Propositions

1. Breeding for partial resistance is a must for sustainable agriculture.

2. The much stronger clustering around the centromere on genetic linkage maps of AFLP markers relative to RFLP markers is due to the higher sensitivity of the AFLP technique in sampling DNA variation compared to the RFLP technique.

3. The absence of race-specific interactions can be demonstrated for a given set of host genotype / pathogen race combinations, but cannot be proven to hold true generally.

4. A test of the minor gene-for-minor gene hypothesis requires a genetic analysis of both the resistance in the host and the aggressiveness in the pathogen.

5. The proportion of co-migrating but locus-non-specific AFLP markers is too low to hamper the construction of AFLP linkage maps in barley.

6. Screening barley germplasm of common ancestry with a large number of mapped molecular markers may identify QTLs directly from the germplasm without the use of segregating populations.

7. Genes for quantitative resistance may be allelic versions of qualitative resistance genes with intermediate phenotypes. It is incorrect to consider such QTLs "defeated" versions of qualitative resistance genes.(*Young, D. N. Ann Rev. Phytopathol. 1996.34:479-500*)

8. Genes for partial resistance in barley to leaf rust are not allelic versions of quantitative resistance genes.

9. The term "latent period" is widely used in the plant pathology community, but it is linguistically incorrect.

10. If "going Dutch" was common practice in China, possibly more Chinese young scientists would like to return to China from Holland.

11. Three cobblers with their wits combined exceed Zhuge Lang—the master mind (Chinese proverb).

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Identification and mapping of genes for partial resistance to *Puccinia hordei* Otth in barley

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Bibliographic Abstract: This thesis describes the construction of two molecular marker linkage maps in barley by using AFLP markers and the mapping of QTLs for partial resistance to barley leaf rust. In total, 13 QTLs, which are predominantly effective in an additive fashion, were identified in two populations, and most QTLs were mapped to different locations on the barley genome. This indicates that accumulation of many minor genes for partial resistance in a single cultivar is feasible. The expression of most QTLs is development stage specific. Isolate specificity of QTLs for partial resistance was clearly revealed, supporting the hypothesis that partial resistance may be based on minor gene-for-minor gene interaction.

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1

Introduction

In modern agriculture, the use of resistant cultivars is the most favourable choice in protecting crops against diseases. Nowadays, many diseases are efficiently controlled by the use of resistant crop cultivars. Two types of host resistance are usually distinguished, i.e., qualitative and quantitative resistance. Actually, in many plant-pathogen systems, both types of resistance occur. Qualitative resistance is characterised by discrete phenotypes, i.e., resistant or susceptible, and is conferred by a single or a few major gene(s). In many cases, this type of resistance is based on a hypersensitive reaction, and shows race specificity, fitting Van der Plank's concept of 'vertical' resistance (Van der Plank's 1963, 1968). This race specificity has been explained by assuming a gene-for-gene relationship between resistance genes in the host and avirulence genes in the pathogen (Flor 1956; Flor 1971)). Analysis of cloned qualitative resistance genes and their corresponding avirulence genes from several plant-pathogen systems have revealed that this model likely holds true at the molecular level (Van den Ackerveken et al. 1992; Joosten et al. 1994; HammondKosack and Jones 1997; Joosten et al. 1997). Often, this qualitative resistance, associated with the hypersensitive response, is not durable because any mutation in the avirulence gene could lead to a frame shift resulting in virulence on a host genotype with the corresponding resistance gene (Joosten et al. 1994, 1997).

Quantitative resistance is characterised by a continuous phenotypic variation between resistance and susceptibility, possibly conferred by many minor genes, and is generally not based on hypersensitivity. Such a quantitative resistance

was coined ‘partial’ resistance by Parlevliet (Parlevliet 1975). Partial resistance was initially considered to be race-non-specific, and therefore fitted Van der Plank’s concept of ‘horizontal’ resistance, which assumes that all resistance (minor) genes are equally effective to all isolates of a pathogen. However, small but significant cultivar x isolate interactions have frequently been observed (Caten 1974; Clifford and Clothier 1974(Parlevliet 1976a; Parlevliet 1977). Consequently, Parlevliet and Zadoks (Parlevliet and Zadoks 1977) then proposed a ‘minor gene-for-minor gene’ model, that is similar to the system known in qualitative resistance, to explain these interactions in quantitative resistance. However, the polygenic nature of both the resistance in the host and the aggressiveness in the pathogen, as well as the sensitivity to environmental variation not only make unambiguous testing of this model impossible, but also hamper the application of quantitative resistance in breeding programmes.

Mapping QTLs for quantitative disease resistance. The first attempts to identify individual polygenes involved in quantitative traits, via linkage analysis, date back to the 1920s (Sax 1923). Later attempts, using genetic markers linked to quantitative trait loci (QTLs) to map QTLs or to assign polygenes to chromosomes, were made by a.o. (Sax 1923; Jayakar 1970; Soller et al. 1976). However, accurate and systematic mapping of QTLs has come to maturity only after molecular markers, in particular restriction fragment length polymorphism (RFLP,(Botstein et al. 1980), became widely available (Lander and Botstein 1989). Today, besides RFLP, several other types of molecular marker, e.g., simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD, (Welsh and Mcclelland 1990; Williams et al. 1990), AFLP(Vos et al. 1995) are widely used in almost all important crop species. Quantitative trait loci, including those involved in quantitative disease resistance, could be mapped on the plant genomes by using molecular DNA marker linkage maps (Paterson et al. 1988; Tanksley 1993). The QTL mapping approach is now intensively applied for analyses of quantitative disease resistance in many important crops. At least 35 plant-pathogen systems have been studied in 14 agriculturally important crops species (Table 1). These studies concerned resistance to fungi, bacteria, viruses and nematodes.

Number and effects of QTLs. In most studies, only few QTLs, normally two to six, that are involved in quantitative resistance could be identified (Table

1). Occasionally, more than ten QTLs were detected (Bubeck et al. 1993a; Leonards-Schippers et al. 1994; Lefebvre and Palloix 1996; Caranta et al. 1997; Pilet et al. 1998). These QTL mapping studies have demonstrated that the effect of substituting a QTL-allele with its alternative allele (usually referred to as the 'effect' of the QTL) varies among QTLs. Frequently, one or two major-effect QTL(s) predominated the resistance, hampering the detection of minor-effect QTLs (Zamir et al. 1994; Ferreira et al. 1995; Miklas et al. 1996; Dirlewanger et al. 1996; Pecchioni et al. 1996a; Steffenson et al. 1996a; Pilet et al. 1998). Cases where only few QTLs were identified might be due to the use of small population sizes, low-density of molecular maps, inaccurate disease scoring methods, or a high threshold value for declaring a QTL (Young 1996). On the other hand, it is no doubt that a few unlinked genes can result in a quantitative resistance phenotype even in the absence of environmental variation.

Epistatic, dominant or recessive QTLs. The interactions between QTLs for disease resistance may vary greatly. Very often, only additive effects were detected. This is not surprising because the methods which have been widely used in QTL mapping, such as interval mapping (Lander and Botstein 1989) and multiple QTL mapping (Jansen 1993), assume that QTLs have only additive effects. Therefore, they can detect QTLs with additive and/or dominance effects, and do not allow the identification of those QTLs that do epistatically interact with other QTLs whilst their additive effects are negligible. However, the interactions among QTLs with additive effects can be tested. Several studies have provided evidence of epistatic QTLs (Webb et al. 1995; Thomas et al. 1995a; Lefebvre and Palloix 1996; Caranta et al. 1997). Epistatic effects of QTLs both with and without additive effects were detected in pepper-root rot pathosystem (Lefebvre and Palloix 1996). In addition, dominant or recessive QTLs were reported in *Brassica oleracea*-black rot (Landry et al. 1992; Grandclément and Thomas 1996), in maize-grey leaf spot (Saghai Maroof et al. 1996a) and in soybean-peanut root-knot nematode (Tamulonis et al. 1997a) pathosystems. Dominance or recessiveness of QTLs can, of course, not be assessed in the type of mapping population that is most frequently used for QTL identification, i.e., doubled haploid lines or recombinant inbred lines.

QTL by environment interactions. Studies of the interaction between QTLs and environments require large populations and many replications in

different environments. Most studies (Table 1) were conducted in one environment, i.e., in one season and at one location, so QTL by environment interaction could not be analysed. Studies on soybean sudden death demonstrated that the four QTLs for resistance were environmentally stable (Hnetkovsky et al. 1996; Chang et al. 1996). However, in rapeseed resistance to blackleg, one major-effect QTL was effective in all environments tested, the remaining moderate- and minor-effect QTLs were year-specific (Dion et al. 1995; Pilet et al. 1998). Interactions were also observed in common bean resistance to bacterial blight (Miklas et al. 1996) and in peach resistance to powdery mildew (Dirlewanger et al. 1996). Among the more than ten QTLs detected in three maize populations for resistance to grey leaf spot, only one QTL was effective in all populations and environments, the remaining ones showed significant QTL by environment interactions (Bubeck et al. 1993a). In contrast, (Saghai Maroof et al. 1996a) demonstrated that three QTLs (out of four) for resistance to maize grey leaf spot were consistently effective across three disease evaluations over two years and two generations.

Plant developmental stage dependent expression of QTLs. Plant developmental stage dependent expression of QTLs for resistance has received little attention (Table 1). A few studies (Young et al. 1993; Camargo et al. 1995; Ferreira et al. 1995; Steffenson et al. 1996a) addressing this aspect have revealed that QTLs for resistance in the seedling stage need not necessarily be effective in the adult plant stage, and vice versa. Not unexpectedly, some QTLs were consistently effective in all stages. Moreover, organ-specific expression of QTLs for resistance to bacterial blight was detected in common bean (Jung et al. 1996; Miklas et al. 1996). Such organ-specific genes may be the same as resistance genes of which the effect is plant-developmental stage specific.

QTLs effective to disease resistance components. Quantitative resistance can directly be assayed by the amount of diseased leaf area. The reduction in diseased leaf area on a quantitatively resistant plant is the combined effect of several components, e. g., latency period, infection rate, lesion size, infectious period, spore production, etc. Classical genetic studies have revealed that these components are associated, indicating that most resistance genes effect several components (Parlevliet, 1979, 1986, 1992; Young 1996). However, only a few studies have addressed this aspect, mainly due to the difficulties in the

evaluation of some components. In a study of maize *Exserohilum turcicum*, lesion number, lesion size and diseased leaf area (%) were examined (Freymark et al. 1994). In addition to the four QTLs effective to all three measures, one QTL was identified that was effective to lesion size only. Furthermore, in mapping QTLs for partial resistance to rice blast, ten of the QTLs were effective to one of the three parameters measured (lesion number, lesion size and diseased leaf area), seven were effective to two of the parameters, and only two QTLs were effective to all three parameters (Wang et al. 1993).

Isolate-specific QTLs for quantitative resistance. For a long time quantitative disease was considered to be race-non-specific. However, small but significant cultivar by isolate interactions have been identified in many plant-pathosystems (Parlevliet 1976a; Parlevliet 1977), and prompted Parlevliet and Zadoks (Parlevliet and Zadoks 1977) to propose a 'minor gene-for-minor gene' model, explaining these interactions in quantitative resistance. With the QTL mapping approach, this hypothesis can be tested as far as it concerns the resistance genes in the host that have been identified. Several papers cited in Table 1 clearly demonstrate that QTLs for resistance show distinctly different effects against different pathogen races (isolates). In the study of quantitative resistance to potato late blight, six of the eleven detected QTLs showed specificity to two *P. infestans* races (Leonards-Schippers et al. 1994). One QTL for resistance to bacterial wilt in tomato was highly race-specific (Danesh and Young 1994). Three out of four QTLs detected for resistance to the soybean cyst nematode were race-specific (Concibido et al. 1994; Concibido et al. 1997), while none of the four QTLs for resistance to downy mildew in pearl millet was effective against all four pathogen populations in four locations (Jones et al. 1995a). In addition, in *Capsicum annuum-Potyvirus*, isolate-specific effects of QTLs for resistance to PVY were clearly demonstrated (Caranta et al. 1997). All these results indicate that it is very likely that minor gene-for-minor gene interactions occur in quantitative resistance. However, in the studies referred to above, race-non-specific QTLs were also detected. Formally, the question of whether these QTLs may also be race-specific can only be answered when an infinite number of races is tested, but this is practically impossible. In other words, the absence of race-specific interaction can be shown for a given set of

host genotype/pathogen race combinations, but can never be proven to hold true absolutely.

Locations of QTLs on the plant genomes. Comparison of the locations of genes involved in qualitative and quantitative resistance (Table 1) reveals that resistance genes tend to form clusters on the genomes. These are either composed of genes of different specificity (heterospecific) or of genes that condition resistance against a single pathogen species only (homospecific) (Graner 1996). In soybean heterospecific gene clusters were observed comprising QTLs for resistance to cyst nematode (Chang et al. 1997), to Javanese root-knot nematode (Tamulonis et al. 1997b) and to peanut root-knot nematode (Tamulonis et al. 1997a). In tomato, a major-effect QTL for resistance to yellow leaf curl virus was mapped near the *Mi* gene, a root-knot nematode resistance gene (Zamir et al. 1994). Two QTLs for resistance to late blight in potato coincided with two major resistance genes for potato virus, *Rx1* and *Rx2*, respectively (Leonards-Schippers et al. 1994).

The *Mla* locus in barley (Mahadevappa et al. 1994), the *Cf* gene clusters in tomato (Lindhout 1995) and *Dm* genes in lettuce (Paran et al. 1991) are the typical examples of homospecific gene clusters for qualitative resistance. In the analysis of partial resistance to rice blast, three QTLs were mapped to the same chromosome regions as previously mapped qualitative resistance genes (Wang et al. 1993). In potato late blight, one QTL coincided with a gene, *R1*, for race-specific resistance against *Phytophthora infestans* (Leonards-Schippers et al. 1994). Two QTLs for potyvirus resistance were in the vicinity of the *pvr2* and *pvr6* loci, underlying hypersensitive resistance (Caranta et al. 1997). The fact that quantitative and qualitative resistance genes tend to map to the same map positions might support the hypothesis that QTLs for quantitative resistance are allelic versions of qualitative resistance genes with intermediate phenotypes. In this view a qualitative resistance gene can be regarded as an extreme allele of a QTL. However, most studies are not accurate enough to substantiate this hypothesis (Table 1). In contrast, studies on barley leaf rust (Thomas et al. 1995a) and on barley powdery mildew (Heun 1992a) showed no clear evidence that QTLs coincide with the corresponding hypersensitive resistance loci.

Barley-barley leaf rust. In the barley (*Hordeum vulgare* L.)-leaf rust (*Puccinia hordei* Otth) plant-pathosystem, both qualitative and quantitative

Table 1. A list of literatures on mapping of QTLs for quantitative resistance in crop species

Plant-pathosystem	No. of QTLs	Var. exp. (%, locus)[a]	Var. exp. (%, total)[b]	Major Observations	Reference
Barley-bacterial leaf streak (*Xanthomonas campestris*)	2	13-20	30	One QTL with a major effect	(El Attari et al. 1998)
Barley-leaf rust (*Puccinia hordei*)	2	-	-	No clear evidence of coincidence with *Rph12*	(Thomas et al. 1995b)
Barley-leaf stripe (*Pyrenophora graminea*)	4	3-59	77	One major-effect QTL dominates the resistance	(Pecchioni et al. 1996b)
Barley-net blotch (*Pyrenophora teres* f. *teres*)	10	10-31	68	One QTL was effective in the seedling and adult plant stage; the others were stage-specific	(Steffenson et al. 1996b)
Barley-powdery mildew (*Erysiphe graminis*)	2	11- 12	20	Not coincide with *Mla12*	(Heun 1992b)
	2	9-18	-	One QTL in the same region as *mlt*	(Backes et al. 1995; Backes et al. 1996)
	8	-	-	One QTL probably at the *Mla* locus	(Thomas et al. 1995a)
Barley-*Rhynchosporium secalis*	5	-	52	Resistant allele from susceptible parent associated with transgressive segregation	(Backes et al. 1995)
	5-6	-	-	One QTL exerted a large effect	(Thomas et al. 1995a)
Barley-spot blotch (*Cochliobolus sativus*)	3	9-71	70-71	QTLs effective in the two stages were mapped to the different positions	(Steffenson et al. 1996b)
Barley-stripe rust (*Puccinia striiformis*)	2	10-57	61	Natural disease epidemics of race 24; possible different formae	(Chen et al. 1994)
	3	-	-	Interaction between QTLs	(Thomas et al. 1995a)

Plant-pathosystem	No. of QTLs	Var. exp. (%, locus)[a]	Var. exp. (%, total)[b]	Major Observations	Reference
Brassica oleracea-black rot (*Xanthomonas campestris*)	4	18-35	46-73	Two QTLs for field resistance and two additional QTLs for seedling resistance	(Camargo et al. 1995)
	2	15-58	61	Two dominant QTLs; no interactions	(Landry et al. 1992)
	3	8-13	30	One dominant QTL; one QTL is due to a heterozygous genotype	(Grandclément and Thomas 1996)
	2	18-50	68	No evidence of interactions	(Voorrips et al. 1997)
Common bean-bacterial blight (*Xanthomonas campestris*)	4	13-35	75	One QTL mapped to the same region as *Rhizobium* nodule number locus	(Nodari et al. 1993)
	6	14-34	14-29	One organ-specific QTL	(Jung et al. 1996)
	2-3	9-60	46-65	Interaction of QTLs with environments and organs	(Miklas et al. 1996)
	4	5-40	18-53	All QTLs for leaf resistance equally effective to two strains	(Jung et al. 1997)
Common bean-golden mosaic virus	4	18-53	>60	Two major-effect QTLs	(Miklas et al. 1996)
Common bean-web blight (*Thanatephorus cucumeris*)	5	2-10	34	One QTL also effective to bacterial blight; one resistance allele from susceptible parent	(Jung et al. 1996)
Maize-*Exserohilum turcicum*	3-5	7-18	29-45	Some QTLs control lesion size only	(Freymark et al. 1994)
Maize-grey leaf spot (*Cercospora zeae-maydis*)	>10	4-26	Up to 58	One QTL effective in all populations and enviroments	(Bubeck et al. 1993b)

Table 1. *Continued*

Plant-pathosystem	No. of QTLs	Var. exp. (%, locus)[a]	Var. exp. (%, total)[b]	Major Observations	Reference
Maize-grey leaf spot (*Cercospora zeae-maydis*)	4	5-56	68	One dominant and one recessive QTL; consistently effective across evaluations	(Saghai Maroof et al. 1996b)
Maize-northern leaf blight (*Setosphaeria turcica*)	4	10-38	41-48	One QTL mapped to the same region as *Ht2*	(Dingerdissen et al. 1996)
Maize-stalk rot (*Gibberella zeae*)	5	-	20	Minor or moderate effects	(Pè et al. 1993)
Mungbean-powdery mildew (*Erysiphe polygoni*)	3	17-28	58	Two QTLs at 65 days after planting; additional one at 85 days after planting	(Young et al. 1993)
Pea-ascochyta blight (*Ascochyta pisi*)	3	38- 58	71	One resistance allele from susceptible parent	(Dirlewanger et al. 1994)
Peach-powdery mildew (*Sphaerotheca pannosa*)	6	14-78	39-78	One major-effect QTL and five minor-effect QTLs; some QTLs were environment-dependent	(Dirlewanger et al. 1996)
Pearl millet-downy mildew (*Scelerospora graminicola*)	4	-	37-65	No QTLs effective against all four pathogen populations (locations)	(Jones et al. 1995b)
Pepper-potyviruses	11	10-67	66-76	Two significant interactions between QTLs; isolate-specific effects; two QTL coincided with qualitative resistance loci	(Caranta and Palloix 1996; Caranta et al. 1997; Caranta et al. 1997)
Pepper-root rot (*Phytophthora capsici*)	13	17-28	Up to 90	Epistatic effects of QTLs	(Lefebvre and Palloix 1996)
Potato-cyst nematode (*Globodera rostochiensis*)	2	7	14	Two QTLs have additive effects	(Kreike et al. 1993)

Plant-pathosystem	No. of QTLs	Var. exp. (%, locus)[a]	Var. exp. (%, total)[b]	Major Observations	Reference
Potato-late blight (*Phytophthora infestans*)	11	-	-	At least one QTL was race-specific; two QTLs coincided with hypersensitive resistance loci	(Leonards-Schippers et al. 1994)
Rapeseed-blackleg (*Leptosphaeria maculans*)	2	8-72	80	One major QTL effective in all environments; minor QTLs were detected in one year-site assays	(Dion et al. 1995)
	7	10-90	-	One major locus for cotyledon resistance; different effects of QTLs for seedling and for field resistance	(Ferreira et al. 1995)
	13	-	23-57	A major-effect QTL masking more QTL detection; QTLs dependent on disease measures; year-specific QTLs	(Pilet et al. 1998)
Rice-blast (*Pyricularia oryzae*)	10	16-60	76	Three QTLs coincide with hypersensitive resistance loci; different QTLs for lesion size	(Yu et al. 1991; Wang et al. 1993)
Rice-sheath blight (*Rhizoctonia solani*)	6	6-27	47	No clear interactions between QTLs	(Li et al. 1995)
Soybean-cyst nematode (*Heterodera glycines*)	4	10-51	-	One QTL detected in all populations and effective to all races tested; others were race-specific	(Concibido et al. 1994; Concibido et al. 1997)
	3	-	-	Interaction between two QTLs	(Webb et al. 1995)
	2	14-24	47	One QTL in the same region as the sudden death syndrome QTL	(Chang et al. 1997)
Soybean-Javanese root-knot nematode (*Meloidogyne javanica*)	2	13-46	54	One QTL in a cluster of distinct disease resistance loci	(Tamulonis et al. 1997b)

Table 1. *Continued*

Plant-pathosystem	No. of QTLs	Var. exp. (%, locus)[a]	Var. exp. (%, total)[b]	Major Observations	Reference
Soybean-peanut root-knot nematode (*Meloidogyne arenaria*)	2	16-32	51	One dominant QTL; one major-effect QTL in a cluster of eight other resistance loci	(Tamulonis et al. 1997a)
Soybean-sudden death syndrome (*Fusarium solani*)	4	7-24	50-65	QTLs were environmentally stable	(Hnetkovsky et al. 1996; Chang et al. 1996)
Tomato-bacterial canker (*Clavibacter michiganensis*)	5	-	-	One QTL from susceptible parent	(Sandbrink et al. 1995)
	2	-	-	Two QTLs nearly explained the difference between the parents	(Van Heusden et al. 1995)
Tomato-bacterial wilt (*Pseudomonas solanacerum*)	3	24-77	82	Effects of QTLs vary according to inoculation methods; and show race-specificity	(Danesh and Young 1994)
	4	6-20	30-56	Dominance at one QTL	(Thoquet et al. 1996a)
	6	5-20	60	Four major-effect QTLs; two minor-effect QTLs	(Thoquet et al. 1996b)
Tomato-yellow leaf curl virus	3	-	-	One major-effect QTL mapped near *Mi*	(Zamir et al. 1994)

[a] variance explained per locus

[b] total variance explained by all detected QTLs

resistance occur. To date, 14 genes underlying qualitative resistance (*Rph* genes, formerly *Pa*) have been identified in barley and its wild progenitor, *H. vulgare* ssp. *spontaneum* (C. Koch) Thell (Roane and Starling 1967; Feuerstein et al. 1990; Jin et al. 1993, 1996; Franckowiak et al. 1997). Some of these genes have been introduced into barley cultivars in order to control leaf rust. However, rapid adaptation of the *P. hordei* populations has rendered most of the hypersensitive resistance genes ineffective. Hence, this qualitative resistance of barley to leaf rust is not durable.

In 1973, the barley-barley leaf rust was chosen as a model system for the study of quantitative resistance to plant disease at the Department of Plant Breeding of Wageningen Agricultural University. The quantitative resistance in this plant-pathosystem was coined ‘partial’ resistance by (Parlevliet 1975), and has been defined as resistance that results in reduced epidemic development despite a compatible (susceptible) infection type (Parlevliet and Van Ommeren 1975; Parlevliet 1978b). Partial resistance in the field is due to the reduction of rust growth and development, and strongly correlates with several components, e.g., latency period, infection frequency, pustule size, infectious period and spore production. Of these components, latency period on adult plants is the best predictor for the level of partial resistance in the field, and can be evaluated with great accuracy (Neervoort and Parlevliet 1978; Parlevliet 1979; Parlevliet 1986; Parlevliet 1992). The latency periods evaluated in the seedling stage and the adult plant stage were only moderately correlated, suggesting that different genes are involved in resistance in the two plant development stages (Parlevliet 1975; Parlevliet and Van Ommeren 1975; Parlevliet and Kuiper 1977).

Continuous variation of partial resistance is due to its polygenic nature, i.e., many minor genes conferring the resistance, and the sensitivity to environmental differences. Assuming equal and additive effects of the minor genes, six loci were estimated to control partial resistance in an old Dutch barley cultivar, ‘Vada’, and also six loci in ‘Cebada Capa’, by traditional quantitative genetic analysis (Parlevliet and Kuiper 1985a; Parlevliet et al. 1985b). As mentioned above, small but significant interactions between cultivars and isolates (Parlevliet 1976a; Parlevliet 1977; Parlevliet 1978b)) prompted Parlevliet and Zadoks (1977) to hypothesise a minor gene-for-minor gene model.

Objectives and outline of present study. However, the individual genes for partial resistance can not be identified and characterised by classic quantitative genetic analysis. This makes testing the minor gene-for-minor gene hypothesis almost impossible. Further, this unsolved problem has to certain extent obstructed the utilisation of partial resistance in breeding programmes, although Parlevliet and his colleagues (Parlevliet et al. 1980; Parlevliet 1981; Parlevliet and Kuiper 1985a; Parlevliet et al. 1985b) have demonstrated that selection for a high level of partial resistance is fairly easy. Therefore, the objectives of the present study were (i) to identify and characterise individual quantitative loci (QTLs) for partial resistance in the partially resistant barley cultivars by using molecular markers, (ii) to verify whether the minor gene-for-minor gene hypothesis holds for this plant-pathogen system, and (iii) to provide useful information for breeding durably resistant varieties by accumulation of QTLs for partial resistance.

In chapter 2, four existing barley RFLP marker linkage maps are compared and integrated, providing a better understanding of the barley genome, and facilitating further mapping studies in barley. The application of the AFLP technique ((Vos et al. 1995) in barley was the aim of chapter 3. The variation in AFLP patterns in the barley species was investigated with a large number of primer combinations. Forty-eight AFLP profiles were generated from 16 representative barley lines, that will facilitate a wide and systematic use of AFLP markers for genetic studies in barley. In chapter 4, a high-density AFLP marker linkage map was successfully constructed by using a recombinant inbred population (103 RILs, F_9) derived from a cross between a line susceptible to leaf rust, L94, and the partially resistant cultivar, 'Vada'. The same set of RILs was tested for resistance to the barley leaf rust isolate 1.2.1. in the seedling and in the adult plant stage. Subsequently, in chapter 5, QTLs for partial resistance to barley leaf rust were mapped to the barley genome. In chapter 6, QTLs for resistance to the leaf rust isolate 24 were mapped to the same linkage map. Isolate-specific QTLs were reported in this chapter, supporting the minor gene-for-minor gene hypothesis. In chapter 7, another recombinant inbred population (117 RILs F_8), derived from a cross between L94 and 116-5, a partially resistant line derived from 'Cebada Capa', was evaluated for resistance to the leaf rust isolate 1.2.1. and scored for the segregation of AFLP markers. Additional QTLs

for partial resistance in this population were identified, indicating that there are abundant loci for partial resistance are scattered over the barley genome. In the last chapter, several important aspects relevant to the present study are discussed.

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2

Comparison and integration of four barley genetic maps*

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Abstract: Barley (*Hordeum vulgare* L.) is one of the most extensively studied food crops in recent molecular researches. More than 1000 molecular markers have been located on the barley genome by using five independent populations. For the present study, four segregation data sets, 'Proctor' x 'Nudinka', 'Igri' x 'Franka', 'Steptoe' x 'Morex' and 'Harrington' x TR306, were downloaded from the publicly available GrainGenes databank. Since 22% of the markers are common to at least two of the independent data sets, we were able to establish an integrated map, using the computer package JoinMap V2.0. The integrated map contains 880 markers, covers 1060 cM, and removes many large gaps present in the individual maps. Comparison of the integrated map with the individual maps revealed that the overall linear order of markers is in good agreement and that the integrated map is consistent with the component maps. No significant reordering of markers was found. This conservative property of the barley genome makes the integrated map reliable and successful. Except for chromosome 7 (5H), marker clustering was observed in the centromeric regions, probably owing to the centromeric suppression of recombination. Based on this integrated map, geneticists and breeders can choose their favourite markers in any region of interest of the barley genome.

Key words: *Hordeum vulgare*, RFLP, integrated map

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Introduction

Barley (*Hordeum vulgare* L.) is extensively studied as a favourite genetic experimental plant species, mainly owing to its diploid nature (2n= 2x =14), self-fertility, large chromosomes (6-8μm), high degree of natural and easily inducible variation, ease of hybridization, wide adaptability, and relatively limited space requirements, as well as agricultural importance (Kleinhofs and Kilian 1994). Its large genome size, (1C=5.3 x 10^9 base pair (bp)) (Bennett and Smith 1976) has slowed down the development of molecular maps. However, techniques for developing doubled haploid lines and the availability of cytogenetic stocks, such as the barley-wheat addition lines, have facilitated genetic mapping.

The first incomplete barley RFLP map for chromosome 6 was published in 1988 (Kleinhofs et al. 1988). Recently, five more extensive molecular maps covering the entire genome have been generated by using five independent doubled haploid populations. These are 'Proctor' x 'Nudinka' (Heun et al. 1991), 'Igri' x 'Franka' (Graner et al. 1991), 'Vada' x *H. spontaneum* line 1b-87 (Graner et al. 1991), 'Steptoe' x 'Morex' (Kleinhofs et al. 1993b) and 'Harrington' x TR306 (Kasha et al. 1993). Many other segregating populations have also been used to construct partial maps and to determine the location of interesting genes on the genome: for example, the 'Aramir' x *H. spontaneum* derived population was used to generate a map of chromosome 4 (Hinze et al. 1991); 120 F_2 plants obtained from 'Betzes' x 'Golden Promise' and 120 F_2 plants from 'Captain' x *H. spontaneum* were used to map 5S rDNA genes on chromosome 2 (Leitch and Heslop-Harrison 1993); Laurie et al. (1993) located the *denso* dwarfing gene to the long arm of chromosome 3 by using 113 doubled haploid lines from 'Magnum' x 'Goldmarker'; and a photoperiod response gene (*Ppd-H1*) was mapped by using 94 doubled haploid lines from 'Igri' x 'Triumph' (Laurie et al. 1994). Today, more than 1000 markers have been located on the barley genome by using different populations and more markers will be developed and mapped in the near future. The rapid accumulation of markers and mapping populations is a challenge to the merging of separate lines of information to accumulate more valuable information for further research and a better understanding of barley genetics and genome organization.

Recent good communication between North American and European barley mapping efforts has resulted in a frequent exchange of probes. As a consequence, many common markers have been utilized in independent mapping populations. The availability of the mapping software programme JoinMap (Stam 1993), which enables the integration of individual maps into one composite map by using common markers, makes the construction of an integrated barley map possible. The present study aims at the integrating four individual maps into a single map. The combined map provides an easy and convenient way of comparison between the component maps and offers important information about the reliability of marker order and distances between markers.

Materials and methods

Four barley segregation data sets (Table 1) were downloaded from the publicly available GrainGenes databank (Graner 1994; Kleinhofs 1994a, 1994b; Sorrells 1992). The mapping populations of 'Proctor' x 'Nudinka' (P/N) (Heun et al. 1991; Sorrells 1992) and 'Igri' x 'Franka' (I/F) (Graner et al. 1991; Graner 1994) consisted of 113 and 73 doubled haploid lines, respectively, derived by anther culture. In P/N, 154 markers and in I/F, 369 have been located on seven chromosomes. 'Steptoe' x 'Morex' (S/M) (Kleinhofs et al. 1993, 1994a) and 'Harrington' x TR306 (H/T) (Kasha and Kleinhof 1994; Kleinhofs 1994b), used in the North American Barley Genome Mapping Project (NABGMP), both contain 150 doubled haploid lines which were derived by the *Hordeum bulbosum* method. Data for 423 and 190 markers respectively, was available for the two populations.

The new version of JoinMap (Stam 1993), which can handle a wide variety of mapping population types including the doubled haploid type, was used to regenerate linkage maps and to merge these into an integrated map. From the segregation data, the pairwise recombination frequencies were estimated and the corresponding LOD values were calculated. If several estimates of the recombination frequency between a certain pair of markers were available (markers shared by at least two populations), they were replaced by a single value after appropriate weighting (Stam 1993). Based on the recombination frequencies and LOD values, the individual or integrated maps were constructed by running

the JoinMap programme. Kosambi's mapping function was adopted for map distance calculation (Kosambi 1944). Since the gene ordering algorithm of JoinMap does not guarantee the best solution, the "fixed order" option was used in a number of cases where the goodness-of-fit criterion cast doubt on the ordering. (The "fixed order" option allows the user to define fixed orders of (sub)sets of markers; by using various fixed orders a better solution is occasionally obtained, especially with data sets of moderate quality.)

