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Haplotype characterization and markers at the barley *Mlo* powdery mildew resistance locus as tools for marker-assisted selection

G. Tacconi*, V. Baldassarre*, N. C. Collins, D. Bulgarelli*, A. M. Stanca* and G. Valè*[1]**
*****Istituto Sperimentale per la Cerealicoltura, Consiglio per la Ricerca e sperimentazione in Agricoltura, Sezione di Fiorenzuola d'Arda, Via S. Protaso 302, I-29107 Fiorenzuola d'Arda (PC) Italy
******Australian Centre for Plant Functional Genomics, University of Adelaide, Waite Campus PMB1, Glen Osmond SA 5064, Australia
[1]Corresponding author (e-mail: gp.vale@iol.it)

SUMMARY – Recessive mlo alleles of the barley Mlo gene confer resistance to almost all known isolates of the powdery mildew fungal pathogen targeting barley. To characterize haplotypes present in the Mlo chromosomal region of cultivated Mlo and mlo barley genotypes, we conducted a polymorphism search in 3 predicted low-copy sequence regions adjacent to the Mlo gene by examining a sample of 4 Mlo and 3 mlo cultivars. Eight single nucleotide polymorphisms (SNPs) and 1 insertion–deletion (indel) were detected, and easy to use PCR-based markers were developed for typing the SNPs. The PCR markers were used to characterize a collection of 46 Mlo and 25 mlo barley cultivars, identifying 3 distinct mlo-11 haplotypes, 1 mlo-9 haplotype, and 4 Mlo haplotypes. We summarized the haplotype and marker information obtained here and in a previous study to help breeders identify strategies for mlo marker-assisted selection. The ability of the markers to identify mlo-resistant genotypes in segregating populations was demonstrated using 2 resistance-characterized F2 populations derived by 3-way crosses.

Keywords: Barley, powdery mildew resistance, *mlo*, SNPs, marker-assisted selection.

Introduction

The *mlo* locus of barley provides an important source of resistance against the barley powdery mildew fungal pathogen *Blumeria graminis* f sp. *hordei*. The recessive resistance alleles confer essentially complete, pre-haustorial, resistance against nearly all known isolates of the pathogen (Büschges *et al.,* 1997). Since the 1970s, *mlo* resistance alleles have been utilized extensively, being incorporated in to over 142 cultivars, representing over 40% of the area of spring barley cultivation in many European countries. Molecular markers closely linked to the *Mlo* locus would be useful for breeding, because they would obviate the need for resistance assays, would enable selection of heterozygotes carrying the recessive resistance alleles and would make possible multiple-genes pyramiding. The non-random association of alleles at linked positions in individuals from a population limits the number of versions (haplotypes) of a chromosomal region which are present in a population. Haplotype information in crop species could be used for identifying effective strategies for marker-assisted trait selection.

Results

Three regions from the published 60 kb of barley genomic sequence containing the *Mlo* gene (GeneBank Y14573; Panstruga *et al.,* 1998) were targeted to search for sequence polymorphisms. To identify sequence polymorphisms we PCR-amplified and direct-sequenced the genomic fragments from a sample of four *Mlo* containing cultivars, two cultivars containing *mlo-11* resistance alleles, and a cultivar with an unclassified *mlo* allele. Alignment of the sequences revealed eight SNPs and one InDel of 393 bp (Table 1).

The SNPs that alter restriction enzyme sites generating CAPS markers and the InDel are named 'om' markers (*Mlo* markers, summarized in Table 1). Figure 1 shows gel images of the six CAPS markers (om1, and om3 to om7) and the InDel marker (om2) in eight *Mlo* and five *mlo* barley lines.

