On-farm evaluation of QTLs: the case of partial resistance to *Puccinia hordei* Otth in southeast Ethiopia

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INTRODUCTION

The majority of important agronomic characters—yield and its components (grain number and grain weight), plant height, days to flowering, and partial resistance to diseases—are controlled by several genes. The number of genes and their interactions control the expression of quantitative traits and are poorly understood in conventional quantitative genetics analyses. Before the development of molecular markers, attempts to localize quantitative trait loci (QTLs) sought to determine their association with morphological markers, but these attempts were successful only in some loci with large effects on a given quantitative trait (Griffiths *et al.,* 1996). Localization of QTLs to small regions within chromosomes requires closely spaced marker loci along the chromosomes. Moreover, there must be parental lines that differ from each other in the alleles carried at a sufficient number of these marker loci. In most cases, these requirements could not be met because of the limited number of morphological markers. The frequently occurring isozyme markers in breeding lines that compensated for some of the drawbacks of the morphological markers enabled the localization of several loci responsible for quantitative traits (Tanksley and Rick, 1980; Tanksley, Medina-Filho and Rick, 1982; Vallejos and Tanksley, 1983).

The construction of dense linkage maps for QTL identification was greatly facilitated by the development of molecular markers based on variation at the DNA sequence level (Paterson *et al*., 1988; Lander and Botstein, 1989; Edwards *et al.*, 1992; Stuber *et al.*, 1992). Lander and Botstein (1989) developed a strategy known as QTL mapping, which uses molecular markers to map QTLs. Various molecular markers (DNA markers), such as restriction fragment length polymorphisms (RFLPs) (Botstein *et al*., 1980); random amplified polymorphic DNA (RAPD) (Weber and May, 1989); simple sequence repeats (SSRs); and amplified fragment length polymorphism (AFLPs) (Vos *et al*., 1995), have been

developed for the construction of dense linkage maps. Several RFLP, AFLP and SSR markers have been mapped on the barley genome in different populations (e.g. Heun et al., 1991; Becker and Heun, 1995; Qi and Lindhout, 1997). QTL mapping and related developments in molecular marker technology have consumed a substantial amount of resources, with the expectation that identified QTLs and knowledge of their map positions would be used in marker-assisted plant breeding to accumulate QTLs with positive effects.

However, wide use of marker assisted selection (MAS) is far from practical, especially in developing countries that lack facilities and technical capacity. Data for QTL identification comes from specific environments and controlled experiments that are not usually representative of target environments in developing countries, and need to be tested under natural production conditions before the data can be used in a breeding programme.

This paper summarizes the performance of some QTLs for partial resistance to barley leaf rust under natural epidemic development in the southeastern highlands of Ethiopia. The paper also reviews types of resistance against the pathogen, their mechanisms, durability and the polygenic nature of partial resistance.

TYPES OF RESISTANCE TO BARLEY LEAF RUST

Barley leaf rust (*Puccinia hordei* Otth) is a major barley disease in Ethiopia that reduces grain yield by an estimated 14% (Yitbarek Semeane *et al.,* 1996). Resistance to the pathogen exists as hypersensitive or non-hypersensitive mechanisms (Parlevliet, 1976a, b). With complete hypersensitive resistance, usually race-specific, the infection causes little or no macroscopic effect (Niks, 1986). A number of race-specific (*Rph*, synonym *Pa*) genes for resistance to barley leaf rust have been mapped on the barley genome (Table 1). Partial resistance is a non-hypersensitive resistance that reduces epidemic development in a susceptible infection type (Parlevliet and van Ommeren, 1975). Among susceptible host plant genotypes, there are quantitative differences in level of infection severity. Niks (1986) showed that plant cell wall penetration as well as pathogen growth and reproduction are less successful in a partially resistant plant than in a more susceptible one, resulting in reduced infection frequency and growth rate of the fungus, and hence a longer latent period. Parlevliet (1986) concluded that genes for partial resistance to barley leaf rust pleiotropically reduce the infection frequency, increase the latent period and reduce the rate of sporulation. Partial resistance, measured as epidemic progress in the field, is highly correlated with latent period (Parlevliet and van Ommeren, 1975).

TABLE 1
***Rph* genes for resistance to barley leaf rust in barley**

Locus	Chromosome position
Rphx	Long arm of 1 (7H); considered allele of *Rph3*
Rph1	Short arm of 2 (2H)
Rph2	Short arm of 7 (5H)
Rph3	Long arm of 1 (7H); considered allele of *Rphx*
Rph4	Short arm of 5 1H)
Rph5	3(3H)
Rph7	Short arm of 3 (3H)
Rph9	Long arm of 7 (5H); considered allele of *Rph12*
Rph10	Long arm of 3 (3H)
Rph11	Long arm of 6 (6H)
Rph12	Long arm of 7 (5H); considered allele of *Rph9*

SOURCES: Hayes *et al.*, 1996; Roane and Starling, 1989; Feuerstein, Brown and Burdon, 1990.