Table 1. Four doubled haploid mapping populations and their characteristics

Parents	Population Size	Number of markers	Length of map (cM)	Reference
Harrington x TR306	150	190	1278	Kasha and Kleinhofs 1994 Kleinhofs 1994a
Steptoe x Morex	150	423	1227	Kleinhofs et al. 1993a Kleinhofs 1994b
Proctor x Nudinka	113	154	1192	Heun et al. 1991 Sorrells 1992
Igri x Franka	73	369	1387	Graner et al. 1994 Graner 1994

Results and discussion

Source and nomenclature of markers and chromosomes

Probes from several different sources have been used as genetic markers for the barley genome (Kleinhofs and Kilian 1994). In the present study, much attention was paid to ascertaining whether markers with different names in different populations represented the same locus. Alternatively, markers with the same name might represent different loci. Multiple MWG markers in the I/F population were designated with lower case letters, for example, MWG555a and MWG555b (Graner et al. 1991); these were converted into capital letters (MWG555A and MWG555B) for consistency with the other three populations (Table 2; Heun et al. 1991; Kleinhofs et al. 1993b; Kasha and Kleinhofs 1994). Similarly, meaningless zeros in marker names of the S/M and H/T populations

Table 2. Nomenclature of markers

Present Name	Original name	Chromosome number	Mapping population
ABA1	ABA001	7(5H)	S/M
ABA2	ABA002	5(1H)	S/M
ABA3	ABA003	4(4H)	S/M
ABA4	ABA004	5(1H)	S/M
ABA5	ABA005	2(2H)	S/M
ABA6	ABA006	6(6H)	S/M
ABC151A	ABC151a	1(7H)	I/F
ABC151A	ABC151	1(7H)	S/M
ABC156D	ABC156	1(7H)	I/F
ABC167A	ABC167a	1(7H)	I/F
ABC310B	ABC310	1(7H)	I/F
ABG10	ABG010	3(3H)	S/M
ABG11	ABG011	1(7H)	S/M
ABG14	ABG014	2(2H)	S/M
ABG19	ABG019	2(2H)	H/T
ABG19	ABG019	2(2H)	S/M
ABG1A	ABG001A	6(6H)	H/T
ABG1A	ABG1	6(6H)	I/F
ABG1A	ABG001	6(6H)	S/M
ABG1B	ABG001B	1(7H)	H/T
ABG1C	ABG001C	6(6H)	H/T
ABG2	ABG002	2(2H)	S/M
ABG22A	ABG022A	1(7H)	S/M
ABG3	ABG003	4(4H)	S/M
ABG387A	ABG387a	5(1H)	I/F
ABG387B	ABG387b	6(6H)	I/F
ABG4	ABG004	3(3H)	S/M
ABG5	ABG005	2(2H)	S/M
ABG500B	ABG500	4(4H)	I/F
ABG53	ABG053	5(1H)	S/M
ABG54	ABG054	4(4H)	S/M
ABG55	ABG055	5(1H)	S/M
ABG57	ABG057	3(3H)	S/M
ABG57B	ABG057B	7(5H)	H/T
ABG58	ABG058	2(2H)	H/T
ABG58	ABG058	2(2H)	S/M
ABG59	ABG059	5(1H)	S/M
ABG65B	ABG065B	1(7H)	H/T
ABG69	ABG069	7(5H)	S/M
ABG703B	ABG703b	2(2H)	I/F

Table 2. Nomenclature of markers (*Continued*)

Present Name	Original name	Chromosome number	Mapping population
ABG705A	ABG705	7(5H)	S/M
ABG72	ABG072	2(2H)	S/M
ABG74	ABG074	5(1H)	S/M
ABG75	ABG075	1(7H)	S/M
ABG77	ABG077	1(7H)	H/T
ABG8	ABG008	2(2H)	S/M
Act8A	Act8	5(1H)	S/M
BCD351E	BCD351e	7(5H)	I/F
BCD453B	BCD453	2(2H)	P/N
BG123A	BG123a	2(2H)	I/F
BG123A	BG123	2(2H)	P/N
BG123B	BG123b	7(5H)	I/F
CDO348B	CDO348	7(5H)	H/T
CDO474C	CDO474	2(2H)	I/F
Chs1B	Chs1b	2(2H)	I/F
Dhn3	XDhn3,4	6(6H)	P/N
Glx(Wx)	Glx	1(7H)	H/T
Glx(Wx)	Wx	1(7H)	I/F
Glx(Wx)	Glx	1(7H)	S/M
His3A	aHis3a	1(7H)	H/T
Hor2	aHor2	5(1H)	H/T
MWG10	MWG010	3(3H)	I/F
MWG10B	MWG010B	1(7H)	S/M
MWG3	MWG003	1(7H)	H/T
MWG3	MWG003	1(7H)	S/M
MWG36A	MWG036A	5(1H)	S/M
MWG36B	MWG036B	1(7H)	H/T
MWG36B	MWG036B	1(7H)	S/M
MWG41	MWG041	3(3H)	H/T
MWG41	MWG041	3(3H)	S/M
MWG520A	MWG520	2(2H)	H/T
MWG520A	MWG520	2(2H)	I/F
MWG555A	MWG555a	1(7H)	I/F
MWG555B	MWG555b	3(3H)	I/F
MWG57	MWG057	4(4H)	I/F
MWG571A	MWG571a	3(3H)	I/F
MWG58	MWG058	4(4H)	I/F
MWG58	MWG058	4(4H)	S/M
MWG635A	MWG635a	4(4H)	I/F
MWG636(HT)	MWG636	2(2H)	H/T
MWG636(IF)	MWG636	2(2H)	I/F

Table 2. Nomenclature of markers (*Continued*)

Present Name	Original name	Chromosome number	Mapping population
MWG64	MWG064	2(2H)	I/F
MWG65	MWG065	2(2H)	I/F
MWG663-2A	MWG663	6(6H)	H/T
MWG77	MWG077	4(4H)	S/M
MWG798A	MWG798	6(6H)	H/T
MWG798A	MWG798a	6(6H)	I/F
MWG813A	MWG813	7(5H)	H/T
MWG813A	MWG813a	7(5H)	I/F
MWG844A	MWG844	2(2H)	H/T
MWG844A	MWG844a	2(2H)	I/F
MWG85	MWG085	3(3H)	I/F
MWG851A	MWG851a	1(7H)	I/F
MWG851B	MWG851b	7(5H)	I/F
MWG89	MWG089	1(7H)	I/F
MWG89	MWG089	1(7H)	S/M
MWG90	MWG090	2(2H)	I/F
RisBPP161A	RisBPP161	1(7H)	H/T
RisBPP161A	RisBPP161a	1(7H)	I/F
WG789A	WG789	1(7H)	P/N
cMWG652A	cMWG652a	6(6H)	I/F
cMWG706A	cMWG706a	5(1H)	I/F
iEst1	Est1	3(3H)	I/F
iEst5	Est5	1(7H)	I/F

were omitted (e.g., ABA001 becomes ABA1). The prefixes, “i”, “m”, and “d” were added to marker names to indicate the isozyme markers, and morphology markers, and disease resistance genes, respectively. The rest of the marker names remained unchanged and the original datasets were used to generate maps of each population separately and one integrated map. If markers with the same core name were mapped within a 5-cM distance they were considered to represent only one locus and the name was adjusted accordingly. For example, the markers ABC151 and ABC151a from datasets of S/M and I/F, respectively, were mapped on 23.9 and 18.3 cM on chromosome 1 of the “pre-integrated map” (not shown). Subsequently, the names ABC151 and ABC151a were converted into ABC151A and a new map was generated with only one locus position for ABC151A at 24.1 cM (Fig. 1A). All changed gene symbols and their original symbols are listed in Table 2. As in Kleinhofs and Kilian (1994), the chromosome designations 1, 2, 3,

4, 5, 6 and 7 are used in this paper and correspond to 7H, 2H, 3H, 4H, 1H, 6H and 5H, respectively.

Table 3. The number of common markers between or among populations

Mapping Populations	Chromosomes 1	2	3	4	5	6	7	total
H/T and I/F	4	3	2	0	1	2	1	13
H/T and P/N	1	3	1	1	0	2	2	10
H/T and S/M	15	5	7	2	6	7	11	53
I/F and P/N	0	0	0	0	0	0	0	0
I/F and S/M	7	10	14	4	9	4	9	57
P/N and S/M	6	6	1	2	2	0	7	24
Subtotal	33	27	25	9	18	15	30	157
H/T and I/F and P/N	0	0	0	0	0	0	0	0
H/T and I/F and S/M	9	5	2	1	2	8	4	31
H/T and P/N and S/M	2	2	0	1	0	0	1	6
I/F and P/N and S/M	0	1	1	0	0	0	0	2
Subtotal	11	8	3	2	2	8	5	39
H/T and I/F and P/N and S/M	0	0	0	1	0	0	0	1
Total	44	35	28	12	20	23	35	197

[a] H/T, I/F, P/N and S/M represent 'Harrington' x TR306, 'Igri' x 'Franka', 'Proctor' x 'Nudinka' and 'Steptoe' x 'Morex', respectively.

Individual maps

Four individual maps were generated by running JoinMap V2.0. The same gene order was obtained for most linkage groups across populations. In some cases the fixed order option had to be used to obtain the most likely gene order for the four individual maps. In population P/N, fixed gene orders were used in generating maps of chromosomes 1, 2, 4 and 5. Fixed orders were also used for mapping of chromosome 3 in the H/T and I/F crosses. For the S/M map, no improvement was obtained by predefining gene orders, which is not surprising since this is the largest data set.

The individual maps generated by JoinMap were slightly shorter compared with the original published maps (Tables 1 and 3). The original maps were estab-

lished by using MapMaker (Lander et al. 1987). The discrepancy between map lengths obtained with JoinMap and MapMaker results from the different methods of calculating map lengths. MapMaker calculates the map length as the sum of adjacent distances, i. e. using adjacent marker pairs only. JoinMap on the other hand uses all pairwise estimates (above a pre-defined LOD threshold) for calculating the total map length. Whenever the assumed level of interference does not exactly reflecting the true interference, the two methods will produce slightly different total map lengths. The likelihood method applied in MapMaker assumes an absence of interference and recombination frequencies are simply translated into centimorgans, according to the chosen mapping function. The JoinMap package, however, does take interference into account. Therefore, where there is interference JoinMap will produce shorter maps than MapMaker, even when both programmes use the same Kosambi mapping function (Stam 1993).

Integrated map

In total, 190, 369, 154 and 423 markers were assigned to the seven barley chromosomes by using the mapping populations H/T, I/F, P/N and S/M, respectively. Having standardized the different gene symbols (Table 2), 157 markers were found to be common to two populations, 39 markers were shared by three populations, and only one marker, WG622 on chromosome 4, was present in four populations. Populations H/T and S/M, and I/F and S/M had 53 and 57 markers in common, respectively, and 31 markers were shared by these three populations. Only three markers were common to I/F and P/N. The backbone of the integrated map consists, of course, of the common markers. Together with the markers that are unique to one of the four populations, the combined map contains 880 markers, including four morphological markers and six disease resistance genes. For the construction of the integrated map, no fixed orders were required. Gene orders on the integrated map are identical to the orders on the four component maps.

The number of common markers is listed in Table 3 and the map data, i.e., length of chromosomes, number of markers and number of gaps >10 cM, are summarized in Table 4. The integrated maps of seven barley chromosomes is presented by Figure 1A-G.

Table 4. Summary of individual and integrated mapping data

	H/T			I/F			P/N			S/M			Integrated		
Chromo-some	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]
1(7H)	180	49	8	156	76	3	167	32	6	138	74	4	152	176	2
2(2H)	160	31	6	167	63	3	176	38	6	151	74	2	157	163	1
3(3H)	131	18	3	128	70	5	187	14	6	162	62	3	131	133	1
4(4H)	142	14	6	136	24	5	140	16	4	139	43	5	134	81	0
5(1H)	131	16	5	120	38	5	153	20	4	157	56	2	150	90	2
6(6H)	160	30	4	128	43	6	36	9	1	140	47	5	141	98	3
7(5H)	214	32	9	199	55	8	158	25	3	184	67	7	195	139	1
Total	1118	190	41	1034	369	35	1017	154	27	971	423	28	1060	880	10

Note: H/T, I/F, P/N and S/M represent 'Harrington' x TR306, 'Igri' x 'Franka', 'Proctor' x 'Nudinka' and 'Steptoe' x 'Morex', respectively; Integrated indicates the integrated map.

[a] A gap indicates a distance between two adjacent markers of more than 10 cM.

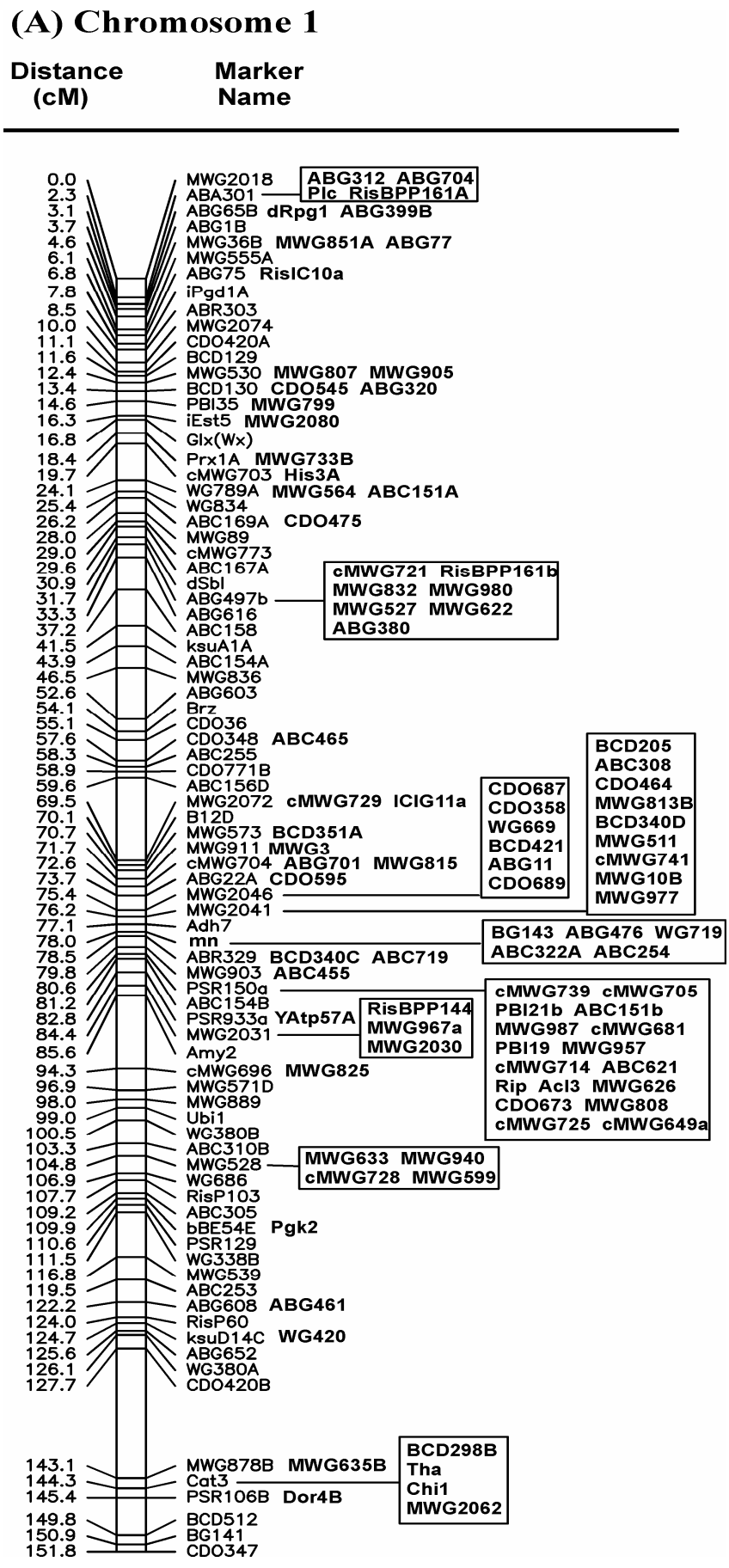


Fig.1. (A-G) Barley integrated molecular linkage map. Chromosomes are oriented with the short arm at the top. The genetic distances are expressed in map distances (cM) according to Kosambi (Kosambi, 1944). Markers in the box are located at the same position as the marker to which it is connected.

Fig. 1. (*Continued*)

(B) Chromosome 2

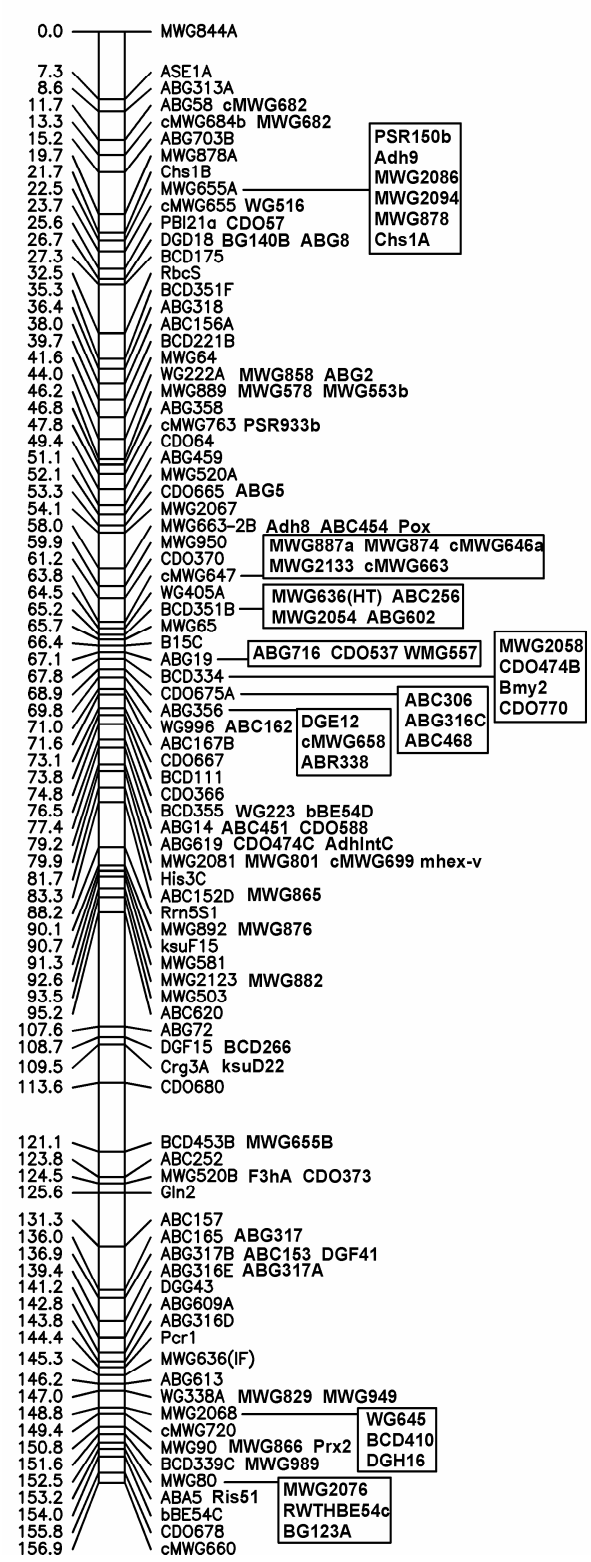


Fig. 1. (*Continued*)

(C) chromosome 3

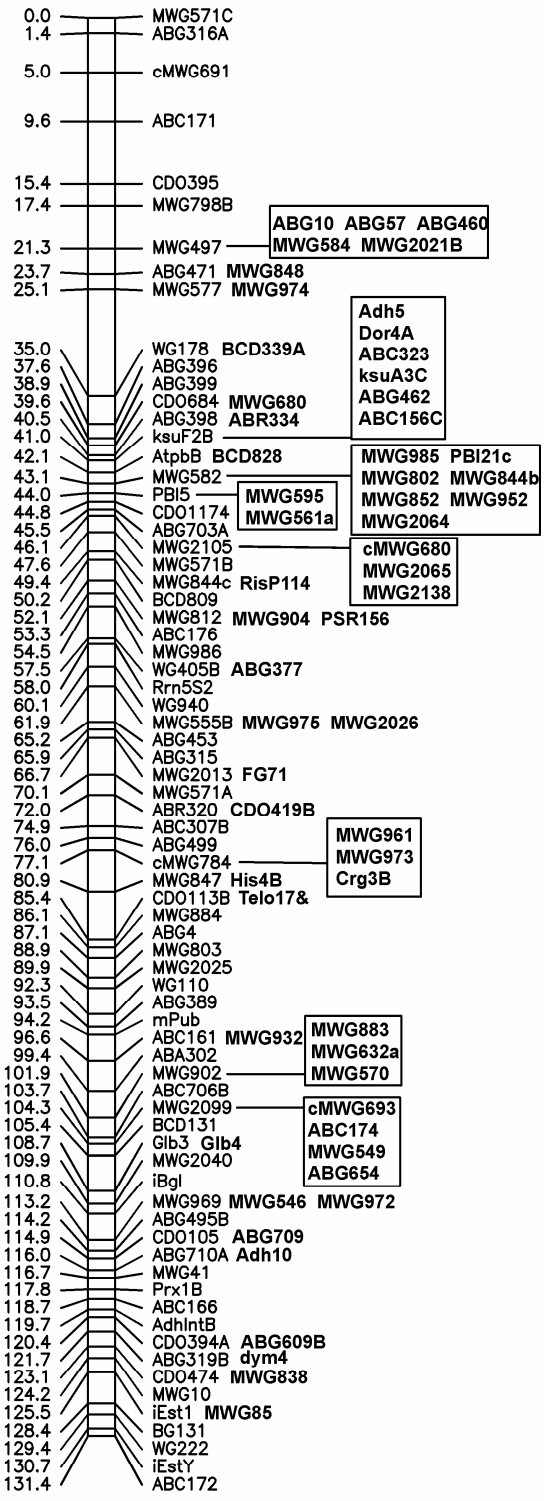


Fig. 1. (*Continued*)

(D) Chromosome 4

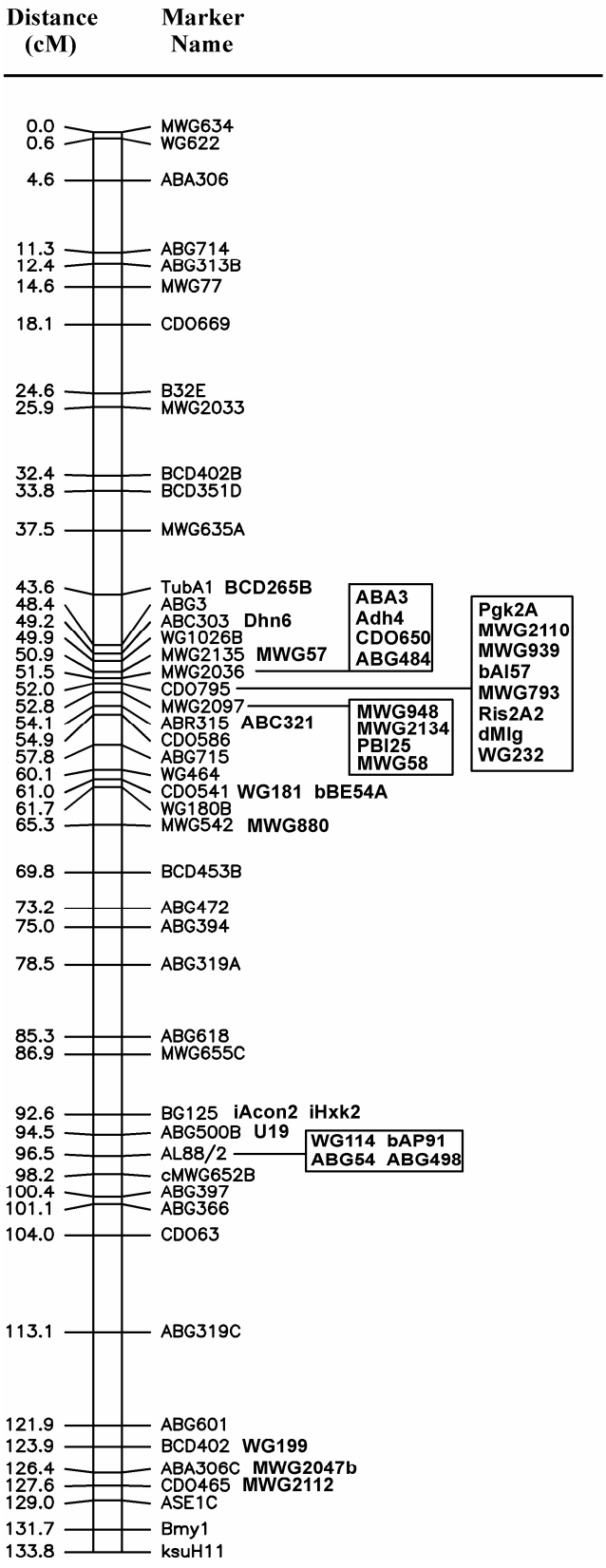


Fig. 1. (*Continued*)

(E) Chromosome 5

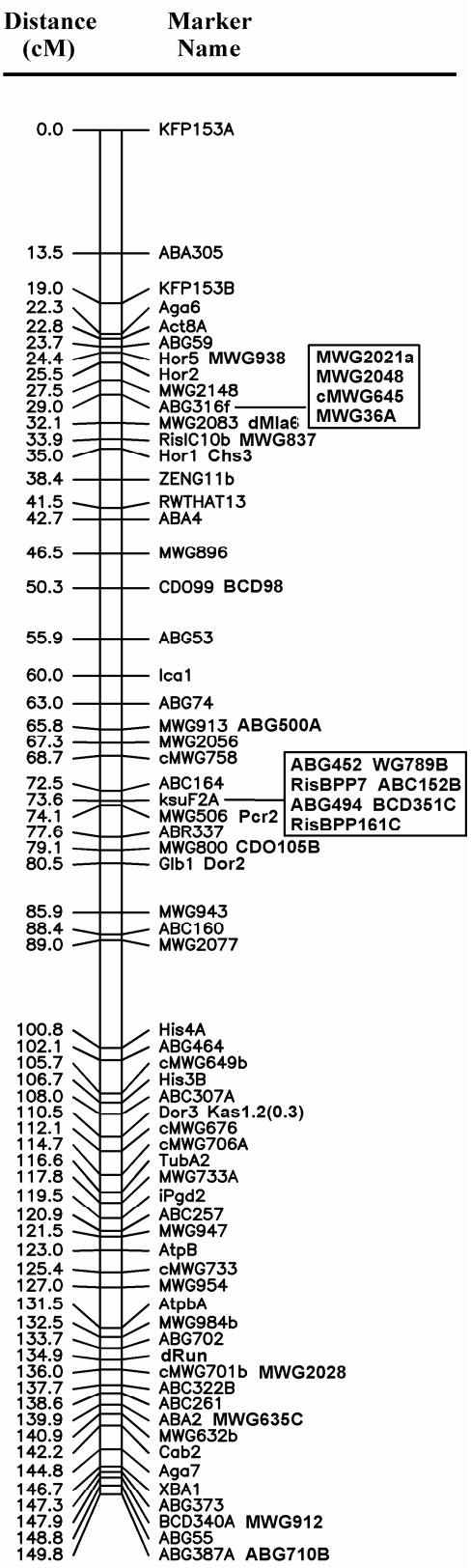


Fig. 1. (*continued*)

(F) chromosome 6

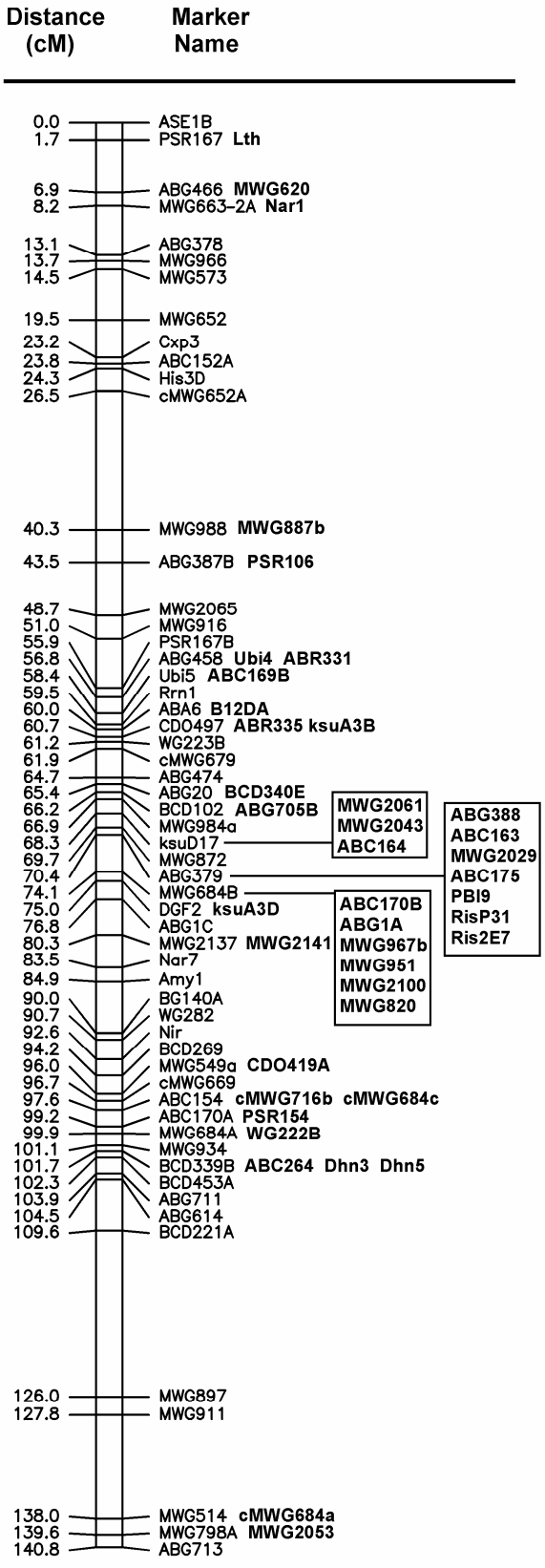
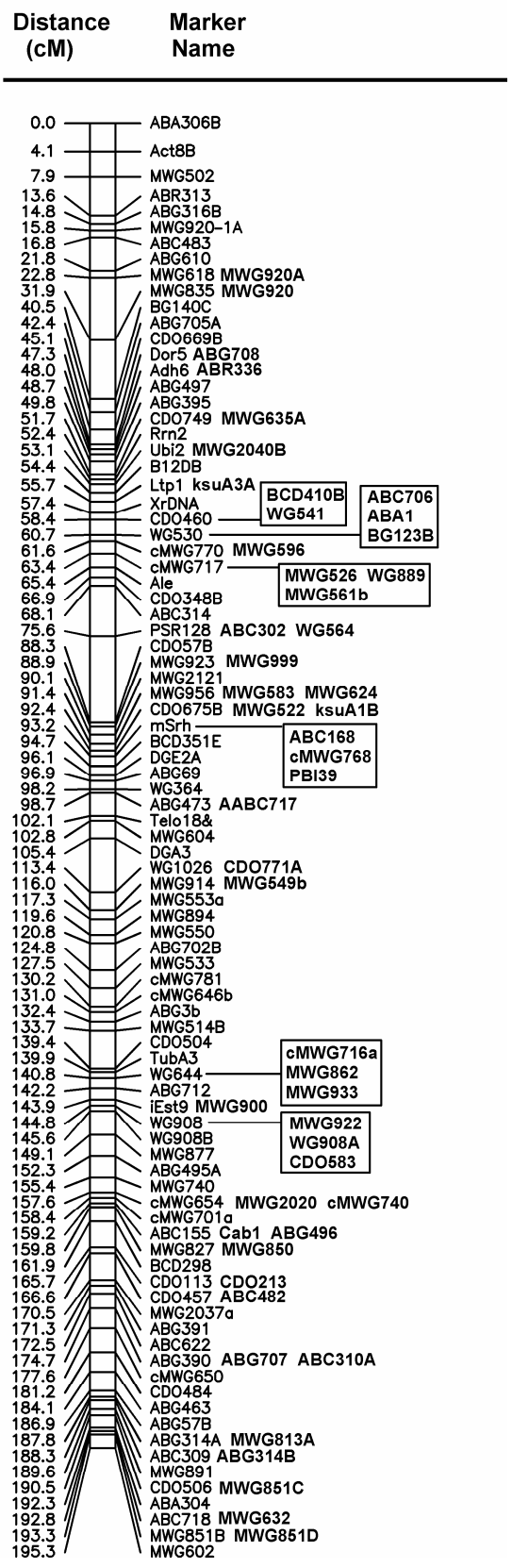


Fig. 1. (*continued*)

(G) Chromosome 7



Chromosome 1. Chromosome 1 contains the largest number of markers (Fig 1A). One morphological marker, naked caryopsis, *mn*, and two disease resistance genes, *dRpg1* and *dSb1*, were mapped on this chromosome. The integrated map shows a fairly uniform distribution of markers. However, clustering of markers occurs in the 70 – 80-cM region, and there is one gap of 15 cM in the 130 – 140-cM region and one of 10 cM in 60 – 70-cM region, whereas the individual maps contained 3 – 8 gaps.

Chromosome 2. With 27 markers shared by two populations and 8 markers common to three populations, the integrated map of chromosome 2 comprises 163 markers with a total map length of 157 cM (Fig. 1B). One morphological marker, *mhex-v*, confering six- or two-rowed spike, mapped to this chromosome. MWG636 was present in both the H/T and I/F populations, but in H/T it was located on the “long” arm near the centromere while in I/F it mapped at the distal end of the “short” arm. Therefore, with the probe MWG636, two different loci may be identified in these populations. To distinguish them, the names MWG636(HT) and MWG636(IF) were used to designate the different loci. The integrated map has only one gap larger than 10 cM, in the 95 – 107-cM region, while the most saturated individual map (S/M) showed two gaps (Table 4). Clustering of markers was observed in the 60 – 70-cM region.

Chromosome 3. For this chromosome 25 and 3 markers were common to two and three populations, respectively. The integrated map, comprising 133 markers and spanning 131 cM, represents the shortest of all chromosomes (Fig. 1C). The “pubescent leaf" gene *mPub* resides on chromosome 3. The recessive gene conferring resistance to barley yellow mosaic and barley mild mosaic virus, *dym4*, also mapped on this chromosome (Graner and Bauer 1993). Only one 10-cM gap remains in the integrated map and one cluster of markers occurs in the 40 – 50-cM region; in other regions the distribution is fairly uniform.

Chromosome 4. A total of 81 markers assigned to chromosome 4 in the four populations, were remapped on the integrated map (Fig. 1D). The powdery mildew resistance gene, *dMlg*, is in the centromeric region of the composite map. The only marker shared by all four populations, WG622, is on chromosome 4. The markers are quite evenly scattered over the chromosome except for one clustering region around 50 – 55-cM. No gap larger than 10 cM remains on the map, while the individual maps have 4 – 6 such gaps.

Chromosome 5. For this chromosome, a total of 20 markers that are non-unique to any of the four populations are available. The P/N population has only two non-unique markers, CDO99 and BCD98 (shared with S/M). Since these are tightly linked, both in P/N and S/M, there is effectively only a single "anchor point" to which to tie the P/N-specific markers. Since their orientation with respect to the "anchor" cannot be established unambiguously, the markers unique to P/N are not included in the composite map. The integrated map based on the data of the other three populations is shown in Fig. 1E. The genes *dMla6* (resistance to powdery mildew) and *dRun* (resistance to *Ustilago nuda)* were located on chromosome 5. Markers were quite uniformly distributed over the integrated map; a small cluster of markers appears around 74-cM region.

Chromosome 6. The individual map of chromosome 6 from the P/N population was very short, with only 36 map units and 9 markers. Maps from the other three populations contained more markers and were longer. The composite map still had three large gaps, one of 14 cM at 26 – 40 cM, one of 16 cM at 110 – 126 cM, and one of 10 cM in the 128 – 138 cM region (Fig. 1F). Clustering of markers was found in the 68 – 75 cM region.

Chromosome 7. Thirty markers were shared by two populations and five were common to three populations. The integrated map of chromosome 7 has 139 markers covering 195 map units, and is the longest map (Fig. 1G). The gene for short rachilla hairs, *mSrh*, resides on this chromosome. A single 10-cM gap (at 76 – 87 cM) remains on the composite map. There is no obvious clustering for this linkage group.

Comparison of maps

Comparison of the integrated map with the individual maps gives insight into the reliability of the integrated map. For illustrative convenience, only the common markers are shown on the maps of Figs. 2A-G. In constructing an integrated map, invariably some regions of the component maps will shrink, while other regions will stretch. This is because a weighted average (over component maps) of recombination frequencies is used for calculating the integrated map. This applies to the non-unique markers that represent the reference positions of an integrated map. Markers that are unique to a particular population can, of course, only be positioned on the basis of the information for

that single population. Therefore, the ordering of unique markers on a composite map is less reliable than the ordering of common markers, especially in regions where the component maps differ in length. Comparison of the integrated map with the individual maps reveals that the overall linear order of markers is in good agreement and that the integrated map is consistent with the component maps. No obvious reordering of markers was found. This is due to the relatively large number of non-unique markers.

