequester plant cell phosphate [18, 20, 38]. Phosphate sequestration would reduce the plant cells potential to synthesise the ATP needed for vital phosphorylation reactions during energy metabolism [23, 56]. This would presumably result in lower energy being available for penetration resistance and lead to increased susceptibility.

4.3. Phenolics

Papillae formed in both resistant and susceptible barley often contain autofluorogenic compounds thought to be phenolics [15, 16, 59]. Phenolics are synthesised de novo in response to powdery mildew attack [16] and are involved in penetration resistance, possibly through strengthening epidermal cell walls and papillae by cross-linking cell wall materials [13, 16, 59]. Compaction of papillae due to phenolic cross-linking, which makes papillae and cell walls resistant to digestion with cellulase, happens at least two hours earlier in *mlo* resistant barley than in susceptible barley [55]. Compacted papillae and cell wall regions where cellulose fibres are bound to phenolics are likely to impede penetration by the fungus. The timing and rapidity of this compaction may be important for the efficiency of *mlo* resistance.

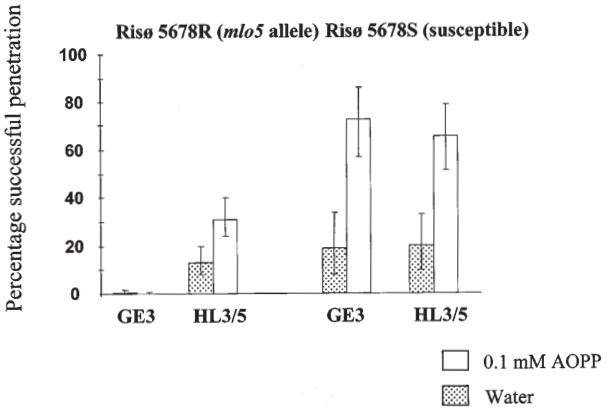

Figure 5. Involvement of phenolic compounds in barley *mlo*-resistance. Mean percentages of appressoria of the powdery mildew isolates GE3 (*mlo* avirulent) and HL3/5 (*mlo* virulent) successfully penetrating (forming haustorium) in leaf epidermal cells of barley isolines Risø 5678R (*mlo5* resistant) and Risø 5678S (susceptible) treated with water (controls) or with 0.1 mM AOPP. Means are based on observation of 100 appressoria on each of three leaves subjected to each treatment. Error bars represent 95% confidence intervals. Modified from Lyngkjær et al. [36].

Phenylalanine ammonia lyase (PAL), the enzyme which catalyses the first committed step in phenolic compound synthesis, can be inhibited by treating leaves with α-aminooxy-β-phenylpropionic acid (AOPP) [3]. Treatment of susceptible barley with AOPP, lead to a marked decrease in papillae autofluorescence and to substantially increased susceptibility [14]. This indicates that in barley, phenolic compounds present in papillae contribute greatly to inherent resistance and to the potential effectiveness of papillae. A soluble phenolic polyamine compound, with fungistatic or anti-fungal properties, has been shown to accumulate differentially in papillae in *mlo* resistant and susceptible barley suggesting that this compound plays an important role in *mlo* resistance [55]. However, when *mlo* barley was treated with AOPP, resistance was maintained despite a reduction in papilla autofluorescence [59]. This suggests that components other than phenolic compounds are largely responsible for the resistance of papillae in *mlo* barley. Nevertheless, tests with *mlo* virulent

powdery mildew have shown that AOPP treatment of *mlo* barley resulted in increased susceptibility to *mlo* virulent powdery mildew (Fig. 5) [37]. This indicates that while phenolics are not the primary cause of resistance in *mlo* barley, they act as a backup defence that contributes to limiting penetration where the primary defence fails.

5. Concluding remarks

The apparently complex function of *mlo* resistance seems to make it difficult for the powdery mildew fungus to develop virulence. This may be because the fungus has to deal with many changed and increased host responses involved in papilla formation and cell wall alterations. This is probably also why several genes are involved in generating *mlo*-virulence. However, the demonstration that the Japanese field isolate is virulent to *mlo* barley [35], supports Schwarzbach's [46] observation that powdery mildew has the genetic potential to overcome, at least partially, *mlo* resistance in the field. If, as seems inevitable, *mlo* cultivars remain widely used, selection pressure will probably lead eventually to the evolution of *mlo*-virulence. Encouragingly, however, although some survey results indicate a small increase in *mlo*-virulence, no evidence yet indicates that widespread breakdown of the resistance is imminent. The fact that failed fungal penetration attempts strongly induce local resistance may help to preserve and prolong the durability of *mlo*. In order to maintain the effectiveness of *mlo* resistance it is desirable that selection pressure should not be increased by further increase in the area of *mlo*-based spring barley. Similarly, the gene should not be introduced to winter barley varieties, which would provide a green bridge selective for virulent isolates. Additionally, combining *mlo* resistance with new race-specific, single genes for powdery mildew resistances should delay selection of *mlo* virulence by demanding the evolution of novel, complex virulence combinations before biotrophy can become established.

Acknowledgements: The authors wish to thank COST Action 817 for supporting travel expenses that were necessary for meeting and to carry out short-term scientific missions.

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