We scored the seven molecular markers *plus* the mlo07646 MITE marker (Piffanelli *et al.,* 2004) in 46 *Mlo* and 25 *mlo* barley cultivars. The marker scores grouped the genotypes in to four haplotypes and haplotypes I, II and III could be seen to be equivalent to haplotype groups I, II and III, respectively from the previous study (Piffanelli *et al.,* 2004), moreover the cvs. 'Balkan' and 'Rebelle' (having common parents), was shown to be part of a distinct group (group IV, Fig. 2). Ethiopian landrace barleys which are the source of the *mlo-11* resistance allele in modern cultivars (Jørgensen 1992) possess a group I *Mlo* locus haplotype. The InDel polymorphism resulting from presence/absence of a MITE transposable element (marker mlo07646; position 7,646 of Y14573) discriminates between the group I haplotype of the Ethiopian *mlo-11* lines and the group I haplotypes of modern *Mlo* and *mlo-9* cultivars (Piffanelli *et al.,* 2004), defining two group I sub-types: Ia and Ib, respectively (Fig. 2). The only *mlo-11* cultivars not possessing the Ia haplotype were 'Ditta' and 'Ferment', possessing a group II haplotype. This was evidently a result of a recombination event that had occurred during the breeding.

Table 1.

Genotype	Position in Y14573								
	8098	8167	8428-8820†	9087	9128	15707	15786	17964	17984
	*Mbo*I	none	InDel	*Mbi*I	*Lwe*I	*Bsm*I	*Xmi*I	*Hpa*II	*Hpa*II
Naturel (*Mlo*)	A	A	In	T	G	C	A	G††	G
Amillis (*Mlo*)	A	A	In	T	G	C	A	G††	G
Diadem (*Mlo*)	G††	G	Del	C††	A††	G††	G††	A	C††
Fp2037 (*Mlo*)	A	A	In	T	G	G††	G††	A	C††
Atem (*mlo-11*)	A	A	In	T	G	G††	G††	A	C††
Krona (*mlo-11*)	A	A	In	T	G	G††	G††	A	C††
Saloon (*mlo*)	A	A	In	T	G	G††	G††	A	C††
Marker	om1	-	om2	om3	om4	om5	om6	om7	om7

†Barley cultivar 'Ingrid', the source of the Y14573 sequence, contains the insertion.
††indicates sequence variant cut by the restriction enzyme.

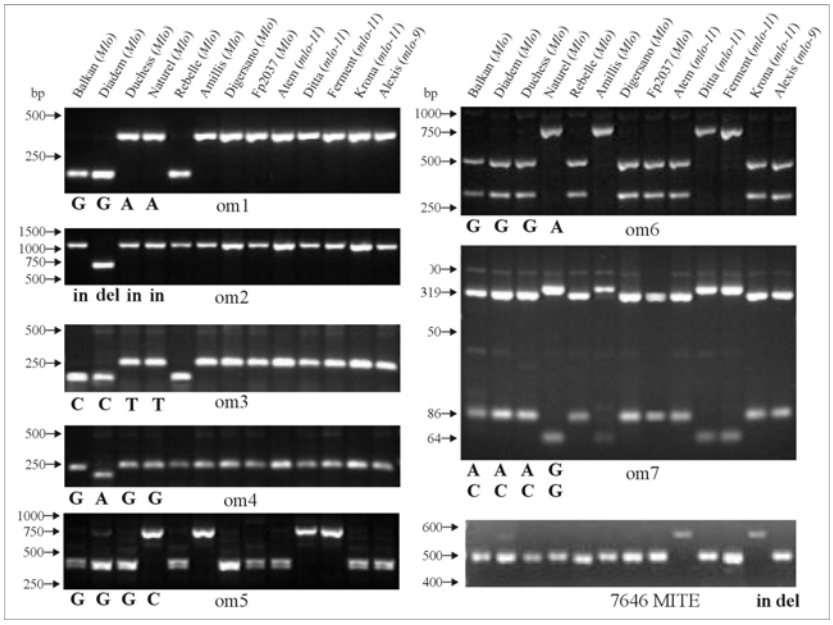

Fig. 1.