Fig. 2. (A-G) Individual maps and their integrated map of seven barley chromosomes. Chromosomes are oriented with the short arm at the top. Only the common markers are presented in the figures. The recombination values were converted into map distance (cM) according to Kosambi (Kosambi, 1944). The small box on the right represents 10 cM distance. Lines between maps connect identical markers. "Gap" in some individual maps indicates very loose linkage. H/T, I/F, S/M, P/N and Integrated refer to populations of 'Harrington' x TR306, 'Igri' x 'Franka', 'Steptoe' x 'Morex', 'Proctor' x 'Nudinka' and to the integrated map, respectively.

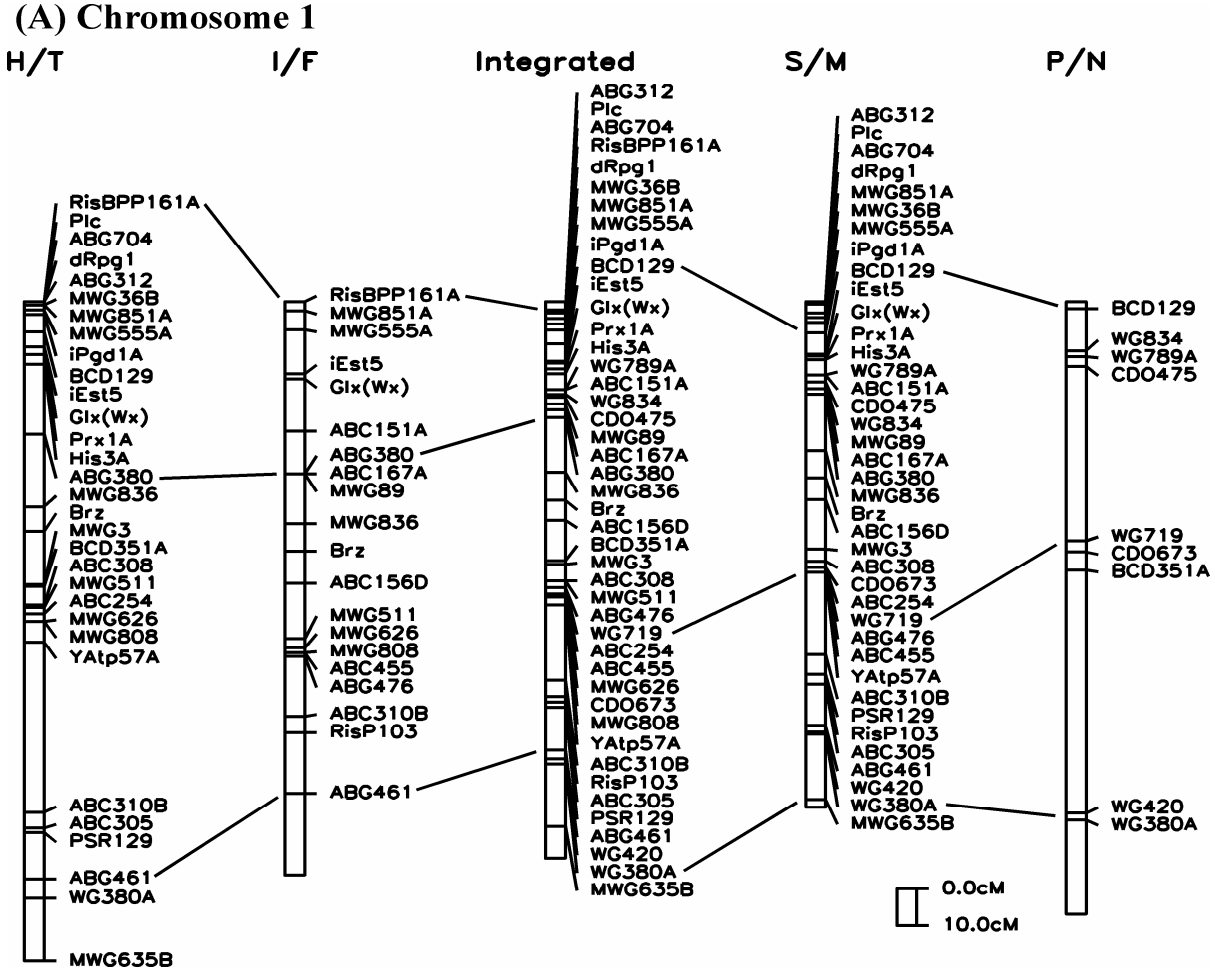


Fig. 2. (*continued*)

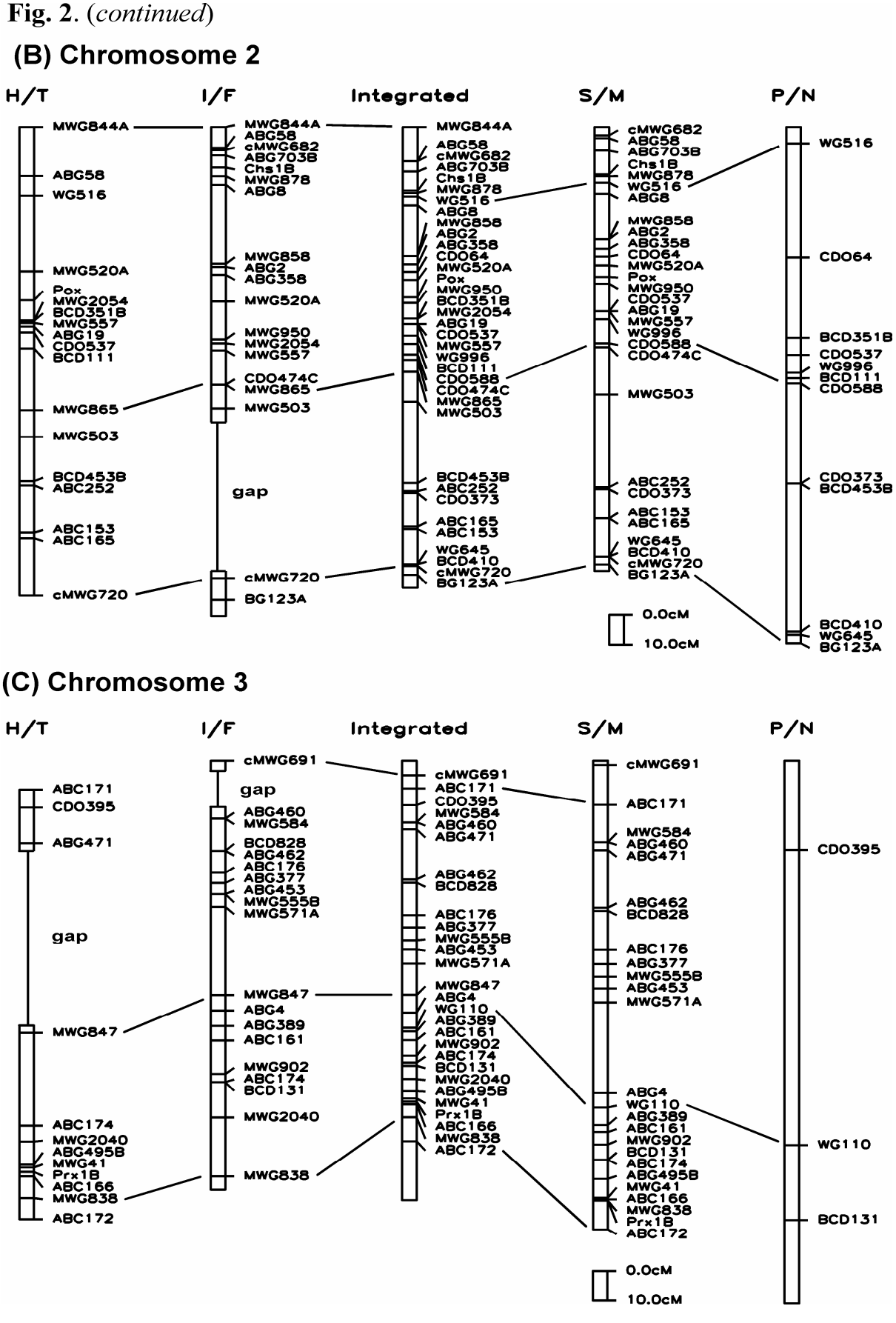
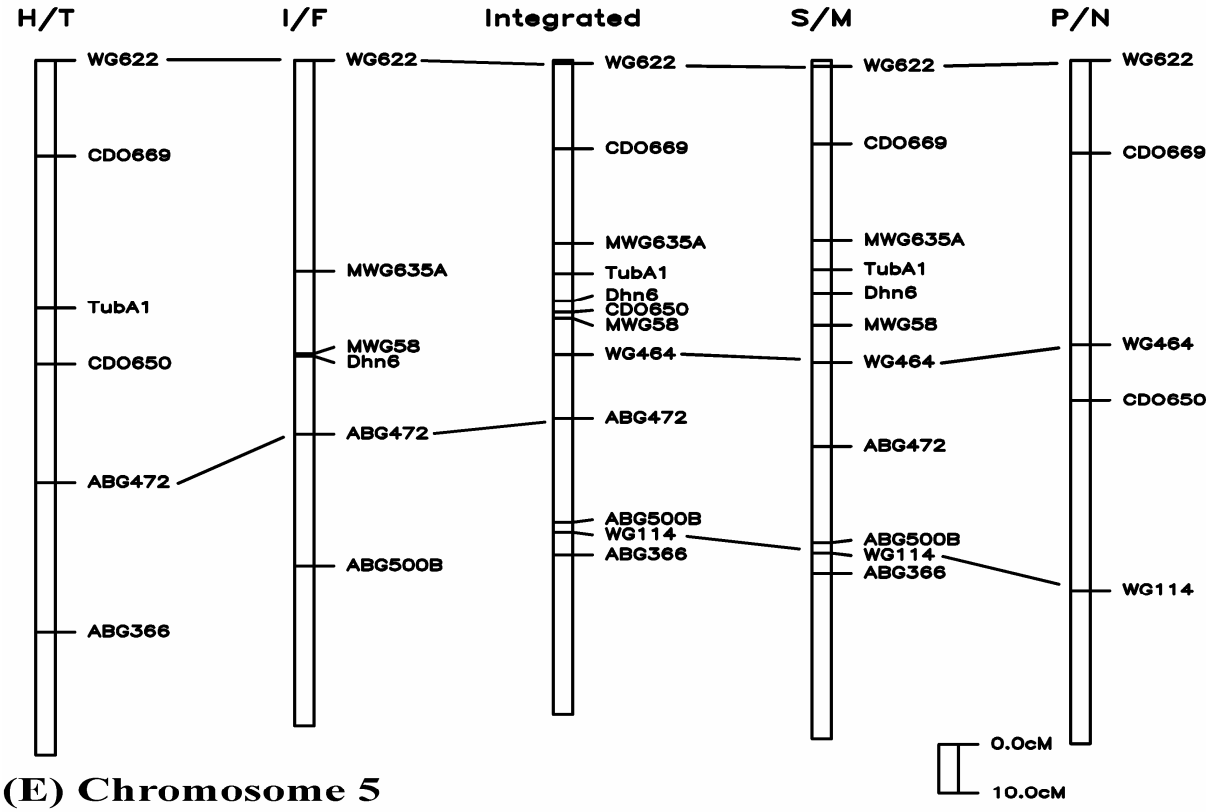


Fig. 2. (*continued*)

(D) Chromosome 4



(E) Chromosome 5

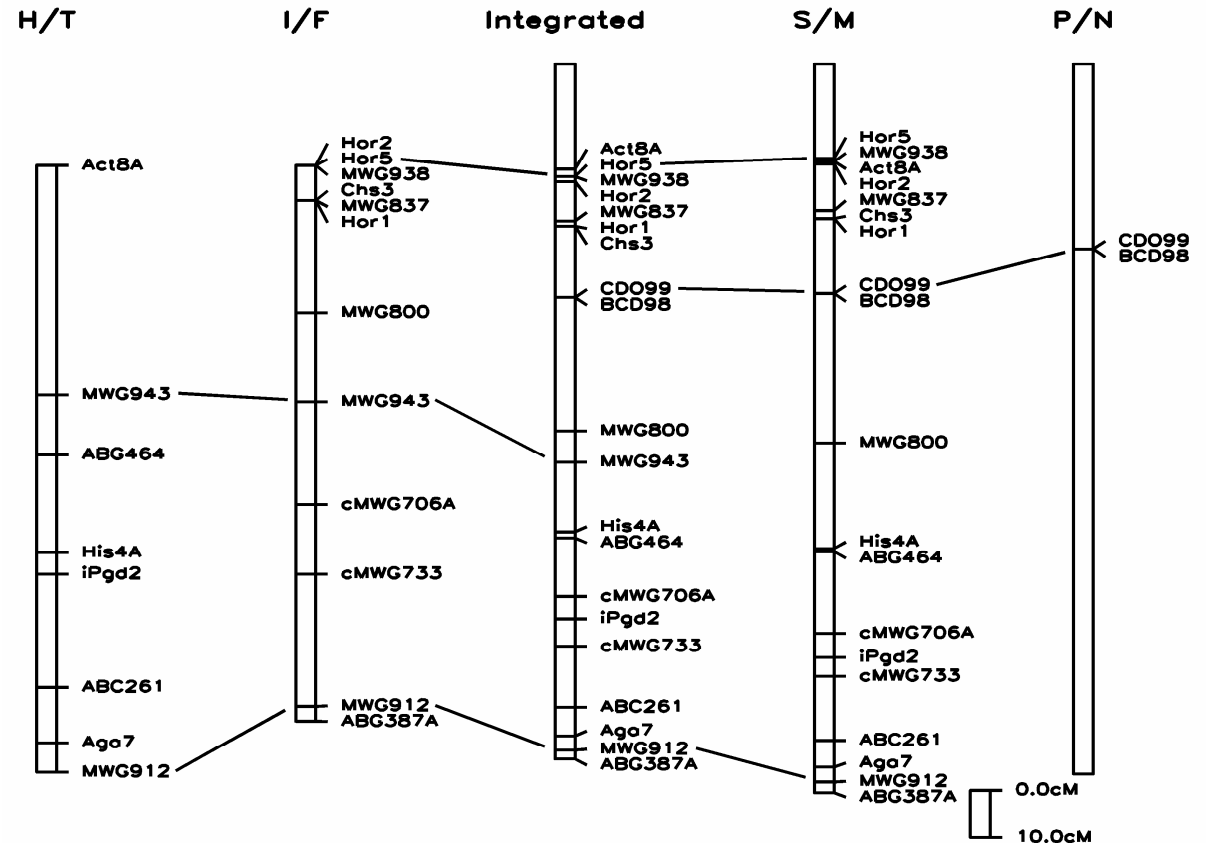
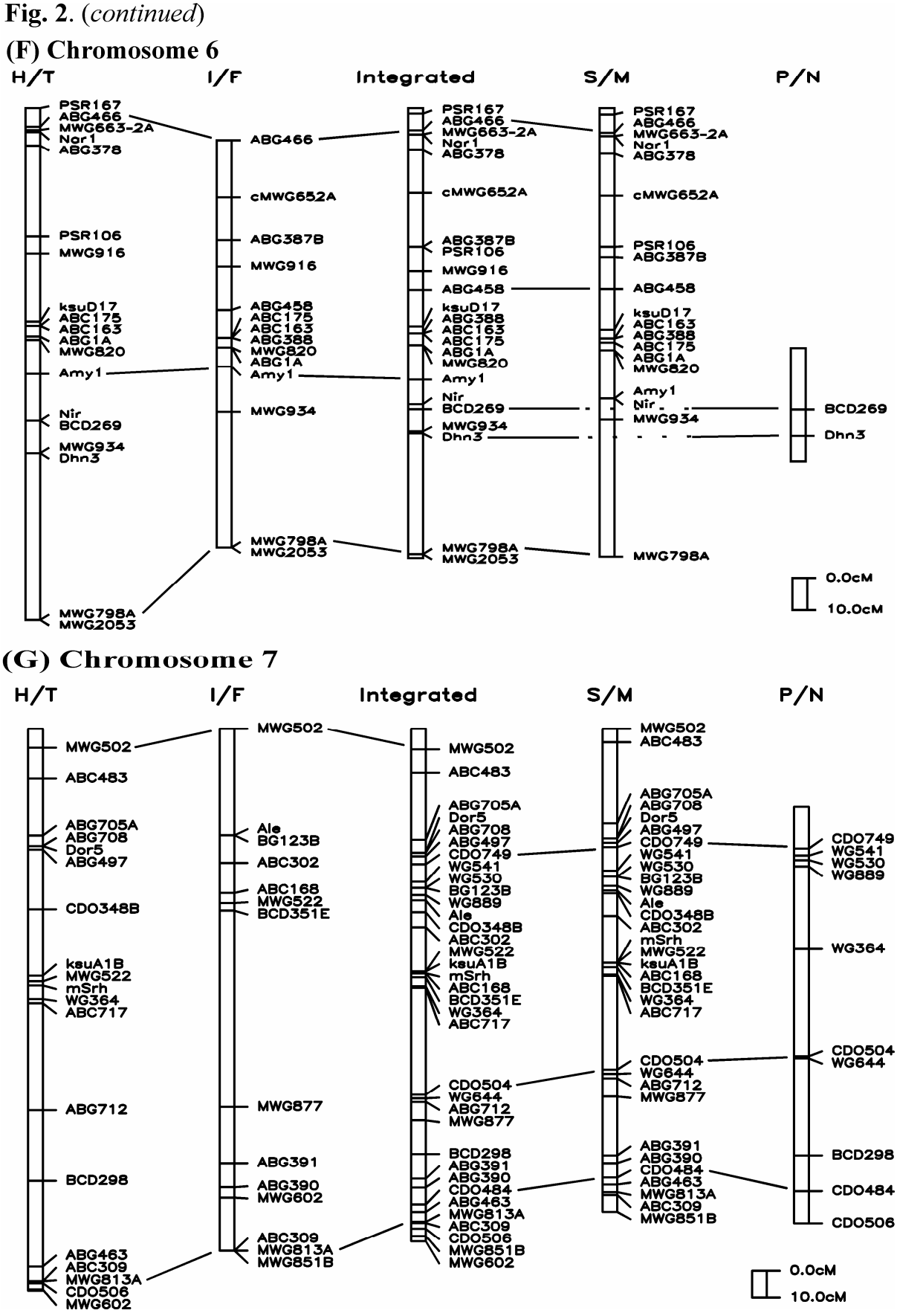


Fig. 2. (*continued*)



Reliability-accuracy of integrated map

Theoretically, any calculated map is only as good as the data allow. Integrated maps strongly depend on the number of common markers shared by the individual maps. The seven integrated maps presented in Figs. 1A-G were established on the basis of 197 common markers. With a total of 880 markers on the integrated map, 22% (197 of 880) of the markers were shared by at least two of the individual mapping populations. The proportions of common markers for each of the seven integrated maps, from chromosome 1 to 7 were 25%, 21%, 22%, 15%, 22%, 24%, and 25%, respectively.

The integration of maps from different population is only feasible if common markers are available. The backbone of the integrated map consisted of 197 markers that were common to at least two populations. The assumption was made that one probe would recognize the same loci in different populations. So, if a probe was used in different populations, it represented a common marker. Also some markers were assigned to one locus if the core name was identical and the separate map position was nearly identical. As all markers were mapped with high likelihood (high LOD-scores) and the X^2 value was low (not shown) these assumptions were valid and the maps were reliable.

It is also clear that the order of unique markers in regions of the genome containing a low density of common markers will be less accurate than in regions with a high density of common markers (Hauge et al. 1993). The distribution and density of the common markers (Figs. 2A – G) indicates that common markers from the four populations were relatively uniformly distributed on the maps. The establishment of the integrated map without much difficulty may be partially due to the large number of common markers and the conservation of gene order in the germplasm represented by the four populations.

Comparative studies of RFLP maps between cereals species have shown an obvious conservation of genome structure (Chao et al. 1989; Devos et al. 1992; Devos et al. 1993; Devos and Gale 1993; Van Deynze et al. 1995; Wang et al. 1992). More extensive analysis of genome organization (Moore et al 1995a) has revealed that the genomes of six major grass species can be aligned by dissecting the individual chromosomes into segments and rearranging these linkage blocks, suggesting there was a single ancestral cereal chromosome (Moore, et al. 1995b). In our study, comparison of four barley individual maps and their integrated maps

indicates that not only the gene orders are identical within the species but also that the distances between genes are quite similar. Apparently, the recombination frequencies in barley are not dependent upon the populations used.

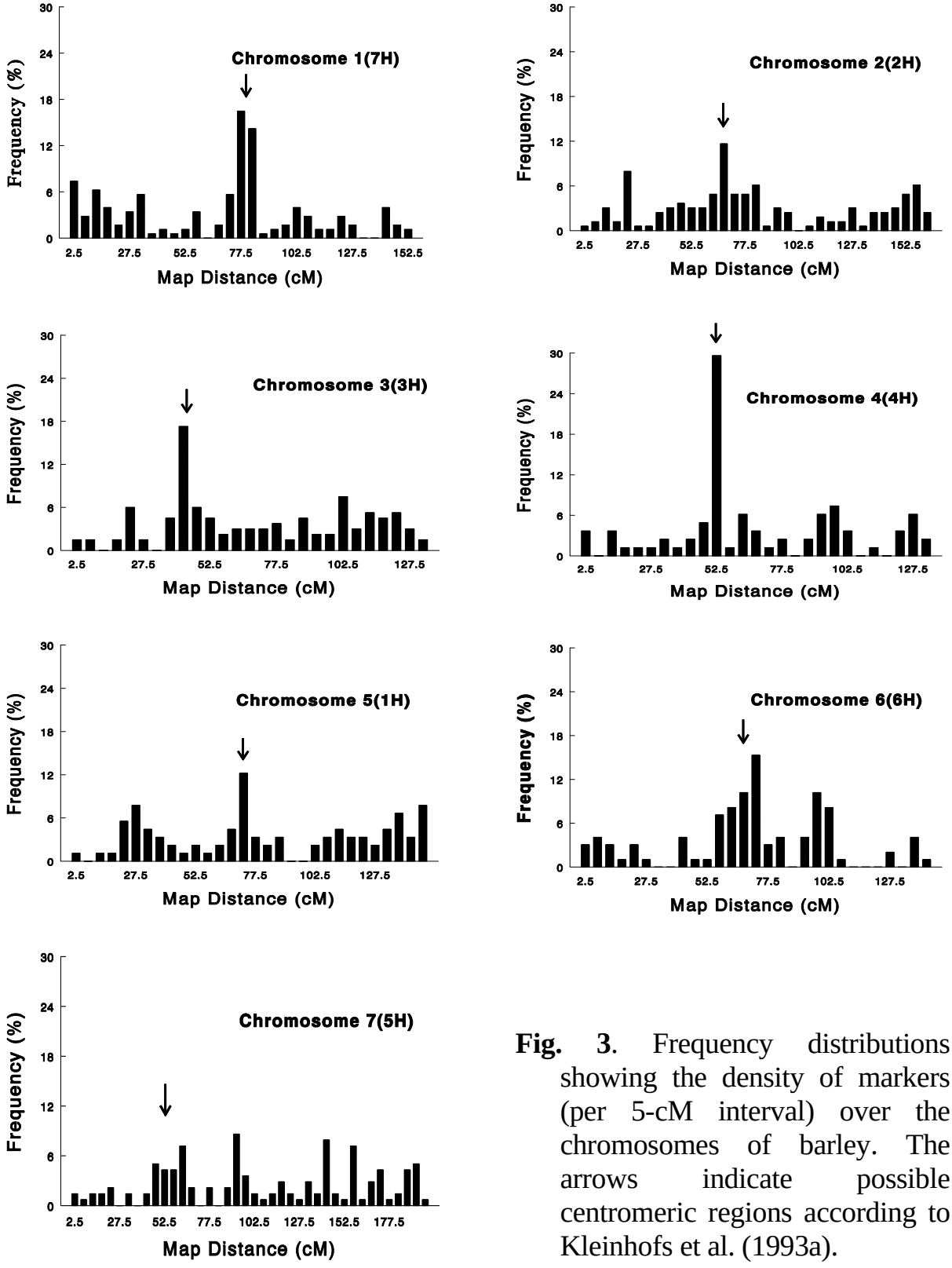


Fig. 3. Frequency distributions showing the density of markers (per 5-cM interval) over the chromosomes of barley. The arrows indicate possible centromeric regions according to Kleinhofs et al. (1993a).

Distribution of markers and centromere region

The study of tomato high density molecular linkage maps (Tanksley et al. 1992) showed that in some regions higher marker density could be identified in all chromosomes and a comparison with the pachytene karyotype of each chromosome suggested that the regions of high marker density corresponded to centromeric areas and, in some instances, to telomeric regions. In *Arabidopsis* there was no indication of clustering of markers in known centromeric regions (Koornneef et al. 1983; Hauge et al. 1993). In maps of wheat, a high degree of clustering of markers around the centromere was a notable feature (Chao et al. 1989; Devos et al. 1992; Hart 1994). Our barley integrated map analysis indicated a clear nonrandom distribution of markers on the maps. Kleinhofs et al. (1993a) identified centromeric regions on each chromosome of barley. An obvious clustering of markers coincided with these chromosome regions (Fig. 3). This result strongly supports the idea of centromeric suppression of recombination (Tanksley *et al*., 1992).

Use of integrated map

The conservative feature of the barley genome has provided us with a fairly reliable integrated map from individual maps that have been constructed in different genetic backgrounds. Compared to the individual maps, the density of markers on the integrated map is much higher and number of gaps (>10 cM) is much lower. Our barley integrated map can serve as a high density map like the tomato high density map (Tanksley et al. 1992), which was based on data from only 67 plants of a single cross.

The integrated map contains about 900 markers, and the various kinds and sources of molecular markers provide a good reference map for further research. New molecular markers and genes of economically importance from different genetic backgrounds can now easily be added to the integrated map by the selection of common markers from the integrated map. In our barley mapping project, the AFLP (amplified fragment length polymorphism) markers will be used to map genes involved in partial resistance to leaf rust on the barley genome. From the integrated map, several RFLP markers, evenly distributed over the genome have been selected as the bridge markers, which will be used for chromosome assignment and adding AFLP markers to the integrated map.

In order to have a chance of detecting all of the quantitative trait loci (QTL) affecting a character in a particular cross, it is necessary to have molecular markers evenly distributed throughout the genome (Tanksley et al. 1992). The integrated map presented in this paper allows selection of evenly spaced polymorphic markers for the detection and mapping of QTLs.

Some agronomic markers *(mn, mhex-v, mPub,* and *mSrh*) and disease resistance genes (*dMla6, dMlg, dym4, dRpg1*, and *dRun*) have been mapped on the integrated map. Compared with the individual maps, more molecular markers are now available around economically important genes on the integrated map, allowing marker-assisted selection in breeding programmes. Also, the composite high density molecular marker map will be useful for more precise mapping of economically important genes in barley, as well as in other cereals, and thus, possibly provide a basis for map-based cloning of those genes from rice small genome (Bennetzen and Freeling 1993; Kilian et al 1995).

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3

Development of AFLP markers in barley*

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Abstract: To investigate the application of amplified fragment length polymorphism (AFLP) markers in barley, 96 primer combinations were used to generate AFLP patterns with two barley lines, L94 and 'Vada'. With seven primer combinations, only a few intense bands were obtained, probably derived from repeated sequences. With the majority of the remaining 89 primer combinations, an average about 120 amplification products were generated, and the polymorphism rate between the two lines was generally over 18%. Based on the number of amplified products and the polymorphism rate, the 48 best primer combinations were selected and tested on 16 barley lines, again including L94 and 'Vada'. Using a subset of 24 primer combinations 2188 clearly visible bands within the range from 80 to 510 bp were generated, 55% of these showed same degree of polymorphism among the 16 lines. L94 versus 'Vada' showed the highest polymorphism rate (29%) and 'Proctor' versus 'Nudinka' yielded the lowest (12%). The polymorphism rates per primer combination showed littler dependence on the barley lines used. Hence, the most efficient and informative primer combinations identified for a given pair of lines turned out to be highly efficient when applied to others. Generally, more than 100 common markers (possibly locus specific) among populations or crosses were easily identified by comparing 48 AFLP profiles of the parent lines. The existence of such a large number of markers common to populations will facilitate the merging of molecular marker data and other genetic data into one integrated genetic map of barley.

Key words: *Hordeum vulgare*, AFLP markers, Genetic variation

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Introduction

The use of restriction fragment length polymorphisms (RFLPs) as DNA markers to construct genetic maps was first proposed by Botstein et al. (1980). Since then, various DNA markers have been developed and applied in many organisms. In plants, DNA markers have been used for genetic and genome studies, and more recently, to facilitate gene cloning and practical breeding. RFLP markers have been particularly suitable for genetic map construction and synteny studies among crop species. The comparison of RFLP maps of several cereal species has identified homologous chromosome segments in many different species (Bennetzen and Freeling 1993). This synteny should facilitate the isolation of genes from species with a large genome, such as wheat, by map-based cloning of the corresponding homologous segments from species with small genomes, like rice (Kilian et al. 1995). In barley (*Hordeum vulgare*), the first molecular marker linkage map (chromosome 6) was generated based on RFLPs by Kleinhofs et al. (1988). So far, more than 1000 molecular markers, predominantly RFLPs, have been mapped on the barley genome. Recently, the genetic linkage maps of four doubled haploid populations: 'Proctor' x 'Nudinka' (Heun et al. 1991), 'Igri' x 'Franka' (Graner et al. 1991), 'Steptoe' x 'Morex' (Kleinhofs et al. 1993) and 'Harrington' x TR306 (Kasha and Kleinhofs 1994) have been integrated into one composite map comprising 880 marker loci (Qi et al. 1996).

The RFLP technique requires a relatively large amount of DNA for optimal results from Southern hybridisations. Due to its large genome size ($1C = 5.1 \times 10^9$ bp; Bennett and Leitch 1995), and the relatively low variation within the barley species, RFLP analyses are labour-intensive and time-consuming. Consequently, other molecular markers, predominantly based on PCR methods, like RAPDs (Welsh and McClelland, 1990; Williams et al. 1990), have also been identified in barley (Kleinhofs et al. 1993). However, poor reproducibility and population specificity have limited the use of RAPDs for genetic studies. In addition, microsatellites or simple sequence repeats (SSR) have been investigated as DNA markers. Saghai-Maroof et al. (1994) identified 71 alleles among 207 accessions of wild and cultivated barley accessions by using only four mircosatellite primer pairs. Becker and Heun (1995) identified 32 alleles among 11 lines by using 15 primer pairs and mapped five microsatellite markers on three barley chromosomes.

With large numbers of alleles at one locus, microsatellite markers are very suitable as universal, locus-specific markers over populations. However, development of a sufficient number of microsatilite markers to cover the entire barley genome is still in its infancy. In a collaborative effort, several European research groups aim to develop another 200 microsatellite markers for the barley genome (Waugh 1995).

More recently, a novel DNA fingerprinting technique called AFLP has been developed (Zabeau and Vos 1993; Vos et al. 1995). The technology is based on the amplification of selected restriction fragments of a total genomic digest by PCR, and separation of labelled amplified products by denaturing polyacrylamide gel electrophoresis. A great advantage of the AFLP technique is that it allows simultaneous identification of a large number of amplification products. One hundred and eighteen AFLP markers have already been mapped on the barley genome by using the 'Proctor' x 'Nudinka' doubled haploid population which had previously been used for construction of an RFLP map (Heun et al. 1991; Becker et al. 1995). In a project to map the genes for partial resistance to barley leaf rust (*Puccina hordei*), we also chose AFLP markers as they allow the construction of a high-density genetic map in the most efficient way. In the present study, the variation in AFLP patterns within the barley species was investigated with a large number of primer combinations. Firstly, AFLP fingerprints of two barley lines were obtained using 96 primer combinations, and, secondly, 16 representative barley lines were used to generate AFLP profiles by using 48 primer combinations. These results may facilitate the wider use of AFLP for extended genetic studies in barley.

Materials and methods

Plant materials

Sixteen barley lines, 'Harrington', TR306, 'Steptoe', 'Morex', 'Igri', 'Franka', 'Proctor', 'Nudinka', 'Apex', 'Prisma', C92, C118, C123, L94, 'Vada' and 116-5, which represent a wide range of the genetic variation in barley (*H. vulgare*), were used in the present research. The first eight lines have been used to generate four doubled haploid populations for the construction of four individual RFLP maps

(Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993; Kasha and Kleinhofs 1994) and two integrated maps (Langridge et al. 1995, Qi et al. 1996). 'Apex' and 'Prisma' are Dutch two-rowed spring barley cultivars with medium and good malting quality, respectively. C92, C118 and C123 are partially resistant to *P. hordei* (Niks 1982) and are derived from the barley composite XXI (Suneson and Wiebe 1962), which was based on intercrossing of 6200 cultivars and lines. L94 is a line from an Ethiopian land race and is extremely susceptible to *P. hordei*. 'Vada' is a commercial cultivar from the Department of Plant Breeding, Wageningen Agricultural University, and has a high level of partial resistance. 116-5 is derived from 'Cebada Capa' (of North-African origin) x L94 and selected for a high level of partial resistance to *P. hordei*.

The AFLP protocol

DNA was extracted from leaf tissue, frozen in liquid nitrogen, of two-week-old seedlings according to the CTAB protocol published by Van der Beek et al. (1992).

The AFLP technique has been described by Zabeau and Vos (1993) and Vos et al. (1995). The procedure was performed essentially as described by Van Eck et al. (1995) for potato, with some minor modifications.

For template preparation, the selection of biotinylated DNA restriction fragments was omitted. After the restriction-ligation reaction, the restriction enzymes and ligase were denatured at 60 ^{o}C for 10 min. Subsequently, products were diluted ten-fold in $T_{0.1}E$ buffer and stored at 4 ^{o}C for pre-amplification.

To obtain good separation of amplified DNA fragments, buffer gradient electrophoresis was conducted with 1 X TBE (100 mM Tris, 100 mM Boric acid, 2 mM EDTA, pH 8.0) in the cathode buffer (-) and 1 X TBE plus 0.5 M sodium acetate in the anode buffer (+).

Adapters, *Mse*I site primers and *Eco*RI site primers used are listed in Table 1.

Data evaluation and nomenclature

The AFLP amplification products were designated according to the restriction enzymes and the primer combination used, and their size estimated with reference to the SequaMark 10 base ladder (Research Genetics, Huntsville, ala.).