DURABILITY AND POLYGENIC NATURE OF PARTIAL RESISTANCE

Partial resistance behaves largely in a race-non-specific manner (VanderPlank, 1963) although small differential interactions may occur in barley (Clifford and Clothier, 1974; Parlevliet, 1977, 1978b). The resistance is more durable than hypersensitive resistance (Clifford, 1972; Parlevliet and van Ommeren, 1975) as there is no major-gene-for-major-gene interaction, and therefore a low probability that all genes become susceptible at the same time. It is inherited polygenically (Parlevliet, 1976a, 1978a). The phenotypic variation in resistance among genotypes shows a continuous range rather than discrete phenotypic classes (Clifford, 1972; Parlevliet and van Ommeren, 1975; Parlevliet *et al.,* 1980). It is therefore difficult to assign a particular genotype unambiguously to a particular phenotype. Phenotypic selection for partial resistance is more difficult than selection for hypersensitive resistance. However, Parlevliet and van Ommeren (1975) and Parlevliet *et al.* (1980) suggest that it should be fairly easy in barley leaf rust, due to the relatively high genetic variation for resistance in the crop.

QTLS FOR PARTIAL RESISTANCE TO BARLEY LEAF RUST

Detailed studies on partial resistance to *P. hordei* in barley began in 1972 at Wageningen University in the Netherlands (Parlevliet and van Ommeren, 1975). Since then, considerable fundamental information has been generated regarding its genetics (Parlevliet, 1976a, 1978b), mechanism (Niks, 1986), selection methods (Parlevliet and van Ommeren, 1975; Parlevliet *et al.,* 1980), and the relationships among components of partial resistance (Parlevliet, 1986).

A number of QTLs have been mapped for quantitative resistance to several barley diseases (Ivandic *et al.,* 2003; Williams, 2003; Backes *et al.,* 1996; Pecchioni *et al.,* 1996). QTLs for resistance to Fusarium head blight (Mesfin *et al.,* 2003) and stripe rust (Castro ***et al.***, 2002, 2003) have been mapped recently. Several QTLs have also been identified for various agronomic traits, including plant height, grain yield, and days to heading (Baum *et al.,* 2003), kernel weight (Nevo *et al.,* 2004), malt quality (Marquez-Cedillo *et al.,* 2000), straw quality (Grando *et al.,* 2005), and for tolerance or resistance to abiotic stresses like aluminium toxicity (Raman *et al.,* 2002).

Fourteen QTLs have been mapped for partial resistance to barley leaf rust in two mapping populations in the Netherlands (Table 2). Three of these QTLs, *Rphq13* (7H), *Rphq10* (4H) and *Rphq3* (6H), were effective at the adult plant stage, with ***Rphq3*** also effective at the seedling stage, and have been mapped in the cross L94 × 116-5 (Qi *et al.,* 2000) and L94 × 'Vada' (Qi *et al.,* 1998, 1999). Two other loci, *Rphq11* and *Rphq12*, effective at the seedling stage, have been mapped in the cross L94 × 116-5 (Qi *et al.,* 2000). One more putative QTL, provisionally named here as *Qch2*, has been described on chromosome 2H in the L94 × 116-5 population.

VERIFICATION OF QTLS UNDER NATURAL EPIDEMIC DEVELOPMENT

Partial resistance to a pathogen works in minor-gene-for-minor-gene interaction and mapping can only be conducted using one specific isolate at a time. However,

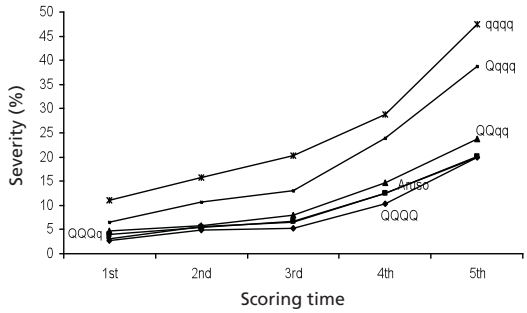
TABLE 2
QTLs mapped for partial resistance to barley leaf rust in two populations[1]