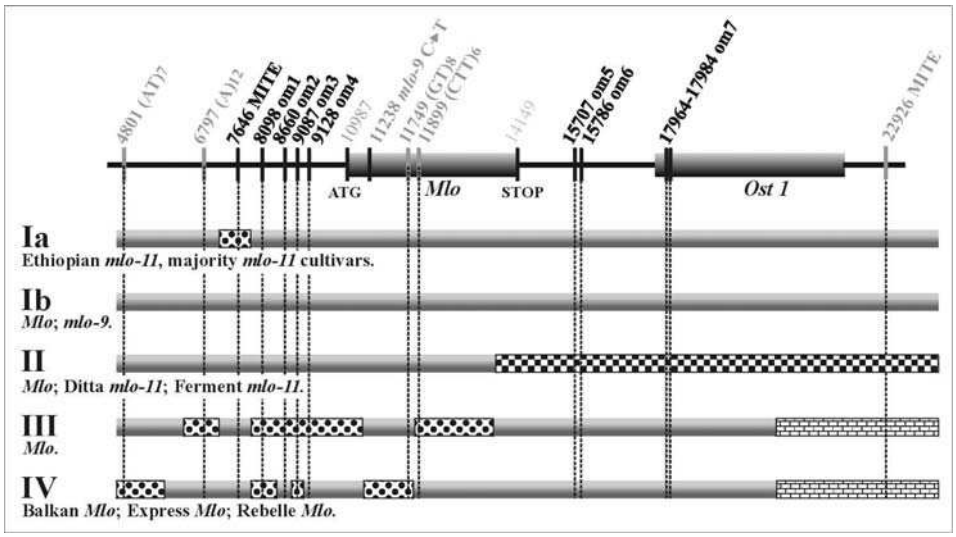

Fig. 2.

Figure 2 illustrates the four *Mlo*-region haplotype groups (and two type I subgroups), incorporating data from this and the previous (Piffanelli *et al.,* 2004) study. To illustrate the utility of the om markers in selecting for *mlo* resistance in segregating populations, we analysed two F_2 populations, each derived from a three-way cross. The CAPS marker om7 was used to analyse 17 F_2 plants derived from a ('Amillis' x 'Naturel') x 'Atem', in which 'Atem' acted as a donor of *mlo-11* (Fig. 3A). The genotype at the *Mlo* locus, as determined by resistance assays with F_3 progeny of the F_2 plants, corresponded completely with the om7 marker genotype, confirming the suitability of this marker for selecting desired *Mlo* genotypes. The markers om2 *plus* om7 multiplexed together were used on a sample of 16 F_2 plants obtained from the cross ('Diadem' x 'Naturel') x 'Krona', in which 'Krona' acted as a donor of *mlo-11* (Fig. 3B). The markers were used in combination, because no single om marker can distinguish both *Mlo* parents. We determined the *Mlo* genotypes of these same F_2 plants by artificial inoculation of the F_3 progeny, and observed complete agreement between the molecular marker genotypes and the *Mlo* resistance locus gentoypes.

Discussion

In the current study, we characterized haplotypes for the *Mlo* chromosomal region in a wider range of barley germplasm suggesting that most, if not all, of the haplotypes present within modern cultivated *Mlo* varieties have been identified. The haplotype information summarized in Fig. 2 should be of help in the selection of markers suitable for following *mlo* segregating in breeding populations arising from particular crosses. The distances of 2.1 kb and 6.7 kb separating the *mlo-9* resistance causing mutation from the closest marker om4 and most distant om marker om7, respectively (Fig. 2), would be expected to correspond to a recombination frequency of one every 2,400 to 746 F_2 plants, respectively. The *mlo-11* resistance would therefore be expected to recombine with the om markers at a frequency similar to that calculated for the *mlo-9* resistance. The om markers described here are all clearly co-dominant (Fig. 1) and can be easily scored using commonly available molecular biology reagents and separation using agarose gel electrophoresis. These PCR markers are currently being used in our own breeding program for introgressing *mlo* alleles into elite spring barley lines.

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