Table 1. Lists of primers and adapters

Primers/adapters		Sequences[a]
*Mse*I adapter		5’-GACGATGAGTCCTGAG-3’ 3’- TACTCAGGACTC AT-5’
M00 (universal primer)		GATGAGTCCTGAG TAA
*Mse*I +1 primer	M02	M00+ C
*Mse*I +3 primers	M47	M00+ CAA
	M48	M00+ CAC
	M49	M00+ CAG
	M50	M00+ CAT
	M51	M00+ CCA
	M54	M00+ CCT
	M55	M00+ CGA
	M58	M00+ CGT
	M59	M00+ CTA
	M60	M00+ CTC
	M61	M00+ CTG
	M62	M00+ CTT
*Eco*RI adapter		5’-CTCGTAGACTGCGTACC-3’ 3’-CTGACGCATGG TTAA-5’
E00 (universal primer)		GACTGCGTACC AATTC
*Eco*RI +1 primer	E01	E00+ A
*Eco*RI +3 primers	E32	E00+ AAC
	E33	E00+ AAG
	E35	E00+ ACA
	E38	E00+ ACT
	E39	E00+ AGA
	E42	E00+ AGT
	E44	E00+ ATC
	E45	E00+ ATG

[a] DNA sequences are always given in the 5’ to 3’ orientation unless indicated otherwise

Results and discussion

AFLP pattern of two barley lines with 96 primer combinations

In the present study, eight *Eco*RI primers and twelve *Mse*I primers, each with three selective bases, were used to generate AFLP fingerprints for two barley lines, L94 and 'Vada'. With the majority of the 96 primer combinations, about one hundred fragments were obtained from each barley line. However, several primer combinations produced rather complex profiles which comprised up to 150 bands (Table 2). With seven primer combinations, namely E32M59, E33M49, E35M51, E38M49, E39M62, E42M49 and E44M51, the majority of the labelled primer was incorporated into a single fragment and other fragments appeared as very faint bands (e.g., E35M51 in Fig. 1). Therefore these seven primer combinations are not useful for genetic studies in barley. The appearance of single intense bands is probably due to a high copy number of one particular DNA restriction fragment in the template (Vos et al. 1995). The large genome size of barley (1C = 5.1 x 10^9 bp; Bennett and Leitch 1995) could well harbour a high proportion of repetitive sequences. Indeed, relatively intense bands were detected by using other primer combinations as well, but they did not obscure other fragments.

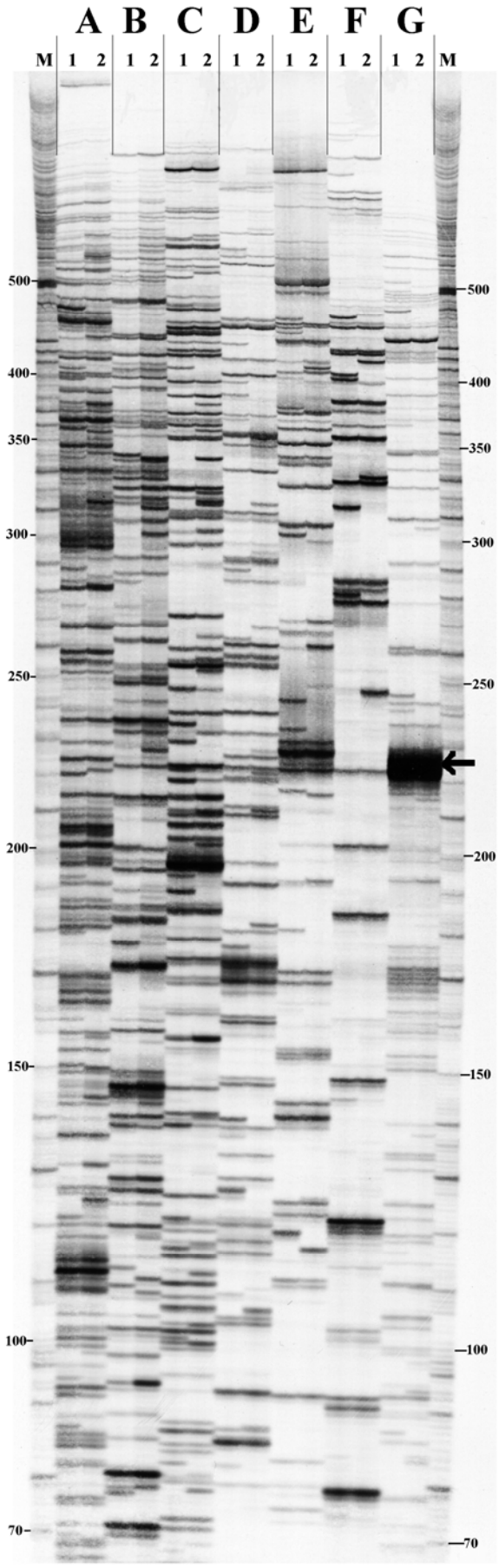
The total number of bands generated by the different primer combinations revealed a large range of variation, from about 50 bands for E42M58 to 180 for E33M50 (Fig. 1 and Table 2). The range in number of bands showed more variation with the 12 *Mse*I primers (67 to 142 bands) than with the 8 *Eco*RI primers (100 to 130 bands). With M58 on average about 70 visible bands were generated, whereas about 150 bands were produced with M47 and with M50. The three selective nucleotides of primer M47 and M50 are CAA and CAT, respectively (Table 1). Most plant DNAs are AT-rich and if the genome size is large, as in barley, it is better to use AT-poor primers with which fewer bands will be amplified. The 20 primers (12 *Mse*I primers and 8 *Eco*RI primers) used in the present research were in fact AT-poor. As more variation in AFLP patterns was observed with *Mse*I primers than with *Eco*RI primers, the selection of the most informative *Mse*I primers is more critical.

The polymorphism rates and total number of bands with the other 89 primer combinations were evaluated per primer combination (Table 2). The most useful primer combinations have a high polymorphism rate and generate a reasonable number of total bands, that are clearly visible. Based on our results, 48 primer combinations were used to generate AFLP profiles for 16 representative barley lines.

Table 2 Evaluation of 96 primer combinations based on two barley lines (L94 and 'Vada')

Polymorphism rates	Number of bands <90	90 -120	120 - 150	>=150
	Fair	**Fair**	**Fair**	**Poor**
<18%	E45M58	E32M49, E32M54 E33M51, E35M60 E38M62, E39M51 E42M59, E45M60	E33M62, E38M50 E44M54, E44M60 E44M62, E45M48 E45M50	E32M47, E44M47 E44M50, E45M62
	Good	**Good**	**Fair**	**Poor**
18%-23%	E32M58, E38M58	E32M51, E32M60 E33M59, E38M47 E38M48, E38M51 E38M60, E39M47 E39M49, E39M59 E39M60, E42M62 E44M61	E32M50, E35M62 E39M50, E44M48 E44M49, E45M51 E45M54, E45M59	E33M47, E33M48 E33M50, E35M47
	Good	**Good**	**Good**	**Fair**
23%-28%	E32M55, E39M55 E42M60, E42M61 E44M58	E32M62, E35M49 E38M59, E39M48 E39M54, E42M47 E42M50, E45M49 E45M61	E32M48, E33M60 E35M54, E35M59 E44M55, E44M59	E35M50,E45M47
	Excellent	**Excellent**	**Good**	**Fair**
>28%	E33M58, E35M55 E35M58, E38M55 E38M61, E39M58 E42M51, E42M54 E42M55, E42M58	E32M61, E33M55 E33M61, E35M48 E35M61, E38M54 E39M61, E42M48 E45M55	E33M54	--

Evaluations are based on polymorphism rates and number of bands. E32M59, E33M49, E35M51, E38M49, E39M62, E42M49, E44M51 were not included due to their excessively amplified single fragment.

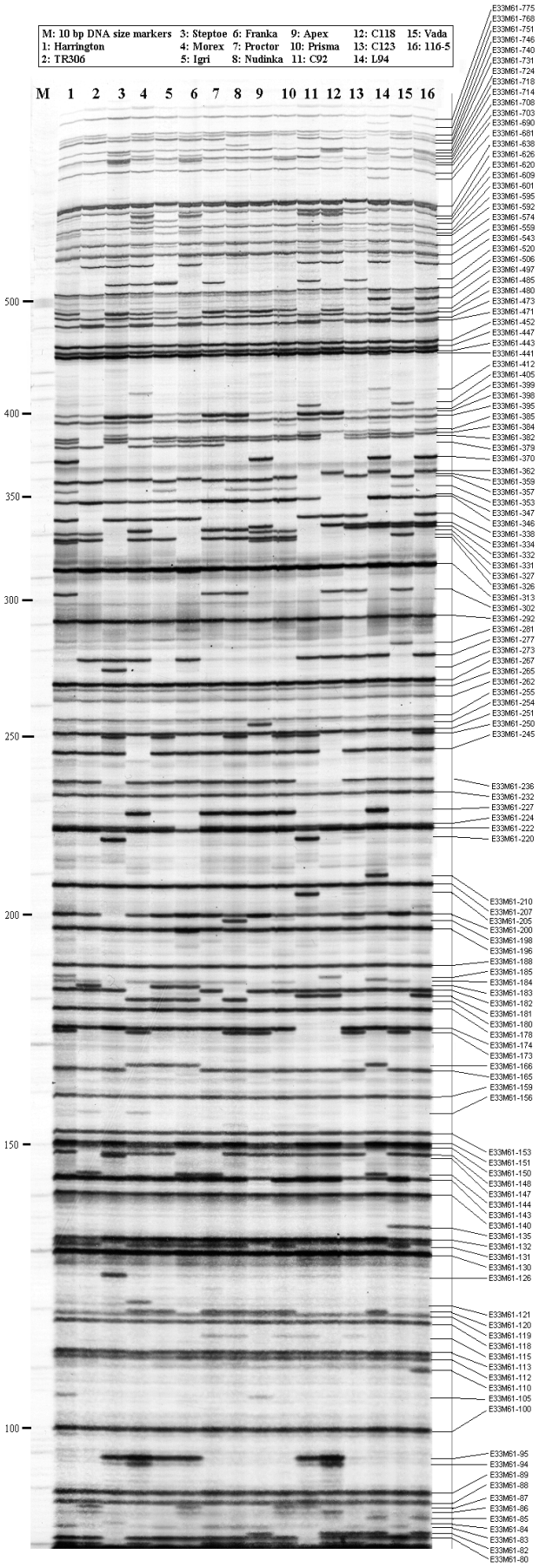


AFLP profiles of sixteen selected barley lines

The AFLP profiles of 16 barley lines were analysed to estimate the sizes of clearly visible bands. Band names were assigned and indicated on images, which are available in GrainGenes on Internet (http://grain.jouy.inra.fr/ggpages/).

The sizes of fragments generated with 96 primer combinations ranged from about 70 bp to 1 kb (Figs. 1, 2), but most fragments were smaller than 500 bp. Using buffer gradient electrophoresis the larger fragments up to 500 bp were well separated, while only limited information from the smaller bands was lost. Fragments larger than 500 bp were not well separated and were beyond the size marker range used. Consequently, their size were estimated by extrapolation and hence are not very

Fig. 1. Variation in AFLP patterns between two barley lines. AFLP patterns obtained with the seven primer combinations E33M47, E33M48, E33M59, E35M61, E35M55, E35M58 and E35M51 (A – G, respectively). M is a marker lane with 10 bp DNA size markers, 1 and 2 represent L94 and 'Vada'. A – F show AFLP patterns ranging from complex to simple. G is a primer combination showing one very intense fragment (indicated by the *arrow*) and many faint bands.



accurate. In addition, larger fragments normally gave weaker signals and were more dependent on the quality of templates. So only bands of 80 - 510 bp were taken into account as summarised in Table 3. Twenty-four primer combinations were selected to study polymorphism rates among 16 barley lines. These primer combinations were recommended by KeyGene, Wageningen or were chosen on the basis of our initial survey (see table 2).

Figure 2 is an example of the AFLP profiles generated, obtained by using the primer combination E33M61: within the size range of 80 to 510 bp, 106 AFLP bands were observed among the 16 barley lines; 35 were present in all 16 lines and the presence of other 71 bands varied over the 16 lines giving 67% polymorphism rate (Table 3). The large number of bands and the high polymorphism rate among the 16

Fig. 2 AFLP fingerprints of 16 barley lines generated with E33M61. 1 to 16 indicate the barley lines listed in the box at the top. M is a marker lane with 10 bp DNA size markers. All clearly visible bands are connected by lines to their corresponding designations on the right of the panel. Band sizes over 500 bp were estimated by extrapolation.

barley lines indicated that AFLP is an extremely efficient technique for marker generation in barley. A parallel study of genetic relationships in barley showed that with a single primer combination sufficient DNA markers could be screened to unambiguously discriminate between 29 related barley lines (not shown). In the present study, data from 24 primer combinations were evaluated to illustrate the usefulness of AFLP markers in barley.

Variation in polymorphism rates

Pair-wise comparisons of six parent pairs, that have been used for generating RFLP maps, showed quite different polymorphism rates (Table 3). The average polymorphism rate per set of two barley lines over 24 primer combinations ranged from only 12.2% between 'Proctor' and 'Nudinka' to 29% between L94 and 'Vada'. Obviously, the genetic distance between L94, a line from an Ethiopian land race, and the European cultivar 'Vada' was larger. 'Proctor' and 'Nudinka' are closer related because both are two-rowed spring barley cultivars bred in England and Germany, respectively. Consequently, these high polymorphism rates between L94 and 'Vada' should facilitate the construction of a high-density AFLP map. By using a large number of AFLP markers, it should be possible to fill in some of the gaps in the integrated RFLP map (Qi et al. 1996).

The average polymorphism rate per primer combination over 6 parent pairs was 20% with a range from 13% for E38M51 to 28% for E33M61. Between L94 and 'Vada', a 41% polymorphism rate was observed with primer combination E42M48 but only 18% with E45M58. The ranking of primer combinations based on polymorphism rates was only weakly dependent upon the barley lines used for comparison. Thus, the most efficient and informative primer combinations identified for a given set of barley lines are likely to be most efficient when applied to other lines also.

A very similar AFLP polymorphism rate (11.3%) was observed between 'Proctor' and 'Nudinka' by Becker et al. (1995) based on 16 different primer combinations. In contrast, RFLP markers, both from genomic clones and cDNA clones showed higher polymorphism rates (27.1% and 15.3%, respectively; Heun et al. 1991), that were also observed between 'Igri' and 'Franka' (28%; Graner et al. 1991). In addition, 35.3% of RFLP clones showed polymorphisms between 'Morex' and 'Steptoe' when *Eco*RI was used as the restriction enzyme. Our

Table 3. AFLP Polymorphism rates among 16 and between six pairs of barley lines

Primer	16 Lines[a]		H/T		S/M		I/F		P/N		A/P		L/V		6 Crosses	
Combinations	TOT[b]	PR(%)[c]	TOT	PR(%)	TOT	PR(%)	TOT	PR(%)	TOT	PR(%)	TOT	PR(%)	TOT	PR(%)	TOT	PR(%)
E32M55	75	62.7	49	22.4	54	33.3	46	13.0	47	10.6	48	20.8	51	23.5	295	21.0
E32M61	79	45.6	58	12.1	65	20.0	65	20.0	59	5.1	59	8.5	67	32.8	373	16.9
E33M54	113	47.8	88	13.6	86	18.6	86	16.3	86	8.1	88	10.2	97	30.9	531	16.6
E33M55	89	49.4	63	4.8	69	17.4	67	14.9	63	12.7	64	17.2	71	21.1	397	14.9
E33M58	65	58.5	40	7.5	49	32.7	44	20.5	45	17.8	46	19.6	50	38.0	274	23.4
E33M61	106	67.0	72	29.2	76	38.2	67	22.4	68	13.2	71	22.5	78	38.5	432	27.8
E35M48	99	56.6	68	16.2	76	30.3	71	22.5	65	7.7	71	14.1	76	34.2	427	21.3
E35M54	84	42.9	68	8.8	68	16.2	71	14.1	66	4.5	71	16.9	72	22.2	416	13.9
E35M55	69	58.0	47	12.8	46	15.2	52	21.2	47	14.9	52	25.0	53	28.3	297	19.9
E35M61	92	58.7	68	17.6	73	23.3	68	20.6	70	11.4	74	24.3	72	29.2	425	21.2
E38M50	118	59.3	82	18.3	91	33.0	86	23.3	83	19.3	83	21.7	92	30.4	517	24.6
E38M51	95	47.4	72	6.9	76	18.4	70	10.0	70	8.6	71	9.9	75	25.3	434	13.4
E38M54	88	56.8	62	12.9	67	16.4	68	23.5	61	8.2	66	21.2	70	30.0	394	19.0
E38M55	64	56.3	48	16.7	49	24.5	44	18.2	47	12.8	50	20.0	49	32.7	287	20.9
E39M55	75	53.3	56	12.5	60	31.7	63	22.2	52	5.8	59	16.9	55	21.8	345	18.8
E39M61	98	59.2	61	8.2	71	23.9	61	16.4	64	20.3	66	22.7	70	32.9	393	21.1
E42M48	93	65.6	57	12.3	62	33.9	60	23.3	59	20.3	60	18.3	71	40.8	369	25.5
E42M51	91	52.7	62	12.9	60	20.0	64	15.6	64	15.6	60	13.3	68	29.4	378	18.0
E44M49	109	51.4	75	6.7	81	22.2	81	21.0	80	12.5	81	12.3	89	23.6	487	16.6
E44M54	118	46.6	94	8.5	100	26.0	91	9.9	92	7.6	94	14.9	98	21.4	569	14.9
E44M58	70	44.3	56	3.6	57	19.3	53	11.3	58	6.9	58	15.5	63	28.6	345	14.5
E45M49	122	59.0	88	13.6	97	33.0	86	19.8	86	15.1	83	16.9	96	29.2	536	21.6
E45M55	101	60.4	65	18.5	76	27.6	73	19.2	69	13.0	71	21.1	81	34.6	435	22.8
E45M58	75	64.0	54	20.4	49	24.5	46	23.9	45	20.0	52	28.8	51	17.6	297	22.6
Total	**2188**	**55.2**	**553**	**13.2**	**1658**	**25.0**	**1583**	**18.5**	**1546**	**12.2**	**1598**	**18.0**	**1715**	**29.0**	**9653**	**19.5**

[a] "16 Lines" data from 16 barley lines; HT, SM, IF, PN, AP and LV represent the parent pairs of 'Harrington' and TR306, 'Steptoe' and 'Morex', 'Igri' and 'Franka', 'Proctor' and 'Nudinka', 'Apex' and 'Prisma', and L94 and 'Vada', respectively; "6 Crosses" lists the accumulated data from these six parent pairs.

[b] Total number of bands

[c] Polymorphism rates

datasets appear to suggest that AFLP markers also show lower polymorphism rates in barley. However, data are still too limited to allow one to generalise this conclusion to other species or populations.

Common markers among mapping populations

Due to the frequent exchange of RFLP probes among barley researchers, many common RFLP probes have been used as locus-specific markers and have been mapped on independent mapping populations. These bridge markers enable the construction of integrated maps and comparison of independent maps (Langridge et al. 1995; Qi et al. 1996). The AFLP profiles generated with 16 barley lines in this paper could serve as standard references. Bands with only 1 bp difference migrate differently in gels and can be distinguished (Vos et al. 1995). Fragments with 16 (6 and 4 bases for *Eco*RI and *Mse*I sites, respectively, and 6 selective bp) identical base pairs and the same mobility in gels are most probably highly homologous and hence locus specific.

The present study indicates that common (assuming locus specificity) markers among populations can clearly be identified and may be used as bridge markers for the comparison of maps or assignment of linkage groups to chromosomes. By comparing the parent pair 'Steptoe' and 'Morex' with L94 and 'Vada', about 150 shared polymorphic AFLP markers were identified based on the 24 primer combinations listed in Table 3. Further survey indicated that 65 and 21 AFLP markers were common to three ('Steptoe' x 'Morex', 'Igri' x 'Franka' and L94 x 'Vada') and four parent pairs ('Steptoe' x 'Morex', 'Harrington' x Tr306, 'Igri' x 'Franka' and L94 x 'Vada') respectively. Three clear AFLP markers, E38M55-618, E39M55-162 and E42M51-94, were common to five parent pairs ('Steptoe' x 'Morex', 'Harrington' x Tr306, 'Igri' x 'Franka', 'Proctor' x 'Nudinka' and L94 x 'Vada'). These common markers are thus extremely useful for bridging maps. If the locus specificity of common markers is confirmed in future, AFLP markers will greatly contribute to merging marker and other genetic data into one integrated genetic map of barley.

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4

Use of locus-specific AFLP markers to construct a high-density molecular map in barley*

Xiaoquan Qi, Piet Stam and Pim Lindhout

Abstract: By using 25 primer combinations, 563 AFLP markers segregating in a recombinant inbred population (103 lines, F_9) derived from L94 x 'Vada' were generated. The 38 AFLP markers in common to the existing AFLP/RFLP combined 'Proctor' x 'Nudinka' map, one STS marker, and four phenotypic markers with known map positions, were used to assign present AFLP linkage groups to barley chromosomes. The constructed high-density molecular map contains 561 AFLP markers, three morphological markers, one disease resistance gene and one STS marker, and covers a 1062-cM genetic distance, corresponding to an average of one marker per 1.9 cM. However, extremely uneven distributions of AFLP markers and strong clustering of markers around the centromere were identified in the present AFLP map. Around the centromeric region, 289 markers cover a genetic distance of 155 cM, corresponding to one marker per 0.5 cM; on the distal parts, 906 cM were covered by 277 markers, corresponding to one marker per 3.3 cM. Three gaps larger than 20 cM still exist on chromosomes 1, 3 and 5. A skeletal map with a uniform distribution of markers can be extracted from the high-density map, and can be applied to detect and map loci underlying quantitative traits. However, the application of this map is restricted to barley species since hardly any marker in common to a closely related *Triticum* species could be identified.

Key words: *Hordeum vulgare*, AFLP markers, Genetic linkage map, Recombinant inbred lines, Locus specificity

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Introduction

In barley (*Hordeum vulgare* L.), restriction fragment length polymorphism (RFLP) has been extensively used for the construction of genetic linkage maps (Kleinhofs et al. 1988; Shin et al. 1990; Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993b; Kasha and Kleinhofs 1994). These have enabled the mapping of important agronomic qualitative and quantitative traits, like the *ym4* virus resistance gene (Graner and Bauer 1993), the *denso* dwarfing genes (Laurie et al. 1993), the liguleless gene (Pratchett and Laurie 1994), a photoperiod-response gene (Laurie et al. 1994), and the quantitative loci for yield, malting quality and disease resistance (Hayes and Iyambo 1994; Han et al. 1995; Kjær et al. 1995; Thomas et al. 1995, 1996). A limitation of the application of RFLPs is the labour- and time-consuming technology of Southern hybridisation that has to be repeated for each RFLP marker. Moreover, due to a large genome size (1C = 5.1 x 10^9 bp) (Bennett and Leitch 1995) and relatively lower variation within the barley species, the progress in map construction by RFLP is slow and expensive. Recently, AFLP markers have been developed and their power as genetic markers has been demonstrated (Zabeau and Vos 1993; Vos et al. 1995). A great advantage of the AFLP technique is the simultaneous identification of a large number of marker loci. Moreover, fragments amplified with the same primer combinations and with the same mobility in gels are most likely homologous and hence locus specific (Qi and Lindhout 1997). Becker et al. (1995) has added 116 AFLP markers to the already existing 'Proctor' x 'Nudinka' RFLP map (Heun et al. 1991). Recently, Waugh et al. (1997) increased the marker density in three barley genetic maps by adding 234, 194 and 376 AFLP markers, respectively.

In a project for mapping QTLs for partial resistance to barley leaf rust, we applied the AFLP technique to generate molecular markers. To assign AFLP linkage groups to barley chromosomes, AFLP markers common to two mapping populations, 'Nudinka' x 'Proctor' and L94 x 'Vada', were identified and subsequently a high-density molecular map was constructed using 103 RILs (F_9) derived from the cross L94 x 'Vada'.

Materials and methods

Plant materials

A population of 103 F_9 recombinant inbred lines (RILs) was obtained from a cross of L94 x 'Vada' by single-seed descent and used as a mapping population. L94 is a line from an Ethiopian land race, with black and covered seeds; it is extremely susceptible to leaf rust (*Puccinia hordei*). 'Vada' is an obsolete commercial cultivar, with white and naked seeds, bred by the Department of Plant Breeding, Wageningen Agricultural University, and has a high level of partial resistance to *P. hordei* (Niks 1982).

The AFLP protocol

The same AFLP procedure as described by Qi and Lindhout (1997) was used in the present study. Restriction enzymes, adapters and primers were as described in Becker et al. (1995) and Qi and Lindhout (1997). In total, the following 25 primer combinations were employed: E37M32, E37M33, E37M38, E40M32, E40M38, E40M40, E41M32, E41M40, E42M32, E42M40, E32M61, E33M54, E33M55, E33M58, E33M61, E35M48, E35M54, E35M55, E35M61, E38M54, E38M55, E39M61, E42M48, E42M51, and E45M55. The first ten primer combinations have been used before to generate AFLP markers for the construction of the 'Proctor' x 'Nudinka' map (Becker et al. 1995), and the other 15 primer combinations were the most informative ones as indicated in the previous study of Qi and Lindhout (1997).

Data analysis and map construction

Segregating markers in the mapping population were designated according to the AFLP profiles of the parent lines (see GrainGenes WWW page, map data; Qi and Lindhout 1997). Clearly visible markers were scored as dominant. Three morphological markers m*n* (naked seeds), m*B* (black seeds) and m*Pau* (purple auricle), and one disease resistance gene d*ml-o* (resistance to *Erysiphe graminis*), were also scored as qualitative traits. The primer pair KV1 and KV9 derived from the sequence of the *Hor2* gene was used as an STS marker for the *Hor2* locus (for sequences, see Kanazin et al. 1993). The amplified products were digested by *Hae*III to reveal polymorphism. Missing data for any marker were very limited in the present study (<2 %).

A software package, JoinMap 2.0 (Stam 1993; Stam and Van Ooijen 1996) was used for linkage grouping and map construction. Linkage groups were assigned to the corresponding barley chromosomes by using the locus-specific common AFLP markers, that had already been mapped on the 'Proctor' x 'Nudinka' map (Becker et al. 1995), morphological markers, and the *Hor2* gene. Kosambi's mapping function was applied for map-distance calculation (Kosambi 1944).

Results

Data scoring

By using 25 primer combinations, 563 easily scored AFLP markers were identified, corresponding to an average of 23 markers per primer combination, ranging from 11 (E40M40) to 33 (E33M61). The number of usable segregating markers was slightly less than observed in a previous study (Qi and Lindhout 1997). This was due to poor separation of amplification products of nearly identical size.

Among 568 markers, 286 were L94-specific and 281 were 'Vada'-specific; one STS marker showed co-dominance. The majority of the markers (92%) showed a 1 : 1 segregation ratio for the two parental alleles ($P \leq 0.05$), as was expected for the F_9 recombinant inbred population. Among the 48 markers with distorted segregation, only three were skewed towards L94 alleles and 45 towards 'Vada' alleles; the latter all mapped on chromosome 6 (Fig. 1).

For mapping, groups of markers with identical segregation were regarded as a single marker; the marker with the fewest missing values was chosen as the representative one for this group. In total, 433 markers, of which 61 co-segregated with at least one other marker and 372 of which showed unique segregation, were applied for the construction of linkage groups (Fig. 2).

Map construction

By using ten primer combinations, 38 AFLP markers were identified in our L94 x 'Vada' mapping population that were identical in the 'Proctor' x 'Nudinka' population (Becker et al. 1995). Markers in common tightly linked in a single

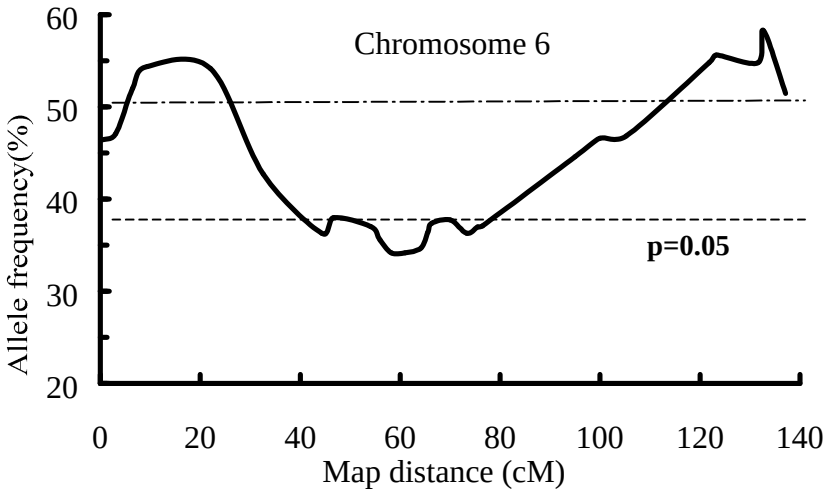


Fig. 1. Frequency distribution of the L94 alleles on chromosome 6 (6H). The fitness test according to a 1:1 ratio which was approximated in the F_9 RI population

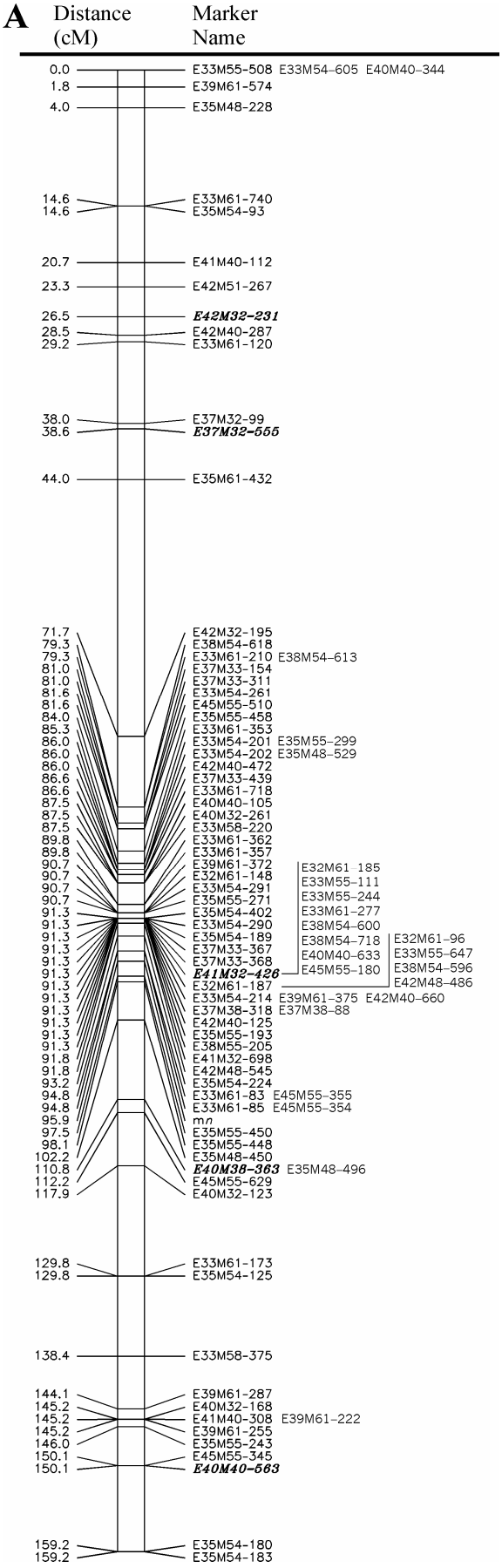
linkage group in our L94 x 'Vada' population also showed linkage in the 'Proctor' x 'Nudinka' population. Similar genetic distances and identical orders of the markers shared by the two mapping populations strongly indicated that these AFLP markers are locus specific and hence their map positions can be used as anchor points across populations (Table 1).