QTL	RLP Expl. (%)	AUDPC Expl. (%)	Population	Isolate	Chromosome
Rphq1	1.9	0.9	L94/Vada	1.2.1	1(7H)
Rphq2	19.9	3.8	L94/Vada	1.2.1/24	2H
Rphq3	14.8	15.7	L94/Vada, L94/116-5	1.2.1/24	6H
Rphq4	11.9	44.7	L94/Vada	1.2.1/24	7(5H)
Rphq5	4.3	3.7	L94/Vada	1.2.1	4H
Rphq6	7.7	1.4	L94/Vada	1.2.1	2H
Rphq7	6.3		L94/Vada	24	7(5H)
Rphq8	9.4		L94/Vada	24	1(7H)
Rphq9	7.1		L94/Vada	24	1(7H)
Rphq10	6.1	5.5	L94/Vada, L94/116-5	1.2.1/24	4H
Rphq11	20.0		L94/116-5	1.2.1	2H
Rphq12	4.5		L94/116-5	1.2.1	2H
Rphq13		9.2	L94/116-5	1.2.1	1(7H)
Qch2			L94/116-5	1.2.1	2H

NOTES: RLP = Relative latent period; AUDPC = Area under the disease progress curve; Expl. = The proportion of the phenotypic variance explained. SOURCES: Compiled from Qi *et al.*, 1998, 1999, 2000.

under natural conditions a given cultivar needs to grow with an unknown pathogen population. Therefore, the effectiveness of a QTL under natural infection across environments needs to be verified before being used in routine breeding programmes. The performances of four QTLs—*Rphq13* (7H), *Qch2* (2H), *Rphq10* (4H), and *Rphq3* (6H)—originally mapped in the cross between L94 (susceptible line) and a partial resistant line 116-5 using isolate 1-2-1 (Qi *et al.*, 2000) were evaluated under natural infection and epidemic development in three environments in the southeastern highlands of Ethiopia. Ninety recombinant inbred lines of barley with varying combinations of the four QTLs were grown in evaluating the QTLs. The experiment was conducted at two locations: Sinana, located 2470 masl in a bimodal rainfall area; and Herero, 2365 masl in a unimodal rainfall area, At Sinana, the experiment was conducted in 1999 in two seasons locally known as ***B**ona/ Meher* season (June–September) and *Ganna/Belg* season (March–July), and at Herero in the same year in ***Meher*** season. The two seasons of Sinana are hereafter referred to as Sinana-*Meher* and Sinana-*Belg*.

FIGURE 1
Leaf rust epidemic development (%) in five genotypes and the local check 'Aruso', an average of three environments under field conditions; Q = 116-5 genotype at each QTL, and q = L94 genotype



The disease epidemic development was measured during the growing season in five genotype groups and the local check 'Aruso' (Figure 1). Disease severity was consistently lowest in the genotype with all its alleles from

the partially resistant parent, and highest in the genotype with all its alleles from the susceptible parent. The severity on 'Aruso' was the same for the genotypes with three QTLs. This indicates that 'Aruso' has some QTLs for partial resistance to the pathogen. These putative QTLs in 'Aruso' could be the same as any three of the four QTLs in 116-5 or could be other unknown type and number of QTLs.

Differences in the area under disease progress curve (AUDPC) between the environments were significant ($P<0.05$) in all gene combinations. Average AUDPC was 437 in Sinana-*Belg*, 263 in Sinana-*Meher*, and 77 at Herero. Table 3 shows the percent reduction in AUDPC due to each QTL. Each QTL significantly reduced AUDPC in all of the three-locus × environment (E) combinations, *Rphq13* × *Qch2* × *Rphq10* × E (referred to as 13×2×10), *Rphq13* × *Qch2* × *Rphq3* × E (13×2×3), *Rphq13* × *Rphq10* × *Rphq3* × E (13×10×3) and *Qch2* × *Rphq10* × *Rphq3* × E (2×10×3), and in the four-locus × E combination, *Rphq13* × *Qch2* × *Rphq10* × *Rphq3* × E (13×2×10×3). Only the alleles from the partially resistant parent, 116-5, reduced AUDPC, with reduction ranging from 4% at *Qch2* locus in the 2×10×3 combination to 47% at the *Rphq3* locus in the four-locus combination analysis (Table 3). *Rphq3* was the most effective locus in all three-locus × E analysis, reducing AUDPC by 45–47%. This locus has been mapped as a plant growth-independent QTL, being effective at both seedling and adult plant stages, with the highest explained variance in the mapping population (Qi *et al.,* 2000). The *Qch2* locus was the least effective.