Table 1. Genetic distances (cM) of tightly linked marker pairs/groups in two mapping populations[a]

Marker pairs/groups	L94 x 'Vada'	'Proctor' x 'Nudinka'
E42M32-231/E37M32-555	12.3	16.8 (1)[b]
E41M32-156/E41M40-110	12.8	25.5 (2)
E42M32-272/E37M38-373	0.5	1.0 (2)
E37M38-199/E37M33-501/E37M32-325	10.6/4.9	13.5/7.2 (2)
E41M40-155/E40M32-180/ E40M32-130	0.5/15.7	2.1/18.8 (4)
E41M40-270/E40M40-358/E40M38-338	2.5/10.0	3.5/8.2 (7)

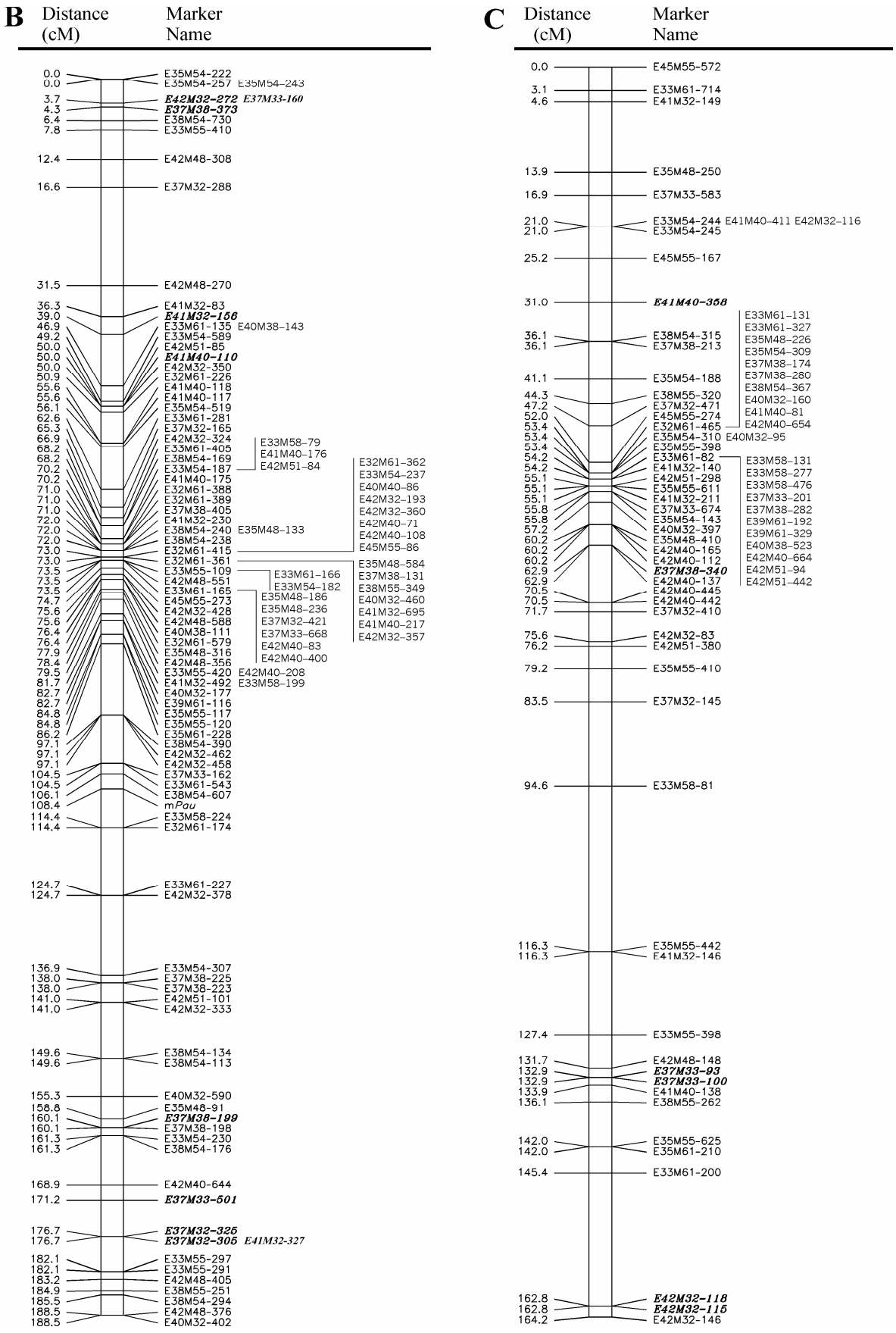
[a] As an example, only six pairs and groups represented in this table
[b] Numbers in parentheses indicated the chromosomes to which these markers were assigned on the 'Proctor' x 'Nudinka' map

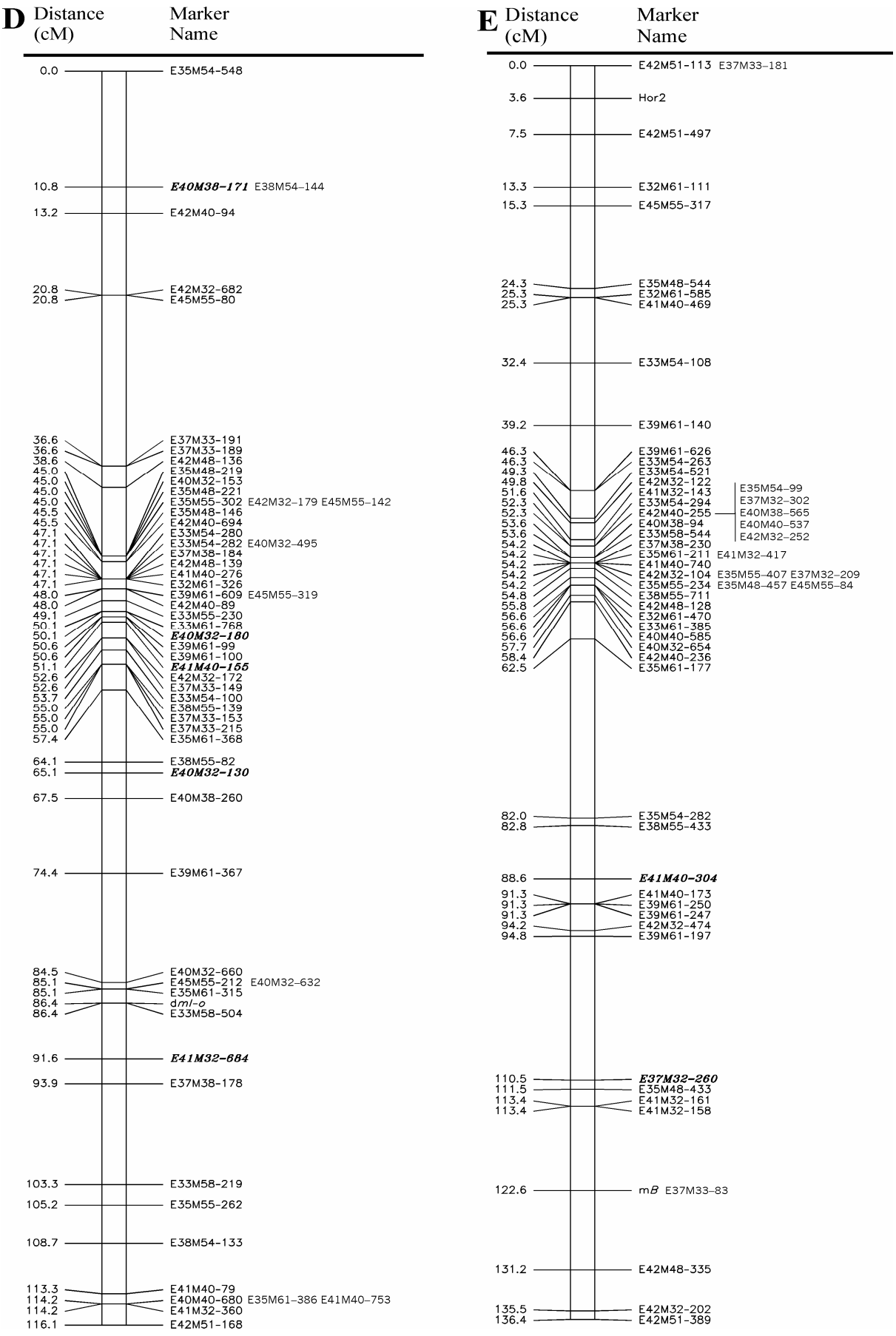
The 563 AFLP markers, four phenotypic markers, and one STS marker, were split into 21 groups at a LOD threshold grouping value of 7.0. Only two markers,

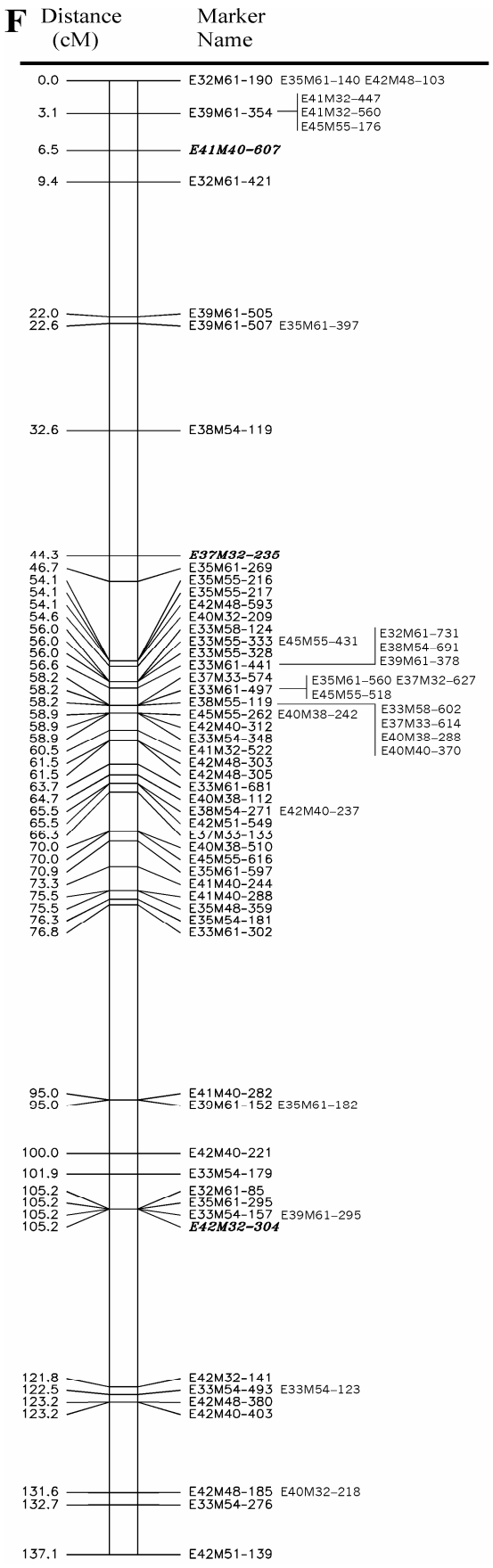


E33M55-191 with 37 missing data and E33M54-310, were not linked to any other marker at a LOD value of 5.0, and one group of three markers remained separated at a LOD threshold value lower than 3.0. The 38 AFLP markers in common, as well as four phenotypic markers (m*n*, m*Pau*, m*B*, *&* d*ml-o***)** and *Hor2*, were used to assign AFLP linkage groups to seven barley chromosomes. Except for the five isolated markers described above, the other 18 groups contained at least one anchor marker and were assigned to the seven barley chromosomes. Chromosomes 1, 2 and 4 were composed of two groups, chromosomes 3, 5, 6 and 7 of three groups. The unassigned group containing three AFLP markers was assigned to chromosome 5 because it showed the tightest linkage (LOD = 2.6 for m*B* and E42M48-335) to the other markers on this chromosome and fitted very well on the map of this chromosome.

Fig. 2 The barley L94 x 'Vada' AFLP map. A – G correspond to barley chromosomes 1 to 7, with the short arm at the top. Markers with a ***bold-italic*** font were common to both the present map and the 'Proctor' x 'Nudinka' map. The markers with identical segregation are aligned to the corresponding representative markers.


B
Distance (cM)
Marker Name
0.0 E35M54-222
0.0 E35M54-257 E35M54-243
3.7 E42M32-272 E37M33-160
4.3 E37M38-373
6.4 E38M54-730
7.8 E33M55-410
12.4 E42M48-308
16.6 E37M32-288
31.5 E42M48-270
36.3 E41M32-83
39.0 E41M32-156
46.9 E33M61-135 E40M38-143
49.2 E33M54-589
50.0 E42M51-85
50.0 E41M40-110
50.0 E42M32-350
50.9 E32M61-226
55.6 E41M40-118
55.6 E41M40-117
56.1 E35M54-519
62.6 E33M61-281
65.3 E37M32-165
66.9 E42M32-324
68.2 E33M61-405
68.2 E38M54-169
70.2 E33M54-187
70.2 E41M40-175
71.0 E32M61-388
71.0 E32M61-389
71.0 E37M38-405
72.0 E41M32-230
72.0 E38M54-240 E35M48-133
72.0 E38M54-238
73.0 E32M61-415
73.0 E32M61-361
73.5 E33M55-109
73.5 E42M48-551
73.5 E33M61-165
74.7 E45M55-273
75.6 E42M32-428
75.6 E42M48-588
76.4 E40M38-111
76.4 E32M61-579
77.9 E35M48-316
78.4 E42M48-356
79.5 E33M55-420 E42M40-208
81.7 E41M32-492 E33M58-199
82.7 E40M32-177
82.7 E39M61-116
84.8 E35M55-117
84.8 E35M55-120
86.2 E35M61-228
97.1 E38M54-390
97.1 E42M32-462
97.1 E42M32-458
104.5 E37M33-162
104.5 E33M61-543
106.1 E38M54-607
108.4 mPau
114.4 E33M58-224
114.4 E32M61-174
124.7 E33M61-227
124.7 E42M32-378
136.9 E33M54-307
138.0 E37M38-225
138.0 E37M38-223
141.0 E42M51-101
141.0 E42M32-333
149.6 E38M54-134
149.6 E38M54-113
155.3 E40M32-590
158.8 E35M48-91
160.1 E37M38-199
160.1 E37M38-198
161.3 E33M54-230
161.3 E38M54-176
168.9 E42M40-644
171.2 E37M33-501
176.7 E37M32-326
176.7 E37M32-305 E41M32-327
182.1 E33M55-297
182.1 E33M55-291
183.2 E42M48-405
184.9 E38M55-251
185.5 E38M54-294
188.5 E42M48-376
188.5 E40M32-402
E33M58-79
E41M40-176
E42M51-84
E32M61-362
E33M54-237
E40M40-86
E42M32-193
E42M32-360
E42M40-71
E42M40-108
E45M55-86
E35M48-584
E37M38-131
E38M55-349
E40M32-460
E41M32-695
E41M40-217
E42M32-357
E33M61-166
E33M54-182
E35M48-186
E35M48-236
E37M32-421
E37M33-668
E42M40-83
E42M40-400
C
Distance (cM)
Marker Name
0.0 E45M55-572
3.1 E33M61-714
4.6 E41M32-149
13.9 E35M48-250
16.9 E37M33-583
21.0 E33M54-244 E41M40-411 E42M32-116
21.0 E33M54-245
25.2 E45M55-167
31.0 E41M40-358
36.1 E38M54-315
36.1 E37M38-213
41.1 E35M54-188
44.3 E38M55-320
47.2 E37M32-471
52.0 E45M55-274
53.4 E32M61-465
53.4 E35M54-310 E40M32-95
53.4 E35M55-398
54.2 E33M61-82
54.2 E41M32-140
55.1 E42M51-298
55.1 E35M55-611
55.1 E41M32-211
55.8 E37M33-674
55.8 E35M54-143
57.2 E40M32-397
60.2 E35M48-410
60.2 E42M40-165
60.2 E42M40-112
62.9 E37M38-340
62.9 E42M40-137
70.5 E42M40-445
70.5 E42M40-442
71.7 E37M32-410
75.6 E42M32-83
76.2 E42M51-380
79.2 E35M55-410
83.5 E37M32-145
94.6 E33M58-81
116.3 E35M55-442
116.3 E41M32-146
127.4 E33M55-398
131.7 E42M48-148
132.9 E37M33-93
132.9 E37M33-100
133.9 E41M40-138
136.1 E38M55-262
142.0 E35M55-625
142.0 E35M61-210
145.4 E33M61-200
162.8 E42M32-118
162.8 E42M32-115
164.2 E42M32-146
E33M61-131
E33M61-327
E35M48-226
E35M54-309
E37M38-174
E37M38-280
E38M54-367
E40M32-160
E41M40-81
E42M40-654
E33M58-131
E33M58-277
E33M58-476
E37M33-201
E37M38-282
E39M61-192
E39M61-329
E40M38-523
E42M40-664
E42M51-94
E42M51-442


D
Distance (cM)
Marker Name
0.0 E35M54-548
10.8 E40M38-171 E38M54-144
13.2 E42M40-94
20.8 E42M32-682
20.8 E45M55-80
36.6 E37M33-191
36.6 E37M33-189
38.6 E42M48-136
45.0 E35M48-219
45.0 E40M32-153
45.0 E35M48-221
45.0 E35M55-302 E42M32-179 E45M55-142
45.5 E35M48-146
45.5 E42M40-694
47.1 E33M54-280
47.1 E33M54-282 E40M32-495
47.1 E37M38-184
47.1 E42M48-139
47.1 E41M40-276
47.1 E32M61-326
48.0 E39M61-609 E45M55-319
48.0 E42M40-89
49.1 E33M55-230
50.1 E33M61-768
50.1 E40M32-180
50.6 E39M61-99
50.6 E39M61-100
51.1 E41M40-155
52.6 E42M32-172
52.6 E37M33-149
53.7 E33M54-100
55.0 E38M55-139
55.0 E37M33-153
55.0 E37M33-215
57.4 E35M61-368
64.1 E38M55-82
65.1 E40M32-130
67.5 E40M38-260
74.4 E39M61-367
84.5 E40M32-660
85.1 E45M55-212 E40M32-632
85.1 E35M61-315
86.4 dmI-o
86.4 E33M58-504
91.6 E41M32-684
93.9 E37M38-178
103.3 E33M58-219
105.2 E35M55-262
108.7 E38M54-133
113.3 E41M40-79
114.2 E40M40-680 E35M61-386 E41M40-753
114.2 E41M32-360
116.1 E42M51-168
E
Distance (cM)
Marker Name
0.0 E42M51-113 E37M33-181
3.6 Hor2
7.5 E42M51-497
13.3 E32M61-111
15.3 E45M55-317
24.3 E35M48-544
25.3 E32M61-585
25.3 E41M40-469
32.4 E33M54-108
39.2 E39M61-140
46.3 E39M61-626
46.3 E33M54-263
49.3 E33M54-521
49.8 E42M32-122
51.6 E41M32-143
52.3 E33M54-294
52.3 E42M40-255
53.6 E40M38-94
53.6 E33M58-544
54.2 E37M38-230
54.2 E35M61-211 E41M32-417
54.2 E41M40-740
54.2 E42M32-104 E35M55-407 E37M32-209
54.2 E35M55-234 E35M48-457 E45M55-84
54.8 E38M55-711
55.8 E42M48-128
56.6 E32M61-470
56.6 E33M61-385
56.6 E40M40-585
57.7 E40M32-654
58.4 E42M40-236
62.5 E35M61-177
E35M54-99
E37M32-302
E40M38-565
E40M40-537
E42M32-252
82.0 E35M54-282
82.8 E38M55-433
88.6 E41M40-304
91.3 E41M40-173
91.3 E39M61-250
91.3 E39M61-247
94.2 E42M32-474
94.8 E39M61-197
110.5 E37M32-260
111.5 E35M48-433
113.4 E41M32-161
113.4 E41M32-158
122.6 mB E37M33-83
131.2 E42M48-335
135.5 E42M32-202
136.4 E42M51-389


F
Distance (cM)
Marker Name
0.0 E32M61-190 E35M61-140 E42M48-103
3.1 E39M61-354 E41M32-447 E41M32-560 E45M55-176
6.5 E41M40-607
9.4 E32M61-421
22.0 E39M61-505
22.6 E39M61-507 E35M61-397
32.6 E38M54-119
44.3 E37M32-235
46.7 E35M61-269
54.1 E35M55-216
54.1 E35M55-217
54.1 E42M48-593
54.6 E40M32-209
56.0 E33M58-124
56.0 E33M55-333 E45M55-431 E32M61-731 E38M54-691 E39M61-378
56.0 E33M55-328
56.6 E33M61-441
58.2 E37M33-574 E35M61-560 E37M32-627
58.2 E33M61-497 E45M55-518
58.2 E38M55-119
58.9 E45M55-262 E40M38-242 E33M58-602 E37M33-614 E40M38-288 E40M40-370
58.9 E42M40-312
58.9 E33M54-348
60.5 E41M32-522
61.5 E42M48-303
61.5 E42M48-305
63.7 E33M61-681
64.7 E40M38-112
65.5 E38M54-271 E42M40-237
65.5 E42M51-549
66.3 E37M33-133
70.0 E40M38-510
70.0 E45M55-616
70.9 E35M61-597
73.3 E41M40-244
75.5 E41M40-288
75.5 E35M48-359
76.3 E35M54-181
76.8 E33M61-302
95.0 E41M40-282
95.0 E39M61-152 E35M61-182
100.0 E42M40-221
101.9 E33M54-179
105.2 E32M61-85
105.2 E35M61-295
105.2 E33M54-157 E39M61-295
105.2 E42M32-304
121.8 E42M32-141
122.5 E33M54-493 E33M54-123
123.2 E42M48-380
123.2 E42M40-403
131.6 E42M48-185 E40M32-218
132.7 E33M54-276
137.1 E42M51-139
G
Distance (cM)
Marker Name
0.0 E38M55-114
7.3 E38M54-247
20.7 E45M55-302
21.7 E33M61-370
22.3 E33M54-218 E45M55-400
22.3 E45M55-402
22.8 E33M55-126
22.8 E35M61-222
32.0 E40M40-354
32.0 E40M40-358
33.3 E41M40-270
33.9 E45M55-304
35.0 E35M61-289
35.0 E32M61-282
35.9 E42M51-519 E35M48-513 E35M61-264
36.9 E37M32-177
37.6 E39M61-360
37.6 E42M51-192
37.6 E37M32-107
39.3 E41M32-416
39.3 E38M55-140
41.6 E32M61-81 E35M48-256 E42M485-478
41.6 E42M40-198
43.2 E35M54-195
43.8 E35M61-82 E45M55-246
43.8 E33M54-308
43.8 E45M55-245
43.8 E42M48-127
45.0 E40M38-338
46.0 E45M55-580 E33M58-278
46.0 E37M38-505
49.9 E35M55-181
56.3 E42M48-129
63.2 E41M32-170
63.2 E38M54-375
65.6 E33M55-533
70.4 E38M55-128
70.4 E38M55-129
71.2 E37M33-334
72.1 E42M48-87
73.6 E42M32-200
74.8 E42M40-482
86.4 E42M48-279
91.3 E42M51-335
91.3 E42M51-333
98.1 E38M55-175
98.8 E42M40-109
101.8 E35M48-160
104.5 E42M48-282
108.1 E41M32-139
112.4 E33M58-306
112.4 E33M58-94
112.4 E33M58-95
123.2 E33M61-595
125.0 E33M55-267
125.7 E40M38-152 E42M32-250
132.8 E42M32-464
138.3 E42M48-203 E41M40-700
145.8 E42M32-87
146.3 E42M51-79
146.3 E40M32-87 E42M51-80
157.0 E37M32-88
159.7 E33M61-148
159.7 E33M61-144
160.7 E35M48-400

The resulting map contains 566 markers covering a total map distance of 1062 cM corresponding to approximately 1.9 cM per marker. Chromosome 2 has the largest number of markers (120) with the longest genetic distance (189 cM), and chromosome 4 is the shortest one. Remarkably, marker clustering was observed on all seven chromosomes (Fig. 2 and Table 2). Using the 'Proctor' x 'Nudinka' AFLP and RFLP combined map (Becker et al. 1995) as a bridge, the present AFLP map was compared with the integrated RFLP map (Qi et al. 1996) which was based on four independent RFLP maps (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993b; Kasha and Kleinhofs 1994) with known centromere regions (Kleinhofs et al. 1993a). The clusters of AFLP markers on the present map were very likely also located around centromeric regions. In the putative centromeric regions, jointly spanning 155 cM, 289 markers were mapped, corresponding to 0.5 cM per marker. In contrast, the chromosome arms, spanning 906 cM, were covered by 277 markers, corresponding to 3.3 cM per marker. Despite this small average genetic distance between markers, chromosomes 1, 3 and 5 still contain a gap larger than 20 cM. Several smaller gaps (10 – 15 cM) are present on the distal parts of the chromosomes (Fig. 2).

Table 2. Summary of L94 x 'Vada' mapping data

				Chromosome arms		Centromeric clusters	
Chromosomes	No. of Markers	Length (cM)	No. of Gaps[a]	No. of Markers	Coverage (cM)	No. of Markers	Coverage (cM)
1 (7H)	96	159	1	33	128 (3.9)[b]	63	31 (0.5)[b]
2 (2H)	120	189	0	59	156 (2.6)	61	33 (0.5)
3 (3H)	77	164	1	38	147 (3.9)	39	17 (0.4)
4 (4H)	61	116	0	30	97 (3.2)	31	19 (0.6)
5 (1H)	60	136	1	29	118 (4.1)	31	18 (0.6)
6 (6H)	77	137	0	42	119 (2.8)	35	18 (0.5)
7 (5H)	75	161	0	46	140 (3.0)	29	21 (0.7)
Total	566	1062	3	277	906 (3.3)	289	156 (0.5)

[a] A gap is a distance between two adjacent markers of more than 20 cM
[b] Numbers in parentheses are the average distances per marker interval

There are no clear indications of uncovered regions on the distal parts of each chromosome though some chromosomes were quite short, such as chromosome 7 in the present map (161 cM) compared to the integrated map (195 cM). Conversely, there are also no clear indications of having covered extra distal parts by the AFLP markers, as compared to the integrated RFLP map (with a 1060-cM total length and 880 markers, Qi et al. 1996).

In conclusion, despite the non-uniform distribution of markers along chromosomes and the presence of three gaps of more than 20 cM, the present AFLP map most likely covers the entire barley genome, or nearly so. From this high-density map a skeletal map with a fairly uniform distribution of markers can be extracted. Such a skeletal map may serve for the detection and mapping of loci underlying qualitative and quantitative traits.

Discussion

Reliability of the map

Genetic maps are calculated from the recombination rates between loci as a result of chromosome crossovers at meiosis. Recombination rates may be influenced by environmental factors (Allard 1963; Powell and Nilan 1963); hence genetic distances may vary from one mapping population to another. But, in general, recombination rates are under genetic control (Paredes and Gepts 1995) and heavily depend on chromosome structure. Comparison of four independent barley RFLP maps indicated that barley genetic linkage maps are quite stable; marker orders are similar and no obvious rearrangements are detectable (Qi et al. 1996). Comparison of the present map with the 'Proctor' x 'Nudinka' map indicated that the orders of all anchor markers (Fig. 2, markers with ***italic bold*** font) on the seven chromosomes were identical and the distances between tightly linked markers were very similar indeed. Moreover, the positions of four phenotypic markers and *Hor2* were also mapped to their correct positions on the barley genome (Franckowiak 1995; Forster 1996; Jensen 1996; Qi et al. 1996).

Non-systematic changes of marker-allele frequencies along a map are indicative of uncertainties in the order of markers. We did not observe any irregular pattern of segregation distortion in our data (Fig. 1). Altogether, our

results indicate that we produced a reliable high-density marker map of the barley genome.

Clustering of markers

A high degree of clustering of markers around the centromere is a notable feature in wheat (Chao et al. 1989; Devos et al. 1992; Hart 1994). The clustering of markers at centromeric, and possibly telomeric areas, was found in the tomato high-density map by Tanksley et al. (1992). Clustering of markers at centromeric regions was also observed on the barley integrated map (Qi et al. 1996). Extreme non-uniform distributions of AFLP markers and strong clustering of markers around the putative centromere were identified in the present AFLP map (Fig. 2 and Table 2). The centromeric suppression of recombination may be the main reason for the clustering of markers (Tanksley et al. 1992; Frary et al. 1996). Surprisingly, clustering is much more pronounced in the present AFLP map than in the RFLP maps. This may be due to differences in the sensitivities of RFLP versus AFLP markers. The AFLP technique is extremely sensitive to polymorphism in the genome, as 1-bp length differences in relatively short DNA fragments (50 – 1000 bp) are already detectable. In species with a large genome, such as barley, a great portion of repetitive sequences occur in the centromeric regions. Small variations such as 1-bp deletion/insertion in repetitive sequences, and/or variable numbers of short sequence repeats (or simple-sequence length polymorphisms, SSPLs), can be detected by the AFLP technique. However, they will probably not be revealed by Southern hybridization with DNA probes, as the repetitive sequences will usually give multiple signals, and multi-copy probes are generally excluded in RFLP map construction. As the amplification products generated by the AFLP technique may contain repeated sequences, there is a higher chance to identify AFLP markers than RFLPs in highly repetitive regions near the centromere. This may be the most plausible explanation for the stronger clustering of AFLP markers.

Locus specificity

If AFLP products show the same mobility in gels, these are very likely to be homologous and locus specific (Qi and Lindhout 1997). This assumption can be verified by comparing the sequences of co-migrating bands and by genetic linkage analyses, respectively. Rouppe van der Voort et al. (1997) sequenced

co-migrating amplification products in potato and showed that this assumption is nearly always valid. Waugh et al (1997) found that 81 co-migrating AFLP markers, segregating in more than one population, mapped to similar loci on the three barley genetic maps and only three markers mapped to different positions. In the present study, all 38 co-migrating bands, segregating in two populations, mapped to the same loci. Altogether, these studies indicate the great probability of the locus specificity of AFLP markers.

To investigate whether less-related populations or species may also show markers in common, the AFLP patterns of barley (*H. vulgare*) were compared with those of three *Triticum* species (data not shown). The lack of co-migrating AFLP products suggests that the genetic distance between these species is too large for markers in common to be identified. Consequently, the use of the locus-specific AFLP markers is limited to populations within species or to very closely related species.

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5

Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley*

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Abstract: The partial resistance to leaf rust in barley is a quantitative resistance that is not based on hypersensitivity. To map the quantitative trait loci (QTLs) for partial resistance to leaf rust, 103 recombinant inbred lines (RILs) were obtained by single seed descent from a cross between the susceptible parent L94 and partially resistant parent 'Vada'. These RILs were evaluated in the seedling and the adult plant stages in the greenhouse for the latency period (LP) of the rust fungus, and in the field for the level of infection, measured as area under the disease progress curve (AUDPC). A dense genetic map based on 561 AFLP markers had been generated previously for this set of RILs. QTLs for partial resistance to leaf rust were mapped by using the 'Multiple Interval Mapping' with the putative QTL markers as cofactors. Six QTLs for partial resistance were identified in this population. Three QTLs, *Rphq1, Rphq2* and *Rphq3*, were effective in the seedling stage and contributed approximately 55% to the phenotypic variance. Five QTLs, *Rph2*, *Rphq3*, *Rphq4, Rphq5*, and/or *Rphq6* contributed approximately 60% of the phenotypic variance and were effective in the adult plant stage. Therefore, only the QTLs *Rphq2* and *Rhpq3* were not plant stage dependent. The identified QTLs showed mainly additive effects and only one significant interaction was detected, i.e., between *Rphq1* and *Rphq2*. The map positions of these QTLs did not

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coincide with those of the race-specific resistance genes, suggesting that genes for partial resistance and genes for hypersensitive resistance represent entirely different gene families. Also, three QTLs for days to heading, of which two were also involved in plant height, were identified in the present recombinant inbred population. These QTLs had been mapped previously to the same positions in different populations. The perspectives of these results for breeding for durable resistance to leaf rust are discussed.

Key words: Partial resistance, Leaf rust, Barley, QTL mapping, *Puccinia hordei*, *Hordeum vulgare*, Latency period

Introduction

Leaf rust caused by the pathogen *Puccinia hordei* Otth is one of the most important diseases in barley (*Hordeum vulgare* L.). Though in most areas the reduction of yield caused by leaf rust is relatively low, in some areas, it may be close to 30% (Arnst et al. 1979; Feuerstein et al. 1990). Barley leaf rust has been controlled primarily by the use of resistant cultivars carrying genes for hypersensitivity resistance, designated as *Rph* (*Pa*) genes. Rapid adaptation of the *P. hordei* populations, however, has rendered most of the resistance genes ineffective. The recently identified resistance genes, *Rph13* and *Rph14* (Jin et al. 1996), are also unlikely to be durable. Furthermore, sources of leaf rust resistance in cultivated barley are limited (Jin et al. 1995; Jin and Steffenson 1994). In contrast, partial resistance to leaf rust, characterised by a reduced rate of epidemic development despite a susceptible infection type (Parlevliet and Van Ommeren 1975), occurs very frequently in West-European spring cultivars (Parlevliet et al. 1980) and Ethiopian barley landraces (Alemayehu and Parlevliet 1996), and is presumably more durable (Alemayehu and Parlevliet 1996; Parlevliet 1983a, 1983b). Partial resistance in the field appears strongly correlated with the latency period (LP) and also with other components, such as infection frequency, pustule size, infectious period and spore production. LP can be evaluated with much greater accuracy than the other components (Parlevliet 1975, 1977, 1979, 1986, 1992; Parlevliet et al. 1985; Neervoort and Parlevliet 1978; Parlevliet and Van Ommeren 1975). Genetic studies indicated

that the longer LP in several partially resistant cultivars was governed by 6 to 7 minor genes with additive effects (Parlevliet 1976, 1977, 1978).

By using a dense molecular linkage map, polygenic quantitative traits can be resolved into discrete Mendelian factors (e.g., Paterson et al. 1988). With QTL mapping, the individual resistance loci can be identified and located on the chromosomes. This is a highly effective tool for studying genetically complex disease resistance such as partial resistance (Young 1996). It will allow the assessment of race-specificity of partial resistance genes, the interactions between resistance genes, and their expression in different growth stages and environments. Many genes conferring hypersensitive resistance to pathogenic fungi and several QTLs for partial resistance to powdery mildew have already been mapped on the barley genome (Graner 1996). Two QTLs for resistance to *P. striiformis* were detected on chromosomes 7L and 4L (Chen et al. 1994) respectively. In the present research, we studied a recombinant inbred population (103 RILs) derived from a cross of L94 (susceptible) x 'Vada' (partially resistant) and mapped QTLs for partial resistance on the barley genome based on a high-density AFLP map (Qi et al. 1998).

Materials and methods

Development of recombinant inbred lines

A population of 103 F_9 recombinant inbred lines (RILs) was obtained from a cross of L94 x 'Vada' by single-seed descent. L94 is a line from an Ethiopian landrace, with black and naked seeds, and is extremely susceptible to leaf rust (*Puccinia hordei*) (Parlevliet 1975). 'Vada' is a commercial West-European cultivar, with white and covered seeds, previously released by the Department of Plant Breeding, Wageningen Agricultural University, and has a high level of partial resistance to *P. hordei*. Both parents have been included in numerous experiments to characterise aspects of partial resistance of barley to leaf rust (Parlevliet 1975, 1976, 1978, 1979, 1983b; Parlevliet et al 1985; Niks 1986). The 103 RILs (F_9) and the two parents were used for AFLP marker analysis (Qi et al. 1998) and for disease tests in the greenhouse and in the field.

Disease evaluations

Seedlings in the greenhouse. Seedlings of 103 RILs, L94 and 'Vada' were inoculated with the leaf rust isolate 1.2.1. Fresh urediospores were diluted 10 times with lycopodium spores and dusted over the adaxial sides of the seedling leaves fixed in a horizontal position. After incubation at a relative humidity of 100% over night, the seedlings were transferred to a greenhouse where the temperature was set at about 18 ^{o}C. The latency period (LP) of each plant was evaluated by estimating the period (hours) at which 50% of the ultimate number of pustules became visible. The relative latency period of seedlings (RLP50S) was calculated relative to the LP of L94 in seedlings, where L94 = 100, as described by Parlevliet (1975). Four experiments were conducted in the course of three years. Each experiment consisted of two replications, each with 5 to 6 plants per line. Because separate analysis of these data did not reveal significant genotype x environment effects and all of the QTLs involved in RLP50S were found in all experiments, the RLP50S values were averaged over these four experiments.

Adult plants in the greenhouse. The rust isolate 1.2.1 was also used for evaluation of the RILs in the adult plant stage in the greenhouse. One experiment was carried out with 5 plants per line. The relative LP of young flag leaves (RLP50A) was measured similar to the RLP50S.

Adult plants in the field. A randomised complete block design with three replications was applied in a field experiment in 1996. Plot size was 0.75 x 1.25 m^2. Plots of barley lines alternated with plots of oats to limit inter-plot interference (Parlevliet and Van Ommeren 1984). One month after sowing, more than 250 L94 plants were inoculated in the greenhouse and after two weeks the pots with sporulating L94 plants were transferred to the experiment field and placed in the alley ways between the plots. When L94 plants in the plots started to sporulate, the spreader-plants were removed. Three samplings with a seven days interval were carried out from the early heading stage to the late grain filling stage. At sampling time, three tillers were sampled from each plot and evaluated for the number of spores according to the scale of Parlevliet and Van Ommeren (1984). The area under the disease progress curve (AUDPC) was calculated by use of the mean values from these three observations. In addition, days to heading was evaluated as the number of days from sowing till 50% of plants in the plot had headed. Plant height was also measured in the final stage of plant development.

Genotyping and map construction

From the high-density AFLP map (Qi et al. 1998), a skeletal map with uniformly distributed markers (approximately 5 cM per marker interval) was extracted (Fig. 3) and used for QTL identification.

Statistical analysis

In both the RLP50S and the field experiment a few observations were missing. Therefore, the least square estimate means of RLP50S, AUDPC, days to heading and plant height, and ANOVAs were calculated by using PROC GLM in SAS programme (SAS Institute 1988). Subsequently, wide sense heritability (h^2) for the four traits were estimated. A computer software package, MapQTL version 3.0 (Van Ooijen and Maliepaard 1996), was used for interval mapping (Lander and Botstein 1989). In the region of the putative QTLs (LOD > 2.5), the markers with the highest LOD values ('peak markers') were taken as co-factors for running a multiple-QTL mapping program, the MQM method (Jansen 1993; Jansen and Stam 1994). When LOD values of some markers on other regions reached a significant level, the MQM was repeated by adding those new 'peak markers' as cofactors until a 'stable' LOD profile was reached. A LOD value of 2.5 was chosen as significant threshold value for declaring a QTL.

Results

Assessment of resistance and plant development traits

The analysis of variance indicated highly significant differences among the 103 RILs for all four evaluated traits (data not shown). Due to the use of one replication, no analysis of variance could be applied for latency period in adult plants in the greenhouse (RLP50A). The frequencies of all three parameters for partial resistance and for days to heading and plant height were approximately normally distributed (Fig. 1). The values of the 103 RILs in three measures for partial resistance were between the parental values indicating absence of transgressive segregation in this population (Figs. 1. A, B, and C). In contrast, transgressive segregation was observed for days to heading and plant height (Figs.

1. D and E).

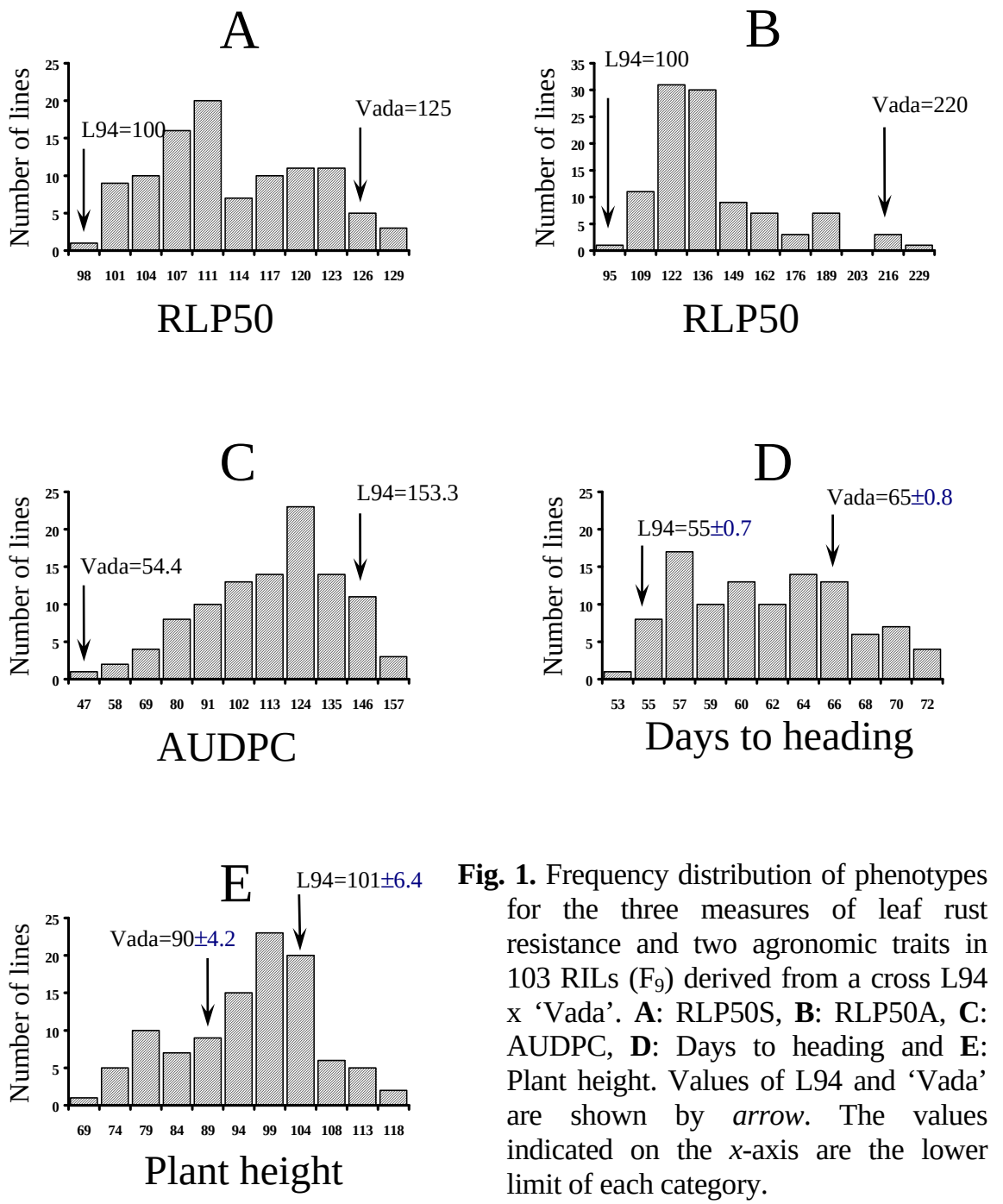


Fig. 1. Frequency distribution of phenotypes for the three measures of leaf rust resistance and two agronomic traits in 103 RILs (F_9) derived from a cross L94 x 'Vada'. **A**: RLP50S, **B**: RLP50A, **C**: AUDPC, **D**: Days to heading and **E**: Plant height. Values of L94 and 'Vada' are shown by *arrow*. The values indicated on the *x*-axis are the lower limit of each category.

The wide sense heritabilities of the two measures for partial resistance and the two traits were estimated. The heritability for RLP50S was about 0.58 and was 0.82 for both plant height and AUDPC. A very high heritability of 0.94 was found for days to heading.

A strong negative correlation was found between RLP50A and AUDPC (Table 1). Moderate correlations were observed between resistance in the seedling stage (RLP50S) and in the adult plant stage (RLP50A and AUDPC). Plant height was strongly correlated with days to heading. No correlation between the three measures of partial resistance and plant height was observed nor between days to heading and resistance in the seedling stage (RLP50S). However, a moderate correlation between days to heading and partial resistance in the adult plant stage (RLP50A and AUDPC) was observed.

Table 1 Correlation coefficients (*r*) among traits in 103 RILs (F_9) derived from the cross L94 x 'Vada'

Traits	RLP50S	RLP50A	AUDPC	Days to heading
RLP50S				
RLP50A	0.43**			
AUDPC	-0.43**	-0.78**		
Days to heading	-0.07	0.40**	-0.34**	
Plant height	-0.15	0.24*	-0.19	0.68**

* P <= 0.5; ** P <= 0.01

QTLs for partial resistance

To map QTLs for partial resistance and plant development traits, interval mapping and MQM methods were applied (Fig. 2). A major improvement in the accuracy of QTL mapping was achieved by using MQM where the 'peak' markers were taken as cofactors. Therefore, QTLs identified by using MQM methods were considered as most reliable (Jansen 1993; Jansen and Stam 1994). In total, six QTLs for partial resistance to leaf rust were identified in this population (Fig. 3). Some QTLs were identified that were common to each of the parameters of partial resistance, often showing the highest LOD score at exactly the same marker loci. Most likely, the same QTL was involved in different parameters of partial resistance. Three QTLs for RLP50S were identified, designated *Rphq1*, *Rphq2* and *Rphq3*. Two major QTLs, *Rphq2* and

Rphq3, located on chromosomes 2 and 6, respectively, explained a large part of the phenotypic variance (Table 2). *Rphq1*, a minor-effect QTL (explaining 3.4% phenotypic variance) on chromosome 1, was detected with a LOD score of 2.5 that was just above the threshold value. The three QTLs together explained 56% of the phenotypic variance. Four QTLs, *Rphq2*, *Rphq3*, *Rphq4* and *Rphq5*, were identified at the adult plant stage, both in the greenhouse and in the field. *Rphq4* and *Rphq3* on chromosomes 7 and 6 respectively explained most of the phenotypic variance; *Rphq2* and *Rphq5*, on chromosomes 2 and 4 respectively, contributed moderately to the partial resistance at adult plant stage. In the field experiment the four QTLs explained 63% of total phenotypic variance. In addition, another QTL, *Rphq6* (R^2 = 0.07) was found to affect the latency period only at the adult plant stage (RLP50A). It was mapped to the same position of a major QTL for days to heading (see next paragraph). In total, 59% of the phenotypic variance for prolonged latency period at the adult plant stage in the greenhouse was accounted for by the five QTLs.

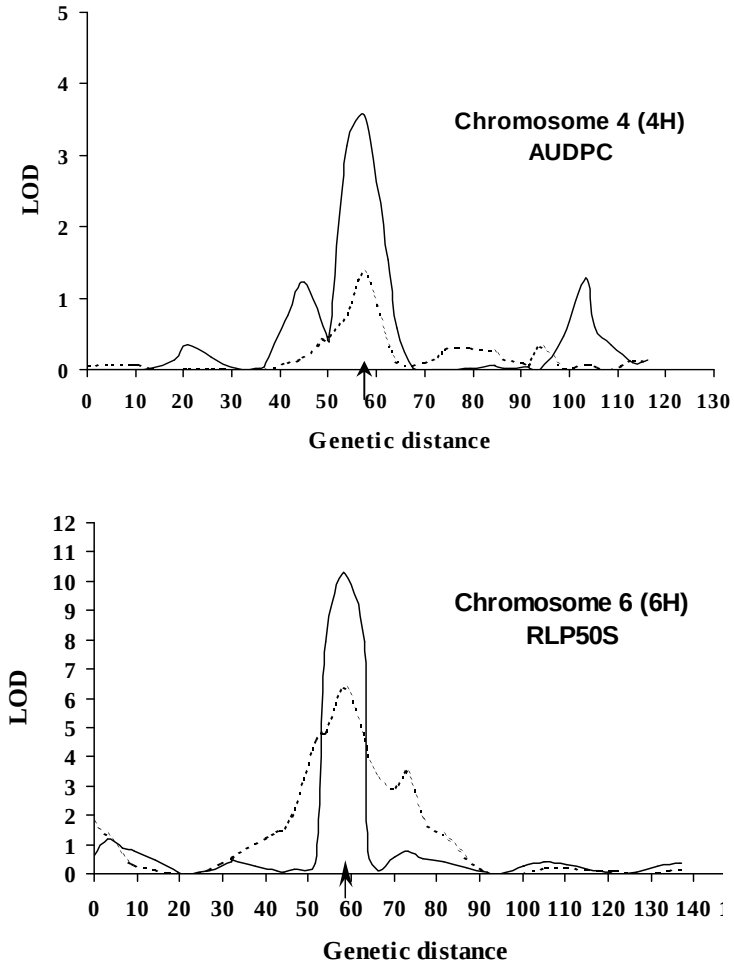


Fig. 2. LOD profiles of QTL on chromosomes 4 (4H) and 6 (6H). Dotted lines are based on interval mapping and the solid lines are MQM. The *arrow* indicates the position of the marker taken as cofactor for the MQM. Maps are oriented with the short arm to the left and correspond to the maps shown in Fig. 3.

Disease evaluations for LP in seedlings (RLP50S) were conducted in four experiments by different persons and in different years. When the four data sets were used separately for QTL mapping, the three QTLs (*Rphq1*, *Rphq2* and *Rphq3*) were always identified with an identical ranking order of the quantitative effects (data not shown). Moreover, the QTLs found to affect RLP50A were also found to affect AUDPC, and had the same ranking order for size of effect for both parameters. These results indicate that these QTLs for partial resistance to barley leaf rust were relatively insensitive to environmental conditions. However, also clear plant stage-specific effects of QTLs were identified. *Rphq4* and *Rphq5* were greatly effective at the adult plant stage (RLP50A and AUDPC), but not in the seedling stage (RLP50S). In contrast, *Rphq2* was largely effective in the seedling stage (RLP50S), but only weakly in adult plant stage (RLP50A and AUDPC). One minor QTL, *Rphq1*, was only effective in the seedling stage. *Rphq3* on chromosome 6 is the only QTL with a substantial effect in the seedling as well as the adult plant stage.

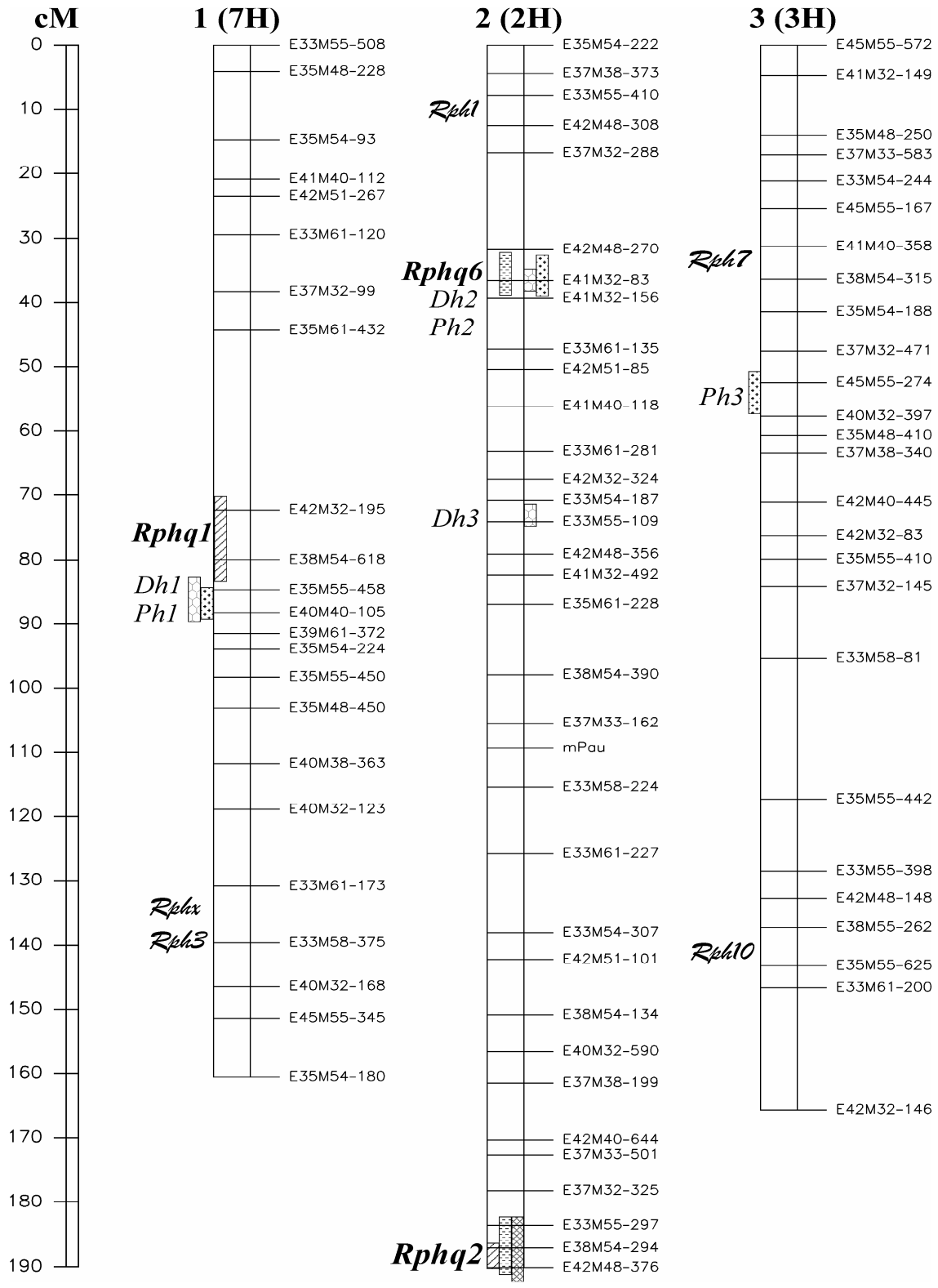
Table 2 Summary of QTLs for partial resistance to barley leaf rust

	RLP50S			RLP50A			AUDPC		
QTLs	LOD	Exp%[a]	Add[b]	LOD	Exp%	Add	LOD	Exp%	Add
Rphq1	**2.5**	**3.4**	**1.5**	0.3	0.4	-1.8	1.2	0.9	-2.6
Rphq2	**18.1**	**35.5**	**4.9**	**3.0**	**4.1**	**5.6**	**4.1**	**3.8**	**-4.9**
Rphq3	**10.3**	**16.7**	**3.5**	**10.7**	**17.4**	**12.0**	**10.3**	**11.1**	**-9.0**
Rphq4	1.0	1.3	0.9	**14.3**	**25.4**	**14.3**	**25.4**	**44.7**	**-17.4**
Rphq5	0	0	0	**3.1**	**4.3**	**5.7**	**3.6**	**3.3**	**-4.6**
Rphq6	0	0	0.1	**5.3**	**7.7**	**7.9**	1.5	1.4	-3.0
Total[c]		**55.6**	**9.9**		**58.9**	**45.5**		**62.9**	**-35.9**

[a] the proportion of the explained phenotypic variance
[b] effects of the alleles from 'Vada'
[c] sum of the values of the significant QTLs (**Bold** font)

QTLs for days to heading and plant height

In a previous study a moderate correlation between days to heading and partial resistance was found (unpublished data). These two traits might be related to partial resistance. In present study, four QTLs were detected for days to heading


cM
0
10
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40
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170
180
190
1 (7H)
E33M55-508
E35M48-228
E35M54-93
E41M40-112
E42M51-267
E33M61-120
E37M32-99
E35M61-432
E42M32-195
Rphq1
E38M54-618
Dh1
Ph1
E35M55-458
E40M40-105
E39M61-372
E35M54-224
E35M55-450
E35M48-450
E40M38-363
E40M32-123
E33M61-173
Rphx
Rph3
E33M58-375
E40M32-168
E45M55-345
E35M54-180
2 (2H)
E35M54-222
E37M38-373
E33M55-410
Rph1
E42M48-308
E37M32-288
E42M48-270
Rphq6
Dh2
Ph2
E41M32-83
E41M32-156
E33M61-135
E42M51-85
E41M40-118
E33M61-281
E42M32-324
E33M54-187
Dh3
E33M55-109
E42M48-356
E41M32-492
E35M61-228
E38M54-390
E37M33-162
mPau
E33M58-224
E33M61-227
E33M54-307
E42M51-101
E38M54-134
E40M32-590
E37M38-199
E42M40-644
E37M33-501
E37M32-325
E33M55-297
Rphq2
E38M54-294
E42M48-376
3 (3H)
E45M55-572
E41M32-149
E35M48-250
E37M33-583
E33M54-244
E45M55-167
Rph7
E41M40-358
E38M54-315
E35M54-188
E37M32-471
Ph3
E45M55-274
E40M32-397
E35M48-410
E37M38-340
E42M40-445
E42M32-83
E35M55-410
E37M32-145
E33M58-81
E35M55-442
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E38M55-262
Rph10
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E33M61-200
E42M32-146

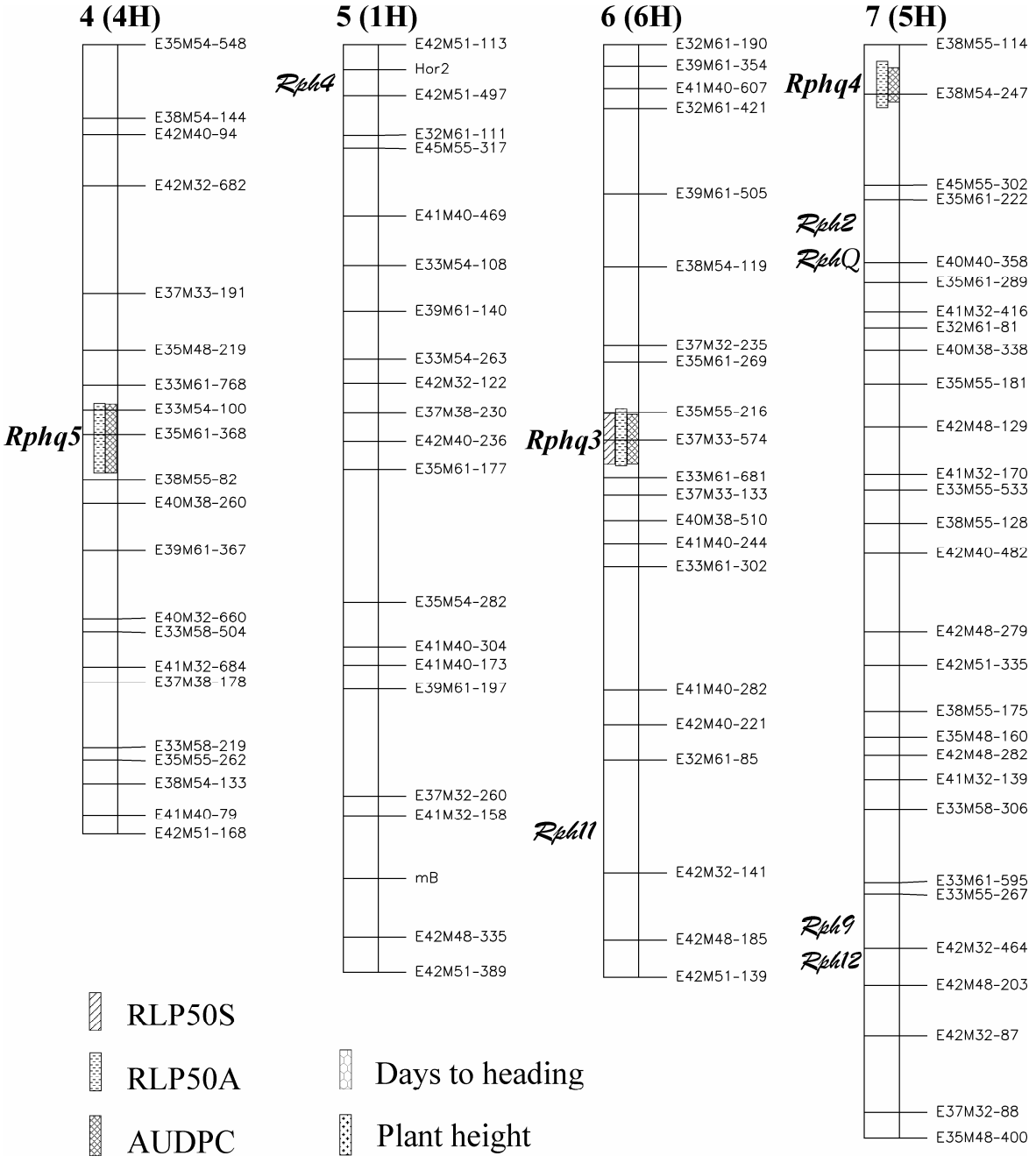

Fig. 3. Locations of QTLs for partial resistance to barley leaf rust and race-specific resistance genes (*Rph*), days to heading and plant height on the skeletal map, based on 103 RILs (F_9) from a cross L94 x 'Vada'. Chromosomes were oriented with the short arms at the top. Kosambi's mapping function was used. Names of QTLs are designated on the left side of each QTL. Boxes inside the chromosome bars are the QTLs for partial resistance (all resistance alleles are from 'Vada'). Boxes outside the chromosome bars are QTLs for days to heading and plant height, and with negative effects of the alleles from 'Vada' on the left side and positive effects of the alleles from 'Vada' on right side. Length of bars corresponds to two LOD support intervals (from peak) based on the results of MQM. The approximate locations of race specific resistance genes (*Rph* genes) are estimated from literatures.

and plant height, two of which were involved in both traits and the other two in only one of these traits (Fig. 3). One major-effect QTL, designated as *Dh2*, on the short arm of chromosome 2 explained 58% of the total phenotypic variance for days to heading (Table 3). A QTL with a moderate effect, *Dh3*, was identified at the putative centromeric region of chromosome 2. The three QTLs for days to heading explained together 70% of the total phenotypic variance. The three QTLs detected for plant height explained 65% of the phenotypic variance. Two main plant height QTLs, *Ph1* and *Ph2*, were mapped to the same positions as *Dh1* and *Dh2* respectively. Another one, *Ph3*, on chromosome 3, affected only days to heading but not plant height.

Table 3 Summary of QTLs for days to heading and plant height

	Days to heading (Dh)			Plant height (Ph)		
QTLs	LOD	Exp%[a]	Add[b]	LOD	Exp%	Add
Dh1, Ph1	**3.6**	**4.2**	**-1.0**	**11.2**	**23.7**	**-5.5**
Dh2, Ph2	**27.5**	**57.8**	**3.7**	**12.7**	**27.9**	**6.0**
Dh3	**7.0**	**8.5**	**1.5**	1.4	2.2	1.7
Ph3	0.2	0.3	-0.2	**7.0**	**13.5**	**-4.1**
Total[c]		**70.5**			**65.1**	

[a] the proportion of the explained phenotypic variance
[b] effects of the alleles from 'Vada'
[c] sum of the values of the significant QTLs (**Bold** font)

Model fitting of QTLs for partial resistance

Model fitting was applied to check to what extent the detected QTLs could account for the observed values (RLP50S and AUDPC). For each QTL, the nearest 'peak' marker (normally a 'cofactor' marker) was used to determine the QTL genotypes of each line (Table 4 and 5). The observed mean values per genotype class fitted well with the predicted values indicating that all major QTLs for partial resistance were correctly identified, despite possible errors of miss-classification of lines by using a single 'peak' marker to define the genotype.

Table 4 Fitted values of three QTLs for partial resistance of seedlings in the greenhouse tests (RLP50S)

Genotype[a]			**No. of**	**Observed**	**Fitted**
Rphq1	*Rphq2*	*Rphq3*	**RILs**	**mean**[b]	**value**[c]
B	B	B	23	122.2d	121.4
A	B	B	9	116.3cd	118.4
B	B	A	5	112.0bc	114.4
B	A	B	14	107.5ab	111.6
A	B	A	9	108.2ab	111.4
A	A	B	20	108.7abc	108.6
B	A	A	5	106.5ab	104.6
A	A	A	13	101.9a	101.6
Mean				111.5	
L94				100.0	
'Vada'				125.0	

[a] genotype classes of QTLs are based on the genotypes of the corresponding 'peak' markers, 'A' indicates L94 genotype and 'B' is 'Vada' genotype

[b] average value of each genotype class, values followed by the same letter do not differ significantly according to Waller-Duncan's test (P <= 0.05)

[c] the theoretical values calculated based on the population mean (μ) and the allelic effect of each QTL, i.e., a genotype class 'A A B' = 111.5 - 1.5 - 4.9 + 3.5 = 108.6

Additive effects of QTL for partial resistance

Three factor (three QTLs for RLP50S) and four factor (four QTLs for AUDPC) analyses of variance (data not shown) based on the values in Tables 4 and 5 gave only one highly significant interaction ($p <= 0.001$) between *Rphq1* and *Rphq2*, the QTLs for partial resistance at the seedling stage (Fig. 4) and no significant interaction among QTLs for resistance at adult plant stage. Previous genetic studies (Parlevliet 1976, 1978) indicated that six unlinked loci could be involved in RLP50A in 'Vada' relative to L94. Furthermore, one of the genes from 'Vada' was supposed to have a larger effect than the others, and with a recessive inheritance. The other genes acted in an additive way. In the present study we detected five QTLs for partial resistance with different quantitative

Table 5 Fitted values of four QTLs for partial resistance of adult plants in the field test (AUDPC)

Genotype[a]				**No. of**	**Observed**	**Fitted**
Rphq2	*Rphq3*	*Rphq4*	*Rphq5*	**RILs**	**mean**[b]	**value**[c]
B	B	B	B	10	74.2a	72.9
B	B	B	A	4	82.8ab	82.1
A	B	B	B	4	71.4a	82.7
B	A	B	B	2	92.0ab	90.9
B	B	A	B	6	107.8bc	107.7
A	B	B	A	6	90.2ab	91.9
B	A	B	A	4	98.6abc	100.1
A	A	B	B	1	100.5	100.7
B	B	A	A	10	117.1cd	116.9
A	B	A	B	5	113.3c	117.5
B	A	A	B	3	125.8cde	125.7
A	A	B	A	8	108.8bcd	109.9
A	B	A	A	15	130.1de	126.7
B	A	A	A	5	129.4de	134.9
A	A	A	B	6	141.3e	135.5
A	A	A	A	2	142.2de	144.7
Mean					108.8	
L94					153.3	
'Vada'					54.4	

[a] genotype classes of QTLs are based on the genotypes of the corresponding 'peak' markers, 'A' indicates L94 genotype, and 'B' is 'Vada' genotype

[b] average value of each genotype class, values followed by the same letter do not differ significantly according to Waller-Duncan's test (P <= 0.05)

[c] the theoretical values calculated based on the population mean (μ) and the allelic effect of each QTL, i.e., a genotype class 'B A B A' = 108.8 - 4.9 + 9.0 - 17.4 + 4.6 = 100.1

effects (RLP50A; Table 2). However, only 55-65% phenotypic variances were explained by the identified QTLs. With respect to the heritabilities of 0.6 to 0.9, most of the genetic variation were explained by these QTLs. Still it is possible

that some QTLs with smaller effects were not identified due to the small population size (103 RILs), and large genetic and environmental noises, or that epistatic loci contributing to partial resistance can not be detected with interval mapping where an additive model is applied.

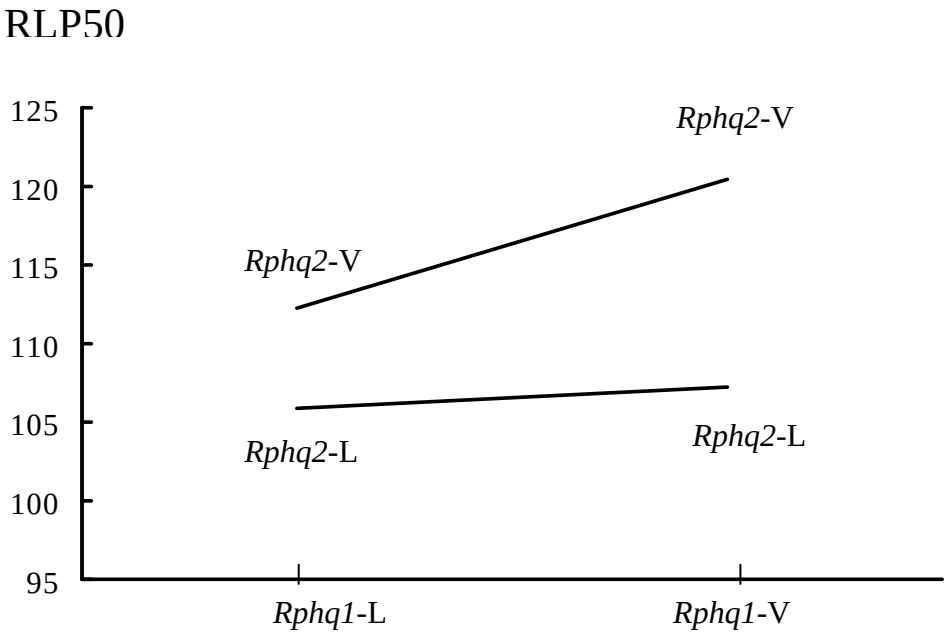

Fig. 4. Interaction of two QTLs, *Rphq1* and *Rphq2* based on seedlings tested in the greenhouse (RLP50S). Letters 'L' and 'V' following the QTL indicate the alleles of the corresponding QTL from L94 and 'Vada', respectively.

Discussion

Resolution of QTL mapping

In the present study, QTLs were identified by using a multiple QTL model which combines the interval mapping method with a multiple linear regression method (Jansen 1993). It is now widely recognised that simultaneous mapping of multiple QTLs is more efficient and more accurate than interval mapping which fits single QTL (Knapp 1991; Jansen and Stam 1994). Indeed, in most cases the QTLs identified by MQM in this research clearly showed higher LOD scores and lower background (sharper peaks) than interval mapping (Fig. 2). Moreover, by using the MQM method, the probability of detecting a QTL may

increase. For instance, *Rphq5* could not be detected by interval mapping, while by MQM, with taking the peak marker for QTL *Rphq4* as a cofactor, it could be identified as significant (LOD 3.6).

Though markers giving 'peak' LOD values in interval mapping are usually taken as cofactors in MQM, there are no good reasons not to take the imminent markers (within 5 cM) as cofactors. A sharp peak in the LOD profile may shift when imminent markers are applied as a cofactor for a QTL. This illustrates that also the sharp LOD peaks obtained with MQM should be taken with some caution when locating QTLs. To maximise the chance of assigning a QTL to the correct interval, one should apply large LOD differences for selection of a support interval or flanking markers when the LOD profiles generated by MQM are used. Therefore, we took two LOD support intervals.

Comparison to known major genes and/or mapped QTLs in barley

By using the 'Proctor' x 'Nudinka' AFLP and RFLP combined map (Becker et al. 1995) as a 'bridge', the present AFLP map of L94 x 'Vada' can be aligned with the integrated RFLP map which contains 880 RFLP markers (Qi et al. 1996). Consequently, previously mapped genes and QTLs can be compared with the presently identified QTLs. The two earliness QTLs, *Dh2* and *Dh3* on chromosome 2 (2H), may correspond to the two QTLs detected in the V. Gold x T. Prentice cross (Kjær et al. 1995). The chromosome region of *Dh2*/*Ph2* with the largest effects for both traits is likely the site of one of the early maturity (*Ea*) loci (Nilan 1964) and/or a photoperiod response gene, *Ppd-H1* (Laurie et al. 1994; Laurie et al. 1995). *Dh3* mapped in the same region as *eps2S*, a QTL for earliness per se, on chromosome 2 based on the genetic map of the Igri x Triumph cross. *Dh1* and *Ph1* on the short arm of chromosome 1 (7H) may correspond to the two very closely linked QTLs for earliness and plant height identified in the Steptoe x Morex cross (Hayes et al. 1993). These two QTLs were also detected in a two-row barley cross, Harrington x TR306 (Tinker et al. 1996). Another QTL (*Ph3*) for plant height on the short arm of chromosome 3 likely mapped to the same region of the plant height QTL detected in the Steptoe x Morex cross (Hayes et al. 1993). In conclusion: the QTLs for plant height and days to heading identified in present L94 x 'Vada' cross were in agreement with the previously mapped QTLs in various barley populations.

In barley, about fourteen race specific resistance genes to leaf rust (designated as *Rph* loci) have been reported (Jin et al. 1996). Recently, several resistance genes have been mapped on barley molecular maps (Fig 3). By using sequence tagged site (STS) and microsatellite markers, *Rph9* and *Rph12* were mapped at the same region of the long arm of chromosome 7 (5H) and later were found to be allelic (Borovkova et al. 1997). Moreover, on chromosome 7 (5H), *RphQ*, a presumed allele at the *Rph2* locus, was mapped on the short arm, near the centromere (B. Steffenson, pers. comm.). This location is quite far from the major QTL, *Rphq4*, mapped at the distal part of the short arm of this chromosome. Another leaf rust resistance locus designated as *RphX* was mapped to the long arm of chromosome 1 (7H) by using RFLP markers (Hayes et al. 1996). It may be allelic to *Rph3* that was also mapped to the similar position on this chromosome by using a morphological marker (Jin et al. 1993). *Rph4* was mapped on the short arm of chromosome 5 (1H) using the *Ml-a* locus as a genetic marker (McDaniel and Hathcock 1969). *Rph1* and *Rph7* were assigned to chromosome 2 (2H) (Tuleen and McDaniel 1971) and chromosome 3 (3H) (Tan 1978; Tuleen and McDaniel 1971) respectively by trisomic analysis, and was localized on the short arm and centromeric region of the corresponding chromosomes by using morphological markers (Roane and Starling 1989). *Rph10* and *Rph11* were assigned to the long arms of chromosome 3 (3H) and chromosome 6 (6H) respectively by using isozyme markers (Feuerstein et al. 1990). Interestingly, there is no indication that map positions are shared between race specific resistance genes (*Rph* loci) and the QTLs for partial resistance identified in the L94 x 'Vada' population. This implies different sets of genes and/or different evolutionary origin of these two types of resistance to barley leaf rust. Histological studies showed that the *Rph* resistance acts post-haustorially with hypersensitivity, whereas partial resistance is based on pre-haustorial mechanisms associated with the formation of papillae (Niks 1986).

Latency period is a major factor (or component) for partial resistance

The severity of rust epidemics in the field measured by AUDPC reflects the joint effects of all components for partial resistance such as infection frequency, latency period, spore production, infectious period and pustule size (Parlevliet

1979; Neervoort and Parlevliet 1978). In the partially resistant parent 'Vada' the barley leaf rust fungus has a lower infection freuqency, longer LP and lower spore production than on the susceptible line L94. LP is regarded the most effective of these components of resistance (Zadoks and Schein 1979; Parlevliet 1979). Indeed, all the QTLs that we detected for affecting the AUDPC were also found to influence the LP of adult plants in the greenhouse. Because of the moderate to high correlation between LP and the other components of partial resistance (Parlevliet 1986, 1992), we presume that some or all of these genes pleiotropically also govern the other components of partial resistance. We did not find QTLs that affect the AUDPC, but not the LP in adult plants. This suggests that in this population there are no genes segregating that substantially affect the epidemic progress without prolonging the LP in adult plants. Therefore, LP of the rust in adult barley plants is indeed a good predictor for partial resistance to leaf rust in the field.