TABLE 3
Reduction of leaf rust AUDPC (%) due to main effects of four QTLs in relation to AUDPC in the susceptible parent in different QTL combinations

QTL combination	Locus			
	Rphq13	*Qch2*	*Rphq10*	*Rphq3*
13×2×10	17	10	39	
13×2×3	15	8		46
13×10×3	11		36	45
2×10×3		4	38	46
13×2×10×3	10	5	35	47

NOTES: E = environment. QTL combinations are 13×2×10 = *Rphq13* × *Qch2* × *Rphq10* × E; 13×2×3 = *Rphq13* × *Qch2* × *Rphq3* × E; 13×10×3 = *Rphq13* × *Rphq10* × *Rphq3* × E; 2×10×3 = *Qch2* × *Rphq10* × *Rphq3* × E; 13×2×10×3 = *Rphq13* × *Qch2* × *Rphq10* × *Rphq3* × E.

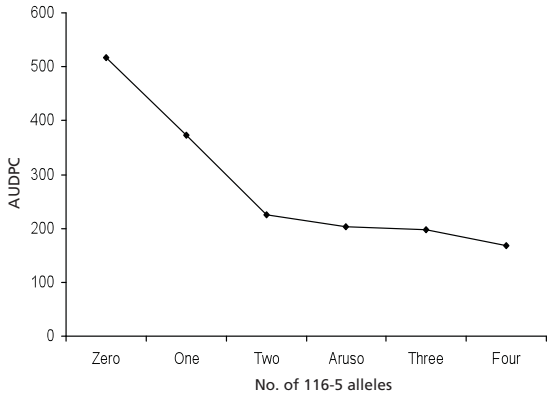
TABLE 4
Effect of two QTLs across environment in different QTLs combinations in reducing AUDPC (%) relative to AUDPC in the susceptible parent

Locus	QTL combination	Environment		
		Sinana-*Belg*	Herero	Sinana-*Meher*
Rphq10	13×2×10	34	35	49
	13×10×3	31	40	44
	2×10×3	34	32	47
	13×2×10×3	31	36	42
Rphq3	13×10×3	49	2	48
	13×2×3	48	6	50
	2×10×3	49	-0.4	49
	13×2×10×3	50	4	50

NOTES: E = environment. QTL combinations: 13×2×10 = *Rphq13* × *Qch2* × *Rphq10* × E; 13×2×3 = *Rphq13* × *Qch2* × *Rphq3* × E; 13×10×3 = *Rphq13* × *Rphq10* × *Rphq3* × E; 2×10×3 = *Qch2* × *Rphq10* × *Rphq3* × E; 13×2×10×3 = *Rphq13* × *Qch2* × *Rphq10* × *Rphq3* × E.

The performances of *Rphq10* and *Rphq3* loci differed with environment in both three-locus and four-locus analyses (Table 4). The range in reduction in AUDPC due to *Rphq10* locus across environments was 31–42% in the 13×2×10×3 gene combination to 34–49% in the 13×2×10 combination. The significant interaction of the locus with environment was merely due to changes in magnitude from one environment to another, and was effective in all environments in all QTL combinations. The *Rphq3* locus reduced AUDPC by about 50% in both seasons at Sinana in all gene combinations, but was not effective at Herero (Table 4).

FIGURE 2
Mean leaf rust AUDPC as affected by the number of alleles in 116-5 at four QTLs. 'Aruso' included as a check



Rphq13 and *Qch2* loci were stable across environments, but there was some two-locus interaction with each other and with other QTLs in one or more combination analyses. The epistatic interactions, however, were only due to changes in magnitude, and therefore do not rule out the incorporation of any two loci into a breeding line. Pyramiding the loci is important to protect a variety by means of a residual resistance in case one of the loci is defeated.

All the three-way, four-way and five-way interactions were non-significant. On average, the highest disease reduction was when all the four loci were present (Figure 2). When at least any three of the four QTLs were present, they acted additively, indicating the possibility of accumulating the loci in a breeding line for a high level of partial resistance that is effective across environments.

In conclusion, breeding for partial resistance is indispensable for developing varieties with durable resistance. The prospect of MAS for partial resistance to barley leaf rust is high, as the majority of QTLs evaluated were effective under natural epidemic development across environments; any interaction was only due to changes in the magnitude of effect. *Rphq3* was the only locus that was not effective at one of the three environments, Herero. The four loci acted additively except for some interactions that were solely due to changes in magnitude of effect, not due to changes in direction. Breeding for quantitative traits in general and for partial resistance in particular could benefit from the various QTLs being mapped in advanced research institutes and international agricultural research centres. Useful breeding lines could be identified through evaluation of QTLs for various quantitative traits under natural production conditions in collaborative projects between these and the national agricultural research system. The collaboration could grow into the area of MAS.

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