Development dependent expression of genes for partial resistance

The often reported moderate correlation coefficient values between seedling data and adult plant data for partial resistance have suggested that during development of the plant, different genes are involved in the latency period and infection frequency of leaf rust in barley (Parlevliet and Kuiper 1977; Parlevliet and Van Ommeren 1975; Parlevliet 1975). By using QTL mapping, we have now resolved the partial resistance (latency period) into six QTLs with different quantitative effects and their dependence on plant development. Three QTLs (*Rphq4*, *Rphq5* and *Rphq6*) contributed to a longer latency period in the adult plant stage only. In contrast, *Rphq1* contributed to longer latency period in the seedling stage, but not in the adult plant stage. These results are in accordance with the previously reported relatively weak correlation between seedling and adult plant data.

Pleiotropic effects of QTLs

As has been reported for yield and its components in maize (Veldboom et al. 1994) and rice (Xiao et al. 1996), correlated traits often are associated with the same QTLs. Also in the present study of barley, we found that the highly correlated earliness and plant height ($r = 0.67$) were governed by the same two

QTLs. Moreover, the allelic effects were in the same directions for the QTLs of both traits (Table 3). Trait correlation may result from either pleiotropic effects of single genes or from tight linkage of several genes controlling the traits.

The same map position on the short arm of chromosome 2 was shared by a major-effect QTL, *Dh2*/*Ph2* for earlier heading and shorter plant height, and a moderate-effect QTL, *Rphq6,* for longer latency period in adult plants in the greenhouse (RLP50A). Minor effects (LOD ca. 1.5) were detected at this position using AUDPC data. However, based on the present results, it is difficult to conclude whether the same gene regulating plant development also affects partial resistance or tightly linked genes are mapped on the same region that can not be resolved by current QTL mapping.

Utilisation of the mapped QTLs for partial resistance in plant breeding

The present study has clearly demonstrated that QTLs prolonging LP in the adult plants are a major factor for partial resistance. Therefore, evaluation of LP in the flag leaf in the greenhouse is an efficient way to select for partial resistance in the progenies (of individual plants). By using marker assisted selection (MAS), molecular markers associated with the favourable QTL alleles for partial resistance can be applied in the early stage of plant development and consequently, improving the efficiency in the selection for partial resistance to leaf rust in the breeding programme. In view of the large number of polymorphisms that can be detected with AFLP, also within the European and North American barley germplasm (Qi and Lindhout 1997; Schut et al. 1997), the transfer of AFLP-tagged QTL-alleles from ‘Vada’ into other cultivars is now a feasible approach. In addition, more QTL alleles for partial resistance from other sources can be easily combined together by using MAS, resulting in a higher level of resistance. Furthermore, in a modern breeding programme, many favourable traits have to be integrated into a cultivar. By use of MAS, QTLs for partial resistance can be more efficiently incorporated in the cultivars to be released, thus offering better prospects for durable resistance as a breeding goal.

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6

Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley*

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Abstract: By using a high-density AFLP marker linkage map, six QTLs for partial resistance to barley leaf rust (*Puccinia hordei*) isolate 1.2.1. have been identified in the RIL offspring of a cross between the partially resistant cultivar 'Vada' and the susceptible line L94. Three QTLs were effective in the seedling stage, and five QTLs were effective in the adult plant stage. To study possible isolate specificity of the resistance, seedlings and adult plants of the 103 recombinant inbred lines from the cross, L94 x 'Vada', were also inoculated with another leaf rust isolate, isolate 24. In addition to the three QTLs that were also effective against isolate 1.2.1., an additional QTL for resistance of seedlings to isolate 24 was identified on the long arm of chromosome 7. Of the eight detected QTLs effective in the adult plant stage, three were effective to both isolates and five were effective to only one of the two isolates. Only one QTL had a substantial effect in both the seedling and the adult plant stage. The expression of the other QTLs was developmental stage specific. The isolate specificity of the QTLs supports the hypothesis of Parlevliet and Zadoks (1977) that partial resistance may be based on a minor gene-for-minor gene interaction.

Key words: Partial resistance, Barley, *Puccinia hordei*, QTL mapping, isolate-specificity, minor gene-for-minor gene

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Introduction

In many plant-pathogen systems two types of resistance occur side by side. One is based on a hypersensitive reaction and is clearly race-specific. This race-specificity has been explained by assuming a gene-for-gene interaction (Flor 1956, 1971). Van der Plank (1963, 1968) called this type of resistance a 'vertical' resistance. Molecular analysis of the cloned vertical resistance genes and the corresponding avirulence genes from several plant-pathogen systems have revealed that this model likely holds true at the molecular level (Van den Ackerveken et al. 1992; Joosten et al. 1994, 1997; Ellis et al. 1997; cf. review of Hammond-Kosack and Jones 1997), although the resistance gene product itself is probably not the receptor for the corresponding avirulence gene product (Kooman-Gersmann et al. 1998). Generally, this vertical resistance is associated with the hypersensitive response and is not durable.

The second type of resistance is quantitative, and in many cases not based on hypersensitivity. Such a quantitative resistance that is not based on hypersensitivity was coined 'partial' resistance by Parlevliet (1975). Partial resistance was initially considered race-non-specific and more durable, and therefore fitted Van der Plank's concept of 'horizontal' resistance. Van der Plank (1963, 1968) presumed that the quantitative resistance genes for horizontal resistance were equally effective to all pathogen isolates. However, more detailed observations showed that small but significant cultivar x isolate interactions may occur (Caten 1974; Clifford and Clothier 1974; Parlevliet 1976a, 1977). According to Parlevliet and Zadoks (1977), these interactions can only be explained by assuming a minor-gene-for-minor-gene interaction, similar to the system known in vertical resistance.

Nowadays, many quantitative traits, including quantitative resistance, have been resolved into discrete genetic loci (QTLs, quantitative trait loci). These QTLs were mapped on plant genomes by using molecular marker linkage maps (Paterson et al. 1988; Tanksley 1993; Young 1996). In barley, two QTLs for quantitative resistance to powdery mildew were identified by using the 'Proctor' x 'Nudinka' RFLP map (Heun 1992) and later, by using the 'Igri' x 'Danilo' map, two QTLs were detected for resistance to powdery mildew based on field data (Backers et al. 1996). One major-effect and one minor-effect QTL for

resistance to barley stripe rust (*Puccinia striiformis* f.sp. *hordei*) were mapped on barley chromosomes 7L and 4L (Chen et al. 1994). Several QTLs for resistance to leaf rust, stripe rust, mildew and *Rhynchosporium* were mapped on barley chromosomes by using 59 doubled haploid lines derived from a spring barley cross between cv Blenheim and a line, E224/3 (Thomas et al. 1995). One major, one moderate and two minor QTLs conferring quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) were identified and mapped on barley chromosomes by Pecchioni et al. (1996). By using the high-density 'Steptoe' x 'Morex' RFLP map, alleles of two or three unlinked loci were found to confer resistance to the net blotch pathogen (*Pyrenophora teres* f. *teres*) in the seedling stage, and seven QTLs were identified for resistance in the adult plant stage. A single gene was found to control resistance to the spot blotch (*Cochliobolus sativus*) pathogen in the seedling stage and two QTLs were detected for resistance in the adult plant stage (Steffenson et al. 1996). Recently, by using a high-density AFLP linkage map (Qi et al. 1998b), six QTLs for partial resistance to barley leaf rust (*Puccinia hordei*) isolate 1.2.1. have been identified in a recombinant inbred population from a cross between cultivar 'Vada' and the line L94 (Qi et al. 1998a). Three QTLs, *Rphq1*, *Rphq2* and *Rphq3*, were effective in the seedling stage, and five QTLs, *Rph2*, *Rphq3*, *Rphq4*, *Rphq5* and *Rphq6* were effective in the adult plant stage. These QTLs acted predominantly in an additive way; all of the resistance-enhancing alleles derived from the partially resistant parent 'Vada'.

In the investigations cited above, the question about race-specificity of partial resistance has not been touched upon. With the QTL approach however we are in a position to investigate to what extent QTLs that contribute to quantitative resistance are isolate- or race-specific in their action. Such an approach may throw light upon the existences of the minor gene-for-minor gene interaction hypothesized by Parlevliet and Zadoks (1977) as a basis for quantitative resistance. The aim of the present study is to resolve this question for the barley-barley leaf rust system. To this end seedlings and adult plants of the mapping population derived from the cross of L94 x 'Vada', used in our earlier study, have been inoculated with another rust isolate, i.e. isolate 24. Comparison of the QTLs for resistance to the two different isolates will reveal possible race-specificity of partial resistance genes.

Materials and methods

Plant material

A set of 103 F_9 recombinant inbred lines (RILs) derived from a cross of L94 x 'Vada' that was used to map QTLs for resistance to barley leaf rust (*Puccinia hordei*) isolate 1.2.1. (Qi et al. 1998a) was also used in this study. L94 is extremely susceptible, and 'Vada' has a high level of partial resistance to *P. hordei* (Parlevliet 1975, 1976b).

Leaf rust

Barley leaf rust isolate 24 was collected about 5 km south-east of Aalten in Achterhoek of the Netherlands in October, 1974. Isolate 1.2.1. which was used in our previous research (Qi et al. 1998a) was a monospore culture derived from isolate 1-2 which was collected in Wageningen in September 1971 (Parlevliet 1976a). A monospore subculture of both isolates was stored in liquid nitrogen. Fresh inoculum was produced on adult plants of the susceptible line L98. The isolates were reproduced in isolated greenhouse compartments in order to maintain their purity. The two isolates were tested on the differential series proposed by Clifford (1977) to which CI 1234, *Pa9* (*Rph9*), and 'Triumph', *Pa12* (*Rph12*), were added.

Map construction

Qi et al. (1998b) constructed a dense linkage map covering the barley genome (1062 cM), containing 566 AFLP markers (Qi and Lindhout 1997). A skeletal map with a uniform distribution of markers at approximately 5-cM distance was extracted and used for mapping QTLs for resistance to leaf rust isolate 24.

Disease evaluations in the seedling and in the adult plant stage

Leaf rust isolate 24 was used to inoculate seedlings and adult plants of the 103 RILs, and the two parents, L94 and 'Vada'. The method of evaluation for resistance to isolate 24 was the same as for that to isolate 1.2.1. (Qi et al. 1998a). Seeds from the mapping population were sown in two rows in small flats (30 x 30 cm). In each flat both parents were included. About 10 days after

sowing, the seedling leaves were fixed horizontally to the soil. Four to 5 seedlings per RIL were used for inoculations. Fresh urediospores (about 150 spores per cm^2 leaf area) were diluted 10 times with lycopodium spores and dusted over the adaxial sides of the seedling leaves in an inoculation tower. After incubation at a relative humidity of 100% overnight, the flats were moved into a greenhouse where temperature was set at about 18 ^oC. The latency period (LP50) of each plant was evaluated by estimating the period (in hours) at which 50% of the ultimate number of pustules became visible. The relative latency period in seedlings (RLP50S) was calculated relative to the LP50 of L94, where L94 = 100 (Parlevliet 1975). Three replications were conducted in the course of two years (1996 and 1997).

Inoculation of adult plants took place when the flag leaves were just unfolded. Fresh urediospores (about 150 spores per cm^2 leaf area) of isolate 24 were diluted 10 times with lycopodium spores and dusted over the plants in the incubation room. Afterwards, a relative humidity of 100% was set and plants were kept in the incubation room overnight. The next day, the plants were placed in a greenhouse at about 15-18 ^oC. The relative latency period in young flag leaves (RLP50A) was measured in the same way as the RLP50S. One experiment with three pots per RIL was carried out in 1997. Three to six young flag leaves per pot were observed for measuring of LP50. Because of the large number of RILs and their differences in earliness, five inoculations were conducted in the course of about one and a half months with one-week interval. The plants were grouped and inoculated according to the stage when the flag leaves were just unfolded. In each inoculation, several L94 and 'Vada' plants were always included as controls.

Statistical analysis

ANOVAs were calculated by using PROC GLM program (SAS Institute 1988). Wide sense heritabilities (h^2) for RLP50S and RLP50A were estimated based on the results from ANOVAs. A computer program, MapQTL version 3.0, developed by Van Ooijen and Maliepaard (1996), was applied for interval mapping (Lander and Botstein 1989) and multiple-QTL mapping (MQM) (Jansen 1993). Firstly, interval mapping was used to detect the region of putative QTLs. The marker with the highest LOD value was taken as co-factor

for running a multiple-QTL mapping program. This was repeated until a 'stable' LOD profile was reached. A LOD value of 3.0 was chosen as significant threshold value for declaring a QTL. In the paper, results obtained with MQM method are presented.

Results and discussion

Partial resistance to two leaf rust isolates

Partial resistance to two *P. hordei* isolates, isolate 1.2.1. and 24, was investigated and compared in this study. The identification of QTLs for partial resistance to isolate 1.2.1. has been described in our previous paper (Qi et al. 1998a). A test on a differential set of barley cultivars (data not shown) indicated

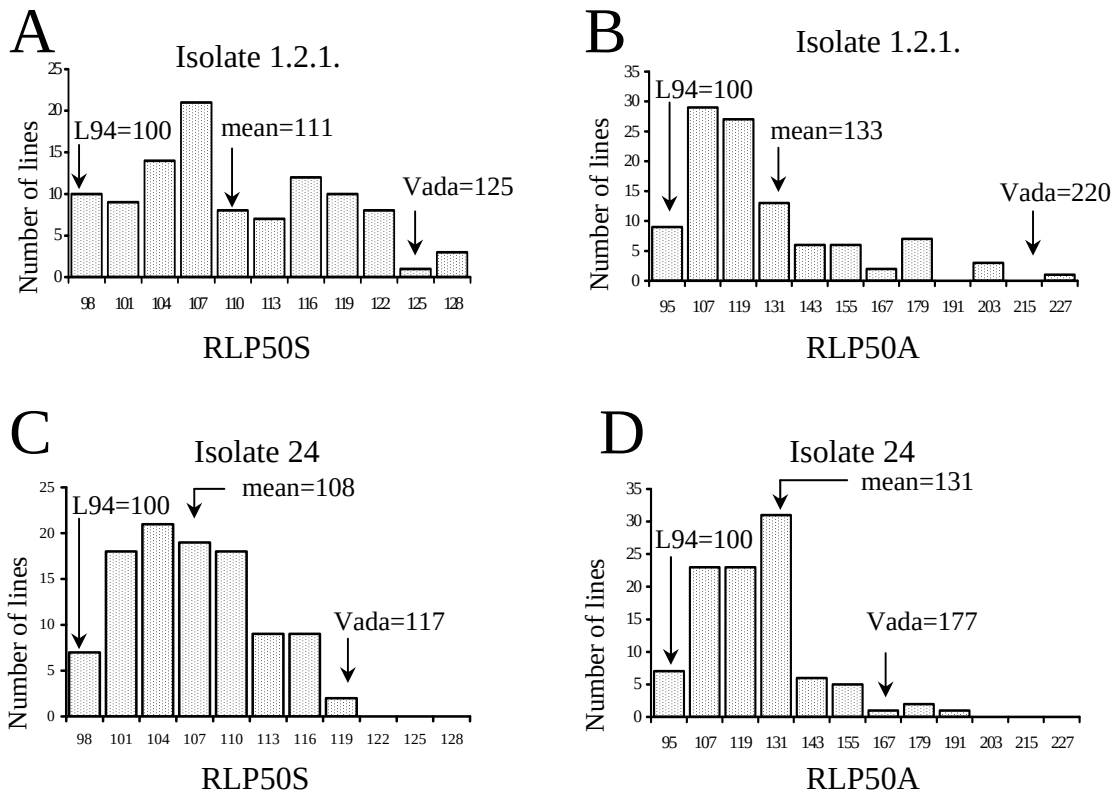


Fig. 1. Frequency distribution of phenotypes for the two measures of leaf rust resistance in 103 RILs derived from a cross L94 x 'Vada'. **A**: RLP50S of isolate 1.2.1., **B**: RLP50A of isolate 1.2.1., **C**: RLP50S of isolate 24, **D**: RLP50A of isolate 24. Values of L94 and 'Vada', and population mean values are shown by *arrow*. The values indicated on the *x*-axis are the lower limit of each category.

that the two isolates differed at least in their virulence spectrum to hypersensitivity resistance genes *Rph5*, *Rph8*, *Rph9* and *Rph12*. The relative latency period of isolate 24 was shorter in both seedlings and adult plants of 'Vada' than that of isolate 1.2.1. The average relative latency period of isolate 24 on the 103 RILs was also lower than that of isolate 1.2.1. (Fig. 1). This indicates that isolate 24 is more aggressive than isolate 1.2.1.

In the 103 RILs, both RLP50S and RLP50A of isolate 24 in the seedling and in the adult plant stage were approximately normally distributed (Figs. 1C and 1D). The relative latency periods of the RILs were between the values of the two parents, indicating absence of transgression. The wide sense heritabilities for RLP50S and RLP50A were 0.61 and 0.70, respectively.

A high correlation was found between RLP50S of isolates 1.2.1. and that of isolate 24 (Table 1). A moderate correlation was observed between RLP50A and RLP50S of isolate 24. The correlation between the RLP50A of the two isolates was weak but statistically significant.

Table 1 Correlation coefficients (*r*) among two measures of partial resistance to two isolates of leaf rust in 103 RILs derived from the cross L94 x 'Vada'

	RLP50S-1.2.1.[a]	RLP50A-1.2.1.[a]	RLP50S-24[b]
RLP50A-1.2.1.[a]	0.42**		
RLP50S-24[b]	0.81**	0.37**	
RLP50A-24[b]	0.66**	0.40**	0.69**

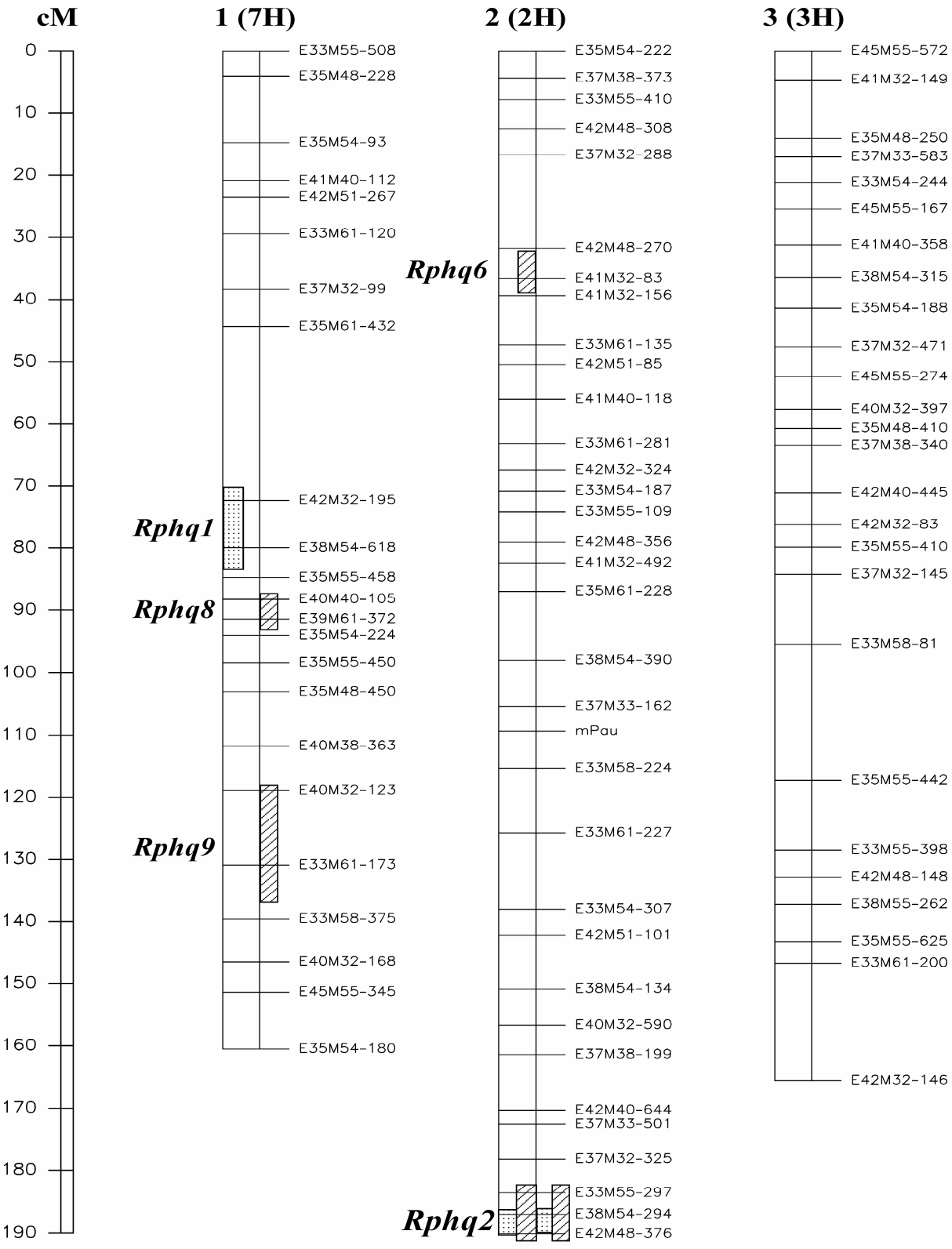
** p <= 0.01

[a] RLP50S-1.2.1. and RLP50A-1.2.1. are the RLP50 of isolate 1.2.1. measured on the 103 RILs in the seedling stage and in the adult plant stage respectively.

[b] RLP50S-24 and RLP50A-24 are the RLP50 of isolate 24 measured on the 103 RILs in the seedling stage and in the adult plant stage respectively.

QTLs for partial resistance to isolate 24

A multiple-QTL mapping method (Jansen 1993) was applied to identify QTLs for partial resistance to leaf rust isolate 24. A LOD value of 3.0 was set as threshold value for declaring a QTL. Seven QTLs (Table 2 and Fig. 2) were


cM
1 (7H)
2 (2H)
3 (3H)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
E33M55-508
E35M48-228
E35M54-93
E41M40-112
E42M51-267
E33M61-120
E37M32-99
E35M61-432
E42M32-195
Rphq1
E38M54-618
E35M55-458
E40M40-105
Rphq8
E39M61-372
E35M54-224
E35M55-450
E35M48-450
E40M38-363
E40M32-123
Rphq9
E33M61-173
E33M58-375
E40M32-168
E45M55-345
E35M54-180
E35M54-222
E37M38-373
E33M55-410
E42M48-308
E37M32-288
E42M48-270
Rphq6
E41M32-83
E41M32-156
E33M61-135
E42M51-85
E41M40-118
E33M61-281
E42M32-324
E33M54-187
E33M55-109
E42M48-356
E41M32-492
E35M61-228
E38M54-390
E37M33-162
mPau
E33M58-224
E33M61-227
E33M54-307
E42M51-101
E38M54-134
E40M32-590
E37M38-199
E42M40-644
E37M33-501
E37M32-325
E33M55-297
Rphq2
E38M54-294
E42M48-376
E45M55-572
E41M32-149
E35M48-250
E37M33-583
E33M54-244
E45M55-167
E41M40-358
E38M54-315
E35M54-188
E37M32-471
E45M55-274
E40M32-397
E35M48-410
E37M38-340
E42M40-445
E42M32-83
E35M55-410
E37M32-145
E33M58-81
E35M55-442
E33M55-398
E42M48-148
E38M55-262
E35M55-625
E33M61-200
E42M32-146

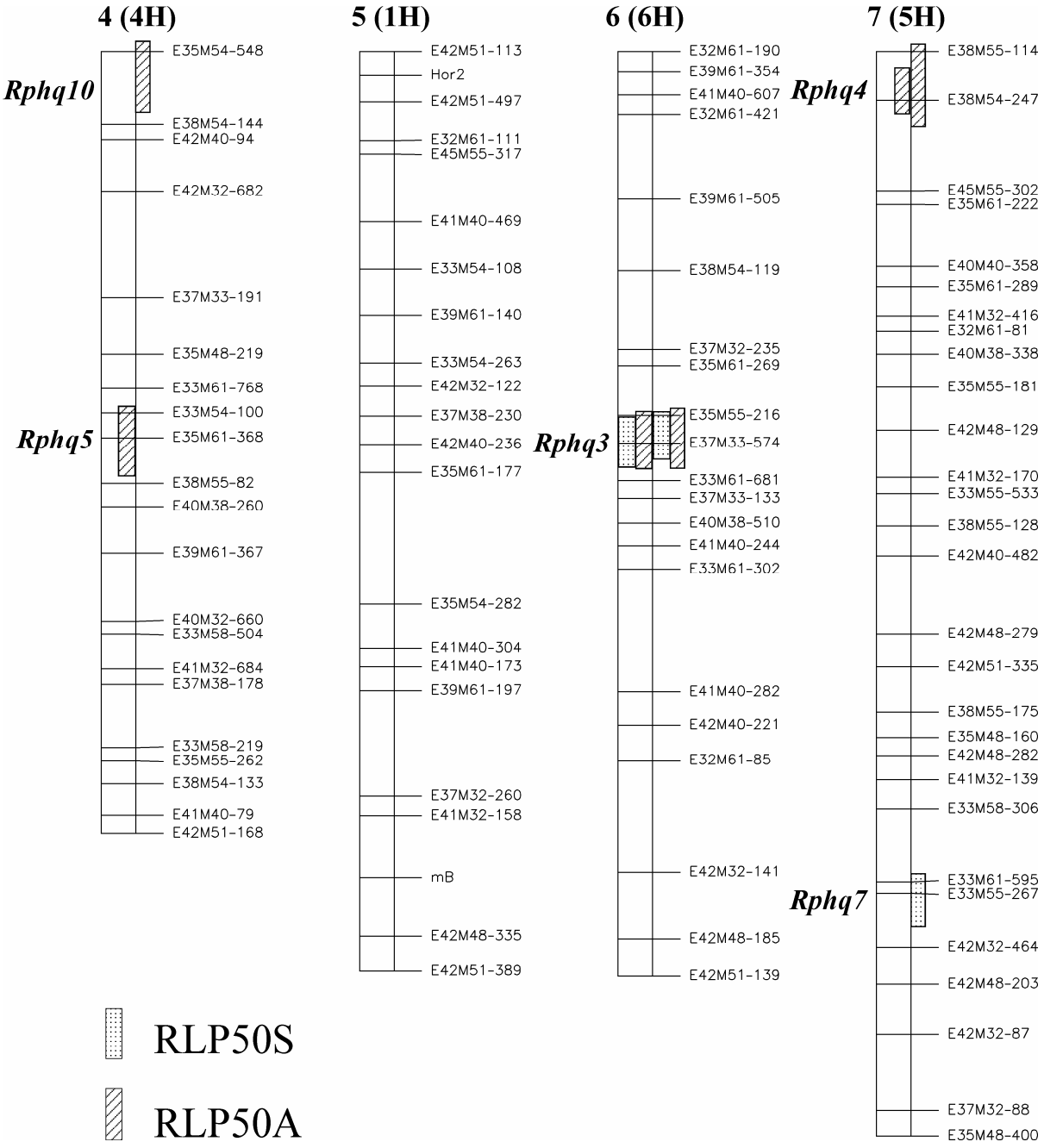


Fig. 2. QTLs for partial resistance to barley leaf rust on the skeletal map, based on 103 RILs from a cross L94 x 'Vada'. Chromosomes were oriented with the short arms at the top. Kosambi's mapping function was used. Names of QTLs are designated on the left side of each QTL. Boxes inside the chromosome bars are the QTLs for partial resistance to leaf rust isolate 1.2.1. Boxes on the right side of the chromosome bars are QTLs for partial resistance to isolate 24. Length of bars corresponds to two LOD support intervals (from peak) based on the results of MQM.

detected by using a skeleton map extracted from a high-density AFLP map (Qi et al. 1998b). Three QTLs for RLP50S were identified, that collectively explained 45% of the phenotypic variance. Six QTLs were detected for RLP50A (Table 2), together explaining 59% of the phenotypic variance. Comparison with the wide sense heritabilities (0.61 and 0.70 for RLP50S and RLP50A respectively) suggested that the most of the genetic variance was explained by these QTLs. The resistance alleles of the seven QTLs all originated from the partially resistant parent, 'Vada'. No resistance allele was identified originating from L94. This result is in accordance with the absence of clear transgression in the RILs.

Table 2 Summary of QTLs for partial resistance to leaf rust isolate 24

	RLP50S			RLP50A		
QTLs	LOD	Exp%[a]	Add[b]	LOD	Exp%	Add
Rphq7	**4.7**	**6.3**	**1.4**	-	-	-
Rphq2	**16.3**	**29.2**	**2.9**	**5.3**	**10.9**	**6.3**
Rphq3	**6.6**	**9.4**	**1.8**	**6.5**	**13.7**	**7.5**
Rphq4	-[d]	-	-	**4.5**	**9.1**	**6.0**
Rphq8	-	-	-	**4.7**	**9.4**	**6.4**
Rphq9	-	-	-	**4.2**	**7.1**	**5.2**
Rphq10	-	-	-	**3.1**	**6.1**	**4.7**
Total[c]		**44.9**	**6.1**		**58.9**	**36.1**

[a] the proportion of the explained phenotypic variance
[b] effects of the alleles from 'Vada'
[c] sum of the values of the significant QTLs (**Bold** font)
[d] only data with a LOD >= 3.0 are presented

Development stage dependent expression of QTLs

One QTL, *Rphq7*, was only effective in the seedling stage while four QTLs, *Rphq4*, *Rphq8*, *Rphq9* and *Rphq10* were only effective in the adult plant stage (Table 2). Such a development-dependent expression of genes for partial resistance was also observed in our previous research (Qi et al. 1998a). It also agrees with the moderate correlation between RLP50S and RLP50A. As was the case with isolate 1.2.1., also now *Rphq2* and *Rphq3* were the only two QTLs

effective in both plant development stages. However, *Rphq2* was strongly effective in the seedling stage (RLP50S) but only moderately in the adult plant stage (RLP50A). *Rphq3* is the only one with a consistent effect in both plant stages.

Isolate-specific QTLs for partial resistance

By applying the QTL mapping approach, the partial resistance to isolates 1.2.1. and 24 has been resolved into ten QTLs. These QTLs were mapped on the barley genome (Fig. 2). Four QTLs were effective in the seedling stage (Fig. 3A). Two of them, *Rphq2* and *Rphq3*, were consistently effective to both isolates, but had smaller effects to isolate 24 than to isolate 1.2.1.. *Rphq1* with a weak effect to isolate 1.2.1 (found in the previous study, Qi et al. 1998a) showed no significant effect to isolate 24. In contrast, *Rphq7* on the long arm of chromosome 7, was only effective to isolate 24. Isolate-specificity of QTLs for partial resistance was evident in the adult plant stage. Among the eight QTLs identified for resistance to the two isolates, two QTLs, *Rphq5* and *Rphq6*, were only effective to isolate 1.2.1. and three, *Rphq8*, *Rphq9* and *Rphq10*, only to isolate 24 (Fig. 3B). Three QTLs, *Rphq2*, *Rphq3* and *Rphq4* were effective to both isolates. The effects of *Rphq3* and *Rphq4* to isolate 24 were smaller than to isolate 1.2.1. while *Rphq2* had a similar effect to both isolates.

The relative latency period of isolate 1.2.1. in ‘Vada’ in both plant stages is much longer than that of isolate 24. Indeed, the isolate-non-specific QTLs, *Rphq2* (except for RLP50A), *Rphq3* and *Rphq4*, contributed smaller effects to isolate 24. However, more QTLs were detected for resistance to isolate 24 than to isolate 1.2.1. It seems that more genes (QTLs), but each with smaller effects were involved in resistance to isolate 24.

The map position on chromosome 1 of *Rphq8*, an isolate-specific QTL with a moderate effect to isolate 24 in the adult plant stage, coincided with a minor QTL for days to heading (*Dh1*) as shown in the previous study (Qi et al. 1998a). So far, it is still obscure whether this reflects two closely linked QTLs or a pleiotropic effect of one QTL.

The present study demonstrates that most QTLs for partial resistance are isolate-specific and show plant stage dependent expressions. Two major-effect QTLs, *Rphq2* and *Rphq3*, are expressed in both development stages and may be

isolate-non-specific. Only *Rphq4* shows development stage specific expression but is isolate-non-specific.

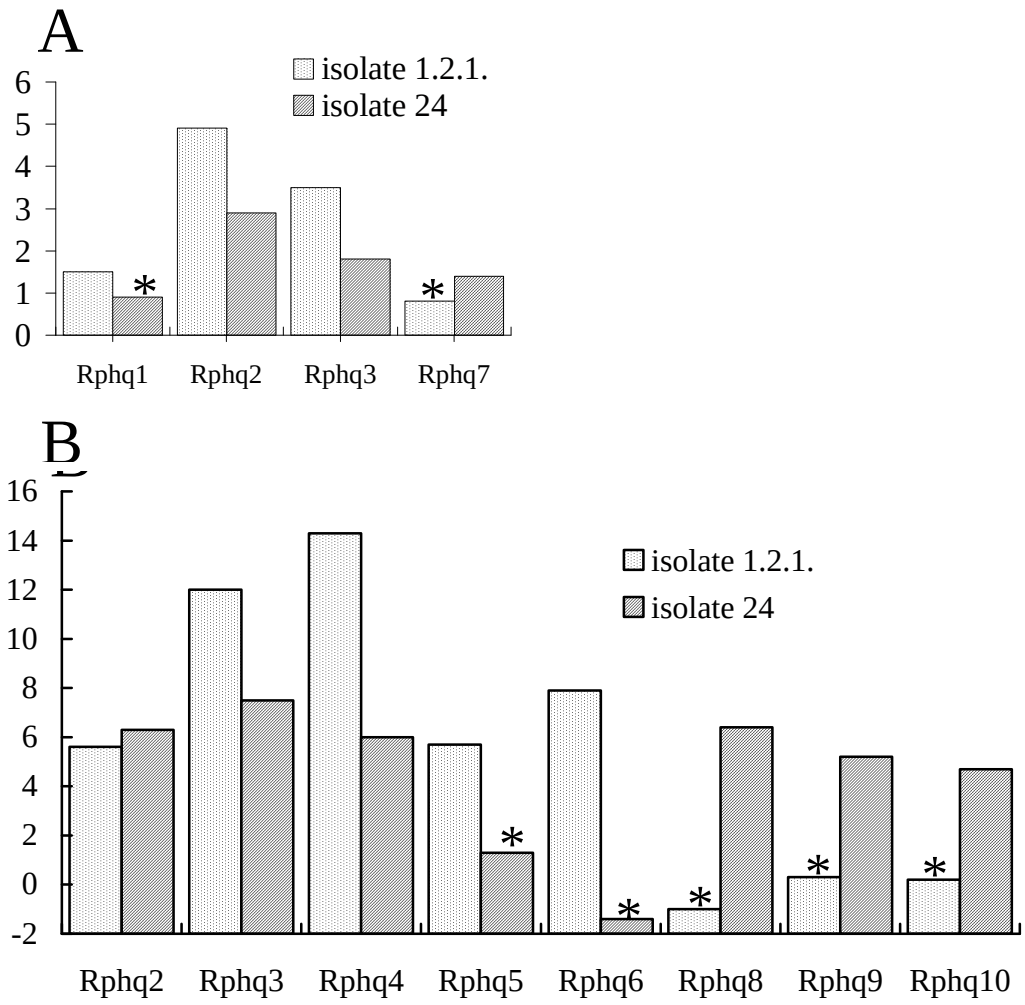


Fig. 3. Histogram of additive effects of each QTL for two leaf rust isolates in the seedling stage (**A**) and in the adult plant stage (**B**). *****: indicates that the effect of the QTL is not significant.

Minor gene-for-minor gene interaction

Isolate-specific QTLs for quantitative resistance to *Phytophthora infestans* were also identified in potato (Leonards-Schippers et al. 1994). Six of the 11 detected QTLs showed specificity to two *P. infestans* races. In mapping QTLs for resistance to bacterial wilt (*Pseudomonas solanacearum*) on the tomato genome, one of two major resistance loci was highly race-specific (Danesh and Young 1994). In addition, in the *Capsicum annuum-Potyvirus* host-pathogen system, isolate-specific effects of QTLs for resistance were clearly demonstrated (Caranta et al. 1997). In the present research, we studied the

barley-barley leaf rust pathosystem and detected clear isolate-specific effects of QTLs for partial resistance. More than 20 years ago, Parlevliet (1976a) reported small but significant cultivar x isolate interactions in partially resistant barley lines. This induced him to propose the 'minor gene-for-minor gene' hypothesis to explain quantitative (horizontal) resistance (Parlevliet and Zadoks 1977). Indeed, the examples mentioned above and the present data indicate that minor-gene-for-minor gene interactions do occur in plant-pathogen systems.

It is still questionable whether all resistance genes (major or minor) in the host population interact in a gene-for-gene manner with genes for virulence or avirulence in the pathogen population. Our study showed that the three major-effect QTLs were effective to both rust isolates and did not show clear isolate-specific effects. Similarly, five of 11 QTLs in potato showed no specificity to two *P. infestans* races (Leonards-Schippers et al. 1994). In pepper, one major-effect QTL was effective to all three potyvirus isolates tested (Caranta et al. 1997). However, it is easy to hypothesize, but hard to prove that all resistance genes are race- or isolate-specific and operate in a gene-for-gene manner. In our on-going studies of the barley-*Puccinia hordei* system, we are developing a series of near-isogenic lines (NILs) for each of the QTLs by using marker assisted selection. Each set of NIL-QTLs with identical genetic background will allow numerous QTL x rust isolate combinations to be tested. In addition, these NILs will serve as starting material for map-based cloning of QTLs for partial resistance.

Durability of partial resistance

The gene-for-gene theory was proposed in studies on the interaction between flax cultivars and flax rust (Flor 1956, 1971). There are numerous examples that testify that hypersensitivity resistance operating on a gene-for-gene basis is not durable. This does not imply, however, that resistance based on the gene-for-gene principle never can be durable. There are at least three considerations to explain durability in a polygenic resistance based on a minor gene-for-minor gene interaction.

Firstly, as Parlevliet and Zadoks (1977) argued, genes operating on a minor gene-for-minor gene basis would result in higher durability of resistance than genes with additive effects that are effective to all genotypes of the pathogen. In

the latter case, a mutant pathogen genotype with an increased aggressiveness would have a selection advantage on all host plants with any QTL for partial resistance, and as consequence, very soon replace the less aggressive pathogen strain in the population. Hence, such a resistance would not be very durable. In case the interaction acts according to the minor gene-for-minor gene principle, a mutation for increased aggressiveness in the pathogen would only increase the fitness of the pathogen on those host genotypes that have the minor gene for quantitative resistance that corresponds with the mutated aggressiveness gene. In genetically diverse host populations, this would lead to a rather mild increase of the pathogen with the mutant minor gene for increased aggressiveness, and hence, the resistance would be quite durable.

Secondly, it is generally accepted that breaking down of hypersensitivity resistance is the consequence of the deletion (Van den Ackerveken et al. 1992) or a mutation (Joosten et al. 1994, 1997) of the avirulence gene in the pathogen. This is a rather unspecific event, e.g. any mutation in the avirulence gene leading to a frame shift should result in virulence on a host genotype with the corresponding resistance gene. In partial resistance, we are concerned with a completely different plant defense system. The *Rphq* do not tend to coincide in the linkage map with the *Rph* genes for hypersensitivity (Qi et al. 1998a) and the resistance mechanism is entirely different, supporting the idea that the *Rph* and *Rphq* genes represent distinct classes of genes or gene families. The *Rph* gene resistance acts post-haustorially with hypersensitivity, whereas partial resistance is based on a pre-haustorial mechanism associated with the formation of papillae (Niks 1986). Therefore, it is very well conceivable that the gene-for-gene specificity in partial resistance is of a different nature. Breaking down this resistance may require very specific, and therefore rare mutations in the pathogen. This scenario would result in higher durability of resistance.

Thirdly, a polygenic resistance *per se* has a higher probability to be durably effective. In case the minor genes each have a different function in the defense, the pathogen can only negate this multiple barrier by step-wise genetic adaptation.

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7

Occurrence of QTLs for partial resistance to *Puccinia hordei* on the barley genome*

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Abstract: Partial resistance is a quantitative trait that is characterized by a reduced rate of epidemic development despite a susceptible infection type. By using AFLP markers, a linkage map was constructed based on a recombinant inbred population (117 RILs, F_8) derived from a cross between a susceptible line, L94, and a partially resistant line, 116-5. The constructed map showed a similar marker distribution pattern as the L94 x 'Vada' map. However, it contained more large gaps, and for some chromosome regions no markers were identified. These regions are most likely derived from L94 because 116-5 was selected from the progeny of a cross of L94 x cv Cebada Capa. Partial resistance to leaf rust isolate 1.2.1 was evaluated in the seedling stage in the greenhouse and in the adult plant stage in the field for the same population. Five QTLs for partial resistance to isolate 1.2.1. were mapped. Three QTLs were effective in the seedling stage, jointly contributing 42% to the total phenotypic variance. Three QTLs were effective in the adult plant stage, collectively explaining 35% of the phenotypic variance. Detection of two linked minor-effect QTLs effective in the adult plant stage was discussed. The major-effect QTL, *Rphq3*, was the only one that was effective in both developmental stages. Moreover, *Rphq3*, was also identified in the L94 x 'Vada' population, being effective to two rust isolates. The other QTLs were detected in either of the two populations, providing evidence of the existence of many loci for partial resistance to leaf rust on the barley genome. As

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already 13 QTLs for partial resistance were mapped, a strategy of accumulating many resistance genes in a single cultivar, resulting in a high level of partial resistance, is feasible.

Key words: Barley, Genetic linkage map, Partial resistance, QTL mapping, *Puccinia hordei*

Introduction

In the barley-barley leaf rust (*Puccinia hordei* Otth) plant-pathosystem, two distinct types of resistance occur. Hypersensitive resistance based on the *Rph* genes, formerly *Pa* genes, (Feuerstein et al. 1990; Jin et al. 1993, 1996; Roane and Starling 1967) has been extensively used in barley breeding programs. However, the great disadvantage of this resistance is its lack of durable effectiveness. As an alternative, partial resistance to leaf rust, defined as resistance that results in reduced epidemic development despite a compatible infection type (Parlevliet 1975; Parlevliet and Van Ommeren 1975), is widely present in barley (Parlevliet et al. 1980; Alemayehu and Parlevliet 1996). Such a partial resistance occurs in numerous biotrophic plant-pathosystems and is presumed to have durable effectiveness. Partial resistance is associated with various components (Parlevliet 1979), such as lower infection rate, longer latency period, smaller pustule size and reduced spore production, that can be measured in monocyclic disease tests in the greenhouse. Of these components the latency period (LP) on mature plants is the best predictor of the level of partial resistance in the field (Parlevliet 1986, 1992).

By use of a high-density AFLP marker linkage map (Qi et al. 1998c), ten QTLs for partial resistance to barley leaf rust in a mapping population from a cross of L94 x ‘Vada’ have been identified (Qi et al. 1998a, 1998b). They are designated as *Rphq* loci. These QTLs act predominantly in an additive fashion. The estimated sizes of the effects of *Rphq* genes differ and the expression of several of these genes are plant stage specific. In addition, most of these QTLs show a differential expression against two rust isolates, supporting the idea that partial resistance operates according to a ‘minor gene-for-minor gene’ model (Parlevliet and Zadoks 1977). The positions of the identified QTLs on the linkage map do not coincide with those of hypersensitive resistance genes (*Rph*

genes). This supports the hypothesis that partial resistance and hypersensitive resistance are two fundamentally distinct types of defense, as was indicated in histological studies (Niks, 1986).

Genetic mapping of quantitative resistance genes has also been conducted in many other plant-pathosystems (see review of Young 1996). In an experiment to detect QTLs for resistance to gray leaf spot in maize, three populations were used (Bubeck et al. 1993). Among more than ten QTLs detected, only one was expressed in all three populations and environments. It indicated that many more QTLs for resistance to this fungus could exist in the maize germplasm. In studies on partial resistance to barley leaf rust (Parlevliet and Kuiper 1985; and Parlevliet et al. 1985), transgression for partial resistance was observed in the offsprings of a cross between cv Vada and cv Cebada Capa. This implies that at least some of the genes for partial resistance in 'Cebada Capa' are at different chromosome positions. 'Cebada Capa' also possesses a gene (*Rph7*) for hypersensitive resistance. One line, 116-5, was derived from a cross between L94 and 'Cebada Capa' by selection against *Rph7* and for a high level of the partial resistance. Using a recombinant inbred (RI) population derived from a cross between this line and the susceptible line L94, an AFLP molecular map was constructed, and more QTLs for partial resistance to barley leaf rust were identified.

Materials and methods

Plant materials

A barley line, L94, which is extremely susceptible to leaf rust (*Puccinia hordei* Otth), was crossed to a partially resistant barley line, 116-5. By applying the single-seed descent (SSD) method, a recombinant inbred (RI) population (F_8) containing 117 lines was derived from this cross. Line 116-5 was derived from a cross between L94 and cv Cebada Capa (Fig. 1). The latter not only has a high level of partial resistance but also has an effective gene for hypersensitive resistance, *Rph7* (Niks and Kuiper, 1983). To eliminate *Rph7*, selection against hypersensitive resistance (for high infection type) was carried out in the F_2 generation. By line selection for a high level of partial resistance, the partially resistant line 116-5 was developed.

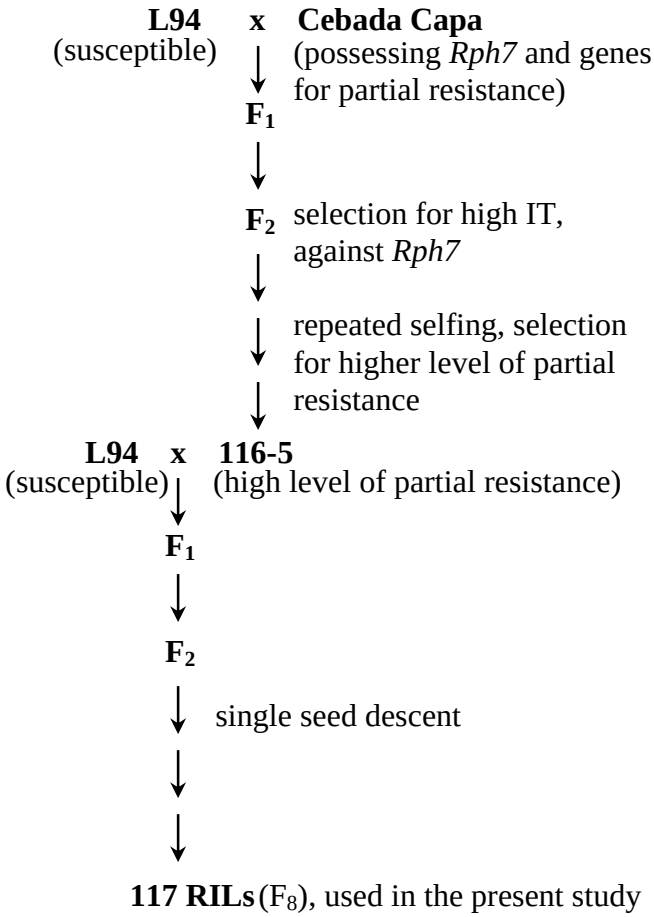


Fig. 1. The ancestry of the partially resistant line 116-5 and the L94 x 116-5 recombinant inbred population.

Disease evaluation in the seedling stage

The procedure for the evaluation of latency period (LP) in the seedling stage was as described in Qi et al. (1998a, 1998b). Seedling leaves of both parental lines, L94 and 116-5, and their progeny, 117 RILs (F_8), were inoculated with the leaf rust isolate 1.2.1 in the greenhouse in three replications. The relative latency period in the seedling stage (RLP50S) was calculated relative to the LP of L94, where L94 = 100, as described by Parlevliet (1975).

Disease evaluation in the field

Two field experiments were carried out, in 1995 and 1997, respectively. The experiment of 1995 had no replications, while in 1997 a randomized complete block design with three replications was applied. Oat was grown between the barley plots to limit inter-plot interference (Parlevliet and Van Ommeren 1984). The inoculation procedure in the field with the monospore culture derived

isolate 1.2.1. was as described by Qi et al. (1998a). In the experiment of 1995, 10 tillers per plot were sampled on July 10, 13 and 19, respectively, for evaluation of the infection frequency according to the scale of Parlevliet and Van Ommeren (1984). In 1997, three tillers per plot were sampled, and five observations were conducted on June 5, 13, 24, 30 and July 6 for all three replications. However, due to dry weather at inoculation time, the epidemics of leaf rust did not develop in some blocks or parts of a block. For the calculation of area under disease progress curve (AUDPC) and for further analyses, these plots/blocks were not taken into account.

Marker generation and map construction

The AFLP protocol was applied as described before (Qi and Lindhout 1997). Genomic DNA was isolated and digested with the restriction enzymes, *Eco*RI and *Mse*I. The corresponding adapters and primers were the same as described in Qi and Lindhout (1997) and are also available via Internet at "GrainGenes WWW Page, map data". Twenty-seven primer combinations were used (Table 1). AFLP marker names were according to the AFLP profiles of 16 reference barley lines (GrainGenes WWW Page, map data). Two qualitative traits, i.e., black/white seeds and two-row/six-row spike (for L94 and 116-5 respectively) were scored as morphological markers, named m*B* and m*hex-v*, respectively. JoinMap 2.0 (Stam 1993; Stam and Van Ooijen 1996) was used to group the linked markers and to construct the genetic map. AFLP markers common to the L94 x 'Vada' population were used to assign linkage groups to the corresponding barley chromosomes. Kosambi's mapping function was applied for map distance calculation (Kosambi 1944).

Statistical analysis

Because of some missing values, the least square estimate means of RLP50S and AUDPC of the 1995 and 1997 experiments, and the ANOVAs were calculated by using PROC GLM of the SAS package (SAS Institute 1988). The wide sense heritabilities (h^2) of two measures of partial resistance were estimated based on the corresponding mean squares from the ANOVA. Both interval mapping (Lander and Botstein 1989) and multiple-QTL mapping (MQM) (Jansen and Stam 1994; Jansen 1996), available in a computer software

package, MapQTL version 3.0 (Van Ooijen and Maliepaard 1996), were used for mapping QTLs. A LOD score of 3.0 was chosen as significance threshold value for declaring a QTL.

Results

Map construction

By using 27 primer combinations, 281 AFLP markers were generated in the present mapping population, yielding an average of 10 markers per primer combination (Table 1). Of these 27 primer combinations, 17 had been used previously for the construction of the L94 x 'Vada' AFLP map (Qi et al. 1998c) resulting in 105 markers in common between the two populations. One marker, E39M61-360 (Fig. 2 indicated by *), was formerly mapped to chromosome 7 of the L94 x 'Vada' map (Qi et al. 1998c), was assigned to chromosome 2. The remaining 104 marker were used as "anchors" to assign marker linkage groups to barley chromosomes. The 283 markers (281 AFLP and two morphological) were split into 16 linkage groups at a LOD threshold grouping value of 4.0. Except one unlinked marker (E38M51-371) and one group of two markers (E32M55-613 and E42M32-490), the remaining 14 linkage groups, containing 280 markers, with at least one anchor marker per group, were assigned to the

Table 1. Number of AFLP markers generated in the L94 x 116-5 population

Primer combinations	No. of Markers[a]	No. of marker in common[b]
E32M55	12	-
E38M50	13	-
E38M51	10	-
E38M59	12	-
E38M60	11	-
E38M62	5	-
E39M55	7	-
E40M32	4	-
E45M49	22	-
E45M58	7	-
E32M61	12	8
E33M54	8	3
E33M55	11	7
E33M61	10	6
E35M48	13	8
E35M54	7	2
E35M55	9	7
E35M61	5	4
E37M33	12	6
E37M38	11	3
E38M54	9	7
E39M61	11	7
E41M32	9	7
E41M40	11	9
E42M32	18	9
E42M40	15	7
E45M55	7	5
Total	281	105

[a] number of AFLP markers in the L94x116-5 population.
[b] number of markers in common with the L94x'Vada' population.

barley chromosomes. By using JoinMap 2.0 (Stam and Van Ooijen 1996), a linkage map was successfully constructed (Fig. 2).

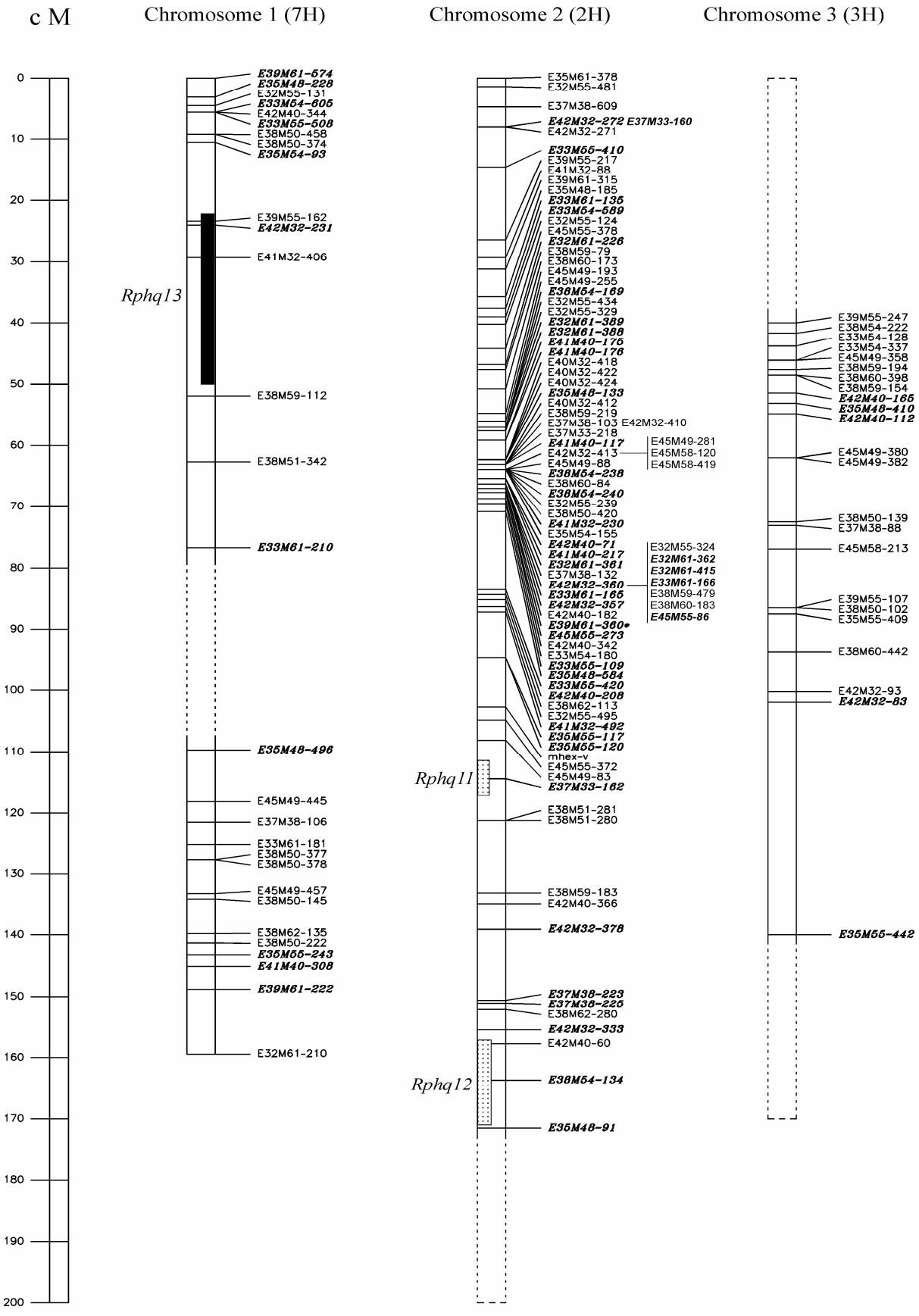
Table 2 Summary of L94 x 116-5 mapping data

Chromosome	No. of markers	Length (cM)
1 (7H), short arm	15	77
1 (7H), long arm	14	50
2 (2H)	93	172
3 (3H)	23	100
4 (4H), short arm	6	37
4 (4H), long arm	13	67
5 (1H)	53	137
6 (6H)	46	84
7 (5H)	17	133
Total	280	857

The linkage map covers a total map distance of 857 cM, corresponding to an average density of 3 cM per marker (Table 2). Markers assigned to chromosomes 1 and 4 were grouped into two linkage groups and two separate linkage maps were constructed for each of the chromosomes. Alignment of the present maps with the L94 x 'Vada' chromosome map revealed large gaps around the putative centromeric regions on chromosomes 1 and 4. (Fig. 2, the dotted lines). In the distal regions of chromosomes 2, 3, 6 and 7, also no dimorphic AFLP markers were found. About one-third of the markers were mapped on chromosome 2. Chromosome 5 was the only chromosome that was equally well-covered in the L94 x 'Vada' map and in the current map. The positions of two morphological markers, *m*hex-v and *m*B, on chromosomes 2 and 5, respectively, were in agreement with earlier reports (Franckowiak 1995, Jensen 1996, Qi et al. 1996, 1998c).

QTLs for partial resistance

Analysis of variance revealed highly significant differences among the 117 RILs for both AUDPC and RLP50S. Since analysis of the AUDPC data did not


c M
Chromosome 1 (7H)
Chromosome 2 (2H)
Chromosome 3 (3H)
0
10
20
30
40
50
60
70
80
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110
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130
140
150
160
170
180
190
200
E39M61-574
E35M48-228
E32M55-131
E33M54-605
E42M40-344
E33M55-508
E38M50-458
E38M50-374
E35M54-93
E39M55-162
E42M32-231
E41M32-406
Rphq13
E38M59-112
E38M51-342
E33M61-210
E35M48-496
E45M49-445
E37M38-106
E33M61-181
E38M50-377
E38M50-378
E45M49-457
E38M50-145
E38M62-135
E38M50-222
E35M55-243
E41M40-308
E39M61-222
E32M61-210
E35M61-378
E32M55-481
E37M38-609
E42M32-272 E37M33-160
E42M32-271
E33M55-410
E39M55-217
E41M32-88
E39M61-315
E35M48-185
E33M61-135
E33M54-669
E32M55-124
E45M55-378
E32M61-226
E38M59-79
E38M60-173
E45M49-193
E45M49-255
E38M54-169
E32M55-434
E32M55-329
E32M61-389
E32M61-388
E41M40-175
E41M40-176
E40M32-418
E40M32-422
E40M32-424
E35M48-133
E40M32-412
E38M59-219
E37M38-103 E42M32-410
E37M33-218
E41M40-117
E42M32-413
E45M49-88
E38M54-238
E38M60-84
E38M54-240
E32M55-239
E38M50-420
E41M32-230
E35M54-155
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E32M61-361
E37M38-132
E42M32-360
E33M61-166
E42M32-357
E42M40-182
E39M61-360*
E45M55-273
E42M40-342
E33M54-180
E33M55-109
E35M48-584
E33M55-420
E42M40-208
E38M62-113
E32M55-495
E41M32-492
E35M55-117
E35M55-120
mhex-v
E45M55-372
E45M49-83
E37M33-162
E45M49-281
E45M58-120
E45M58-419
E32M55-324
E32M61-362
E32M61-415
E33M61-166
E38M59-479
E38M60-183
E45M55-86
Rphq11
E38M51-281
E38M51-280
E38M59-183
E42M40-366
E42M32-378
E37M38-223
E37M38-225
E38M62-280
E42M32-333
E42M40-60
Rphq12
E38M54-134
E35M48-91
E39M55-247
E38M54-222
E33M54-128
E33M54-337
E45M49-358
E38M59-194
E38M60-398
E38M59-154
E42M40-165
E35M48-410
E42M40-112
E45M49-380
E45M49-382
E38M50-139
E37M38-88
E45M58-213
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E42M32-83
E35M55-442

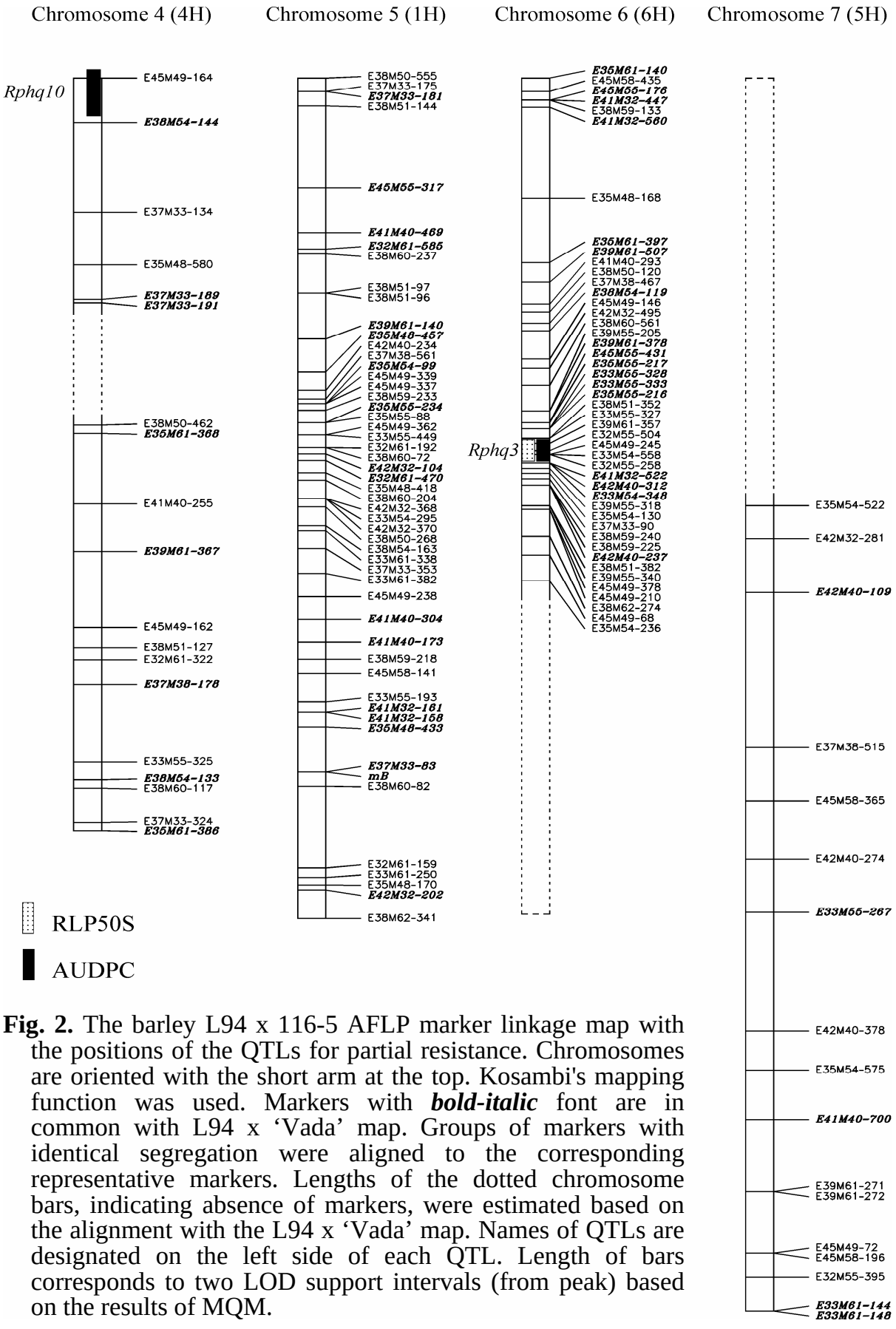


Fig. 2. The barley L94 x 116-5 AFLP marker linkage map with the positions of the QTLs for partial resistance. Chromosomes are oriented with the short arm at the top. Kosambi's mapping function was used. Markers with ***bold-italic*** font are in common with L94 x 'Vada' map. Groups of markers with identical segregation were aligned to the corresponding representative markers. Lengths of the dotted chromosome bars, indicating absence of markers, were estimated based on the alignment with the L94 x 'Vada' map. Names of QTLs are designated on the left side of each QTL. Length of bars corresponds to two LOD support intervals (from peak) based on the results of MQM.

show significant 'Year x RIL' interaction, the 1995 experiment was treated as another replication. Due to some missing observations, the least square estimate means of AUDPC and RLP50S of the 117 RILs were calculated from the four and three replications, respectively. The frequency distribution of AUDPC and RLP50S were approximately normal (Fig. 3). The RLP50S and AUDPC values of the most extreme RILs were similar to those the two parents, indicating absence of transgression for partial resistance. The wide sense heritabilities (h^2) in the seedlings stage (RLP50S) and in the adult plant stage (AUDPC) were 0.72 and 0.51, respectively. A moderate correlation was observed between RLP50S and AUDPC (r = -0.52).

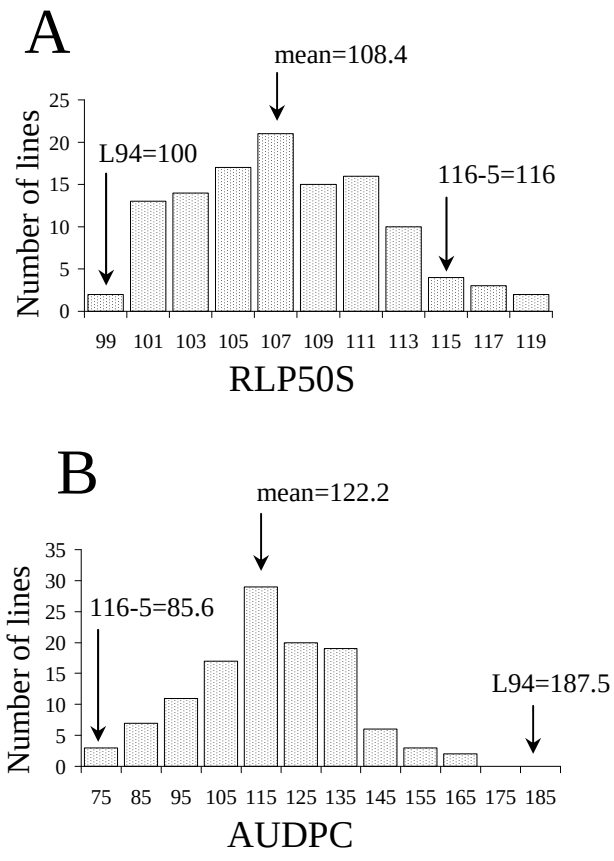


Fig. 3. Frequency distribution of phenotypes for the two componets of leaf rust resistance in 117 RILs derived from the cross L94 x 116-5. **A**: RLP50S, **B**: AUDPC. Values of L94 and 116-5, and population mean values are shown by *arrow*. The values indicated on the *x*-axis are the lower limit of each category.

Five QTLs for partial resistance to isolate 1.2.1. were identified (Fig. 2 and Table 3). Three QTLs were effective in the seedling stage, jointly contributing 42% of the total phenotypic variance. Two of those QTLs, *Rphq3* and *Rphq11*, had relatively large effects, and were mapped on chromosomes 6 and 2, respectively. Three QTLs were effective in the adult plant stage, together explaining 35% of the phenotypic variance. A major-effect QTL, *Rphq3*, explaining 20% of the phenotypic variance, was mapped to the centromeric region of chromosome 6. Also, *Rphq3* was the only QTL that was effective in both plant development stages. *Rphq11* and *Rphq12* were only effective in the seedling stage, and *Rphq10* and *Rphq13* only in the adult plant stage. All of the resistance-enhancing alleles of the five QTLs originated from the partially resistant parent 116-5. This is in accordance with the absence of transgression. Three factor analysis of variance based on

the genotype classes of three QTLs showed that there were no significant two-way and three-way interactions among the identified QTLs for partial resistance in both development stages (not shown). Therefore, the genes have mainly additive effects on the level of partial resistance.

Table 3 Summary of QTLs for partial resistance to barley leaf rust

	RLP50S			AUDPC		
QTLs	LOD	Exp%[a]	Add[b]	LOD	Exp%	Add
Rphq11	14.2	20.0	2.3	-	-	-
Rphq12	3.5	4.5	1.0	-	-	-
Rphq3	12.8	16.9	2.2	10.1	20.2	-8.6
Rphq13	-	-	-	3.7	9.2	-5.7
Rphq10	-	-	-	3.1	5.5	-4.5
Total[c]	-	**41.9**	**5.5**	-	**34.9**	**-18.8**

[a] the proportion of the phenotypic variance explained
[b] effects of the alleles from 116-5
[c] sum of the values of the significant QTLs

Minor-effect QTLs for partial resistance

The three QTLs (LOD >= 3.0) for partial resistance in the seedlings stage explained 42% of the total genetic variance, whereas the three QTLs expressed in the adult plant stage explained 35% of genetic variance. Comparison with the heritabilities (0.72 for RLP50S and 0.51 for AUDPC) showed that 60 – 70% of the genetic variance was explained by the declared QTLs. Actually, in addition to the five declared QTLs, several other chromosome regions showed LOD scores between 2.0 and 3.0, that may correspond to even more minor-effect QTLs.

There is strong evidence for two linked QTLs for AUDPC on chromosome 2 near the map position of 100 cM, within a distance of about 20 cM. One of these QTLs coincides with *Rphq11*, which is also affecting RLP50S. Fig. 4 shows LOD profiles for chromosome 2 obtained with interval mapping and MQM mapping, the latter by using cofactors at varying positions in the region of interest. Interval mapping gives a profile with two, not clearly separated, peaks, both above the threshold value 3.0. In order to verify whether these peaks corresponded to two QTLs, we introduced cofactors at either of these peak positions and also at both peak positions simultaneously. With all these cofactor

configurations, the LOD profile clearly showed two separate peaks. Although the two peaks are not simultaneously above the threshold value (3.0), this pattern was taken as a strong evidence for the existence of two QTLs. Since there is no clear guideline for the significance threshold for such a configuration of QTLs, we have further investigated this by means of simulation. Analysis of the simulated data (using a population of the same size and QTL effects of similar size as the estimated effects) showed that in case of a single QTL, the LOD profiles, obtained by changing the choice of markers as cofactors, do not show two clearly separated peaks. Thus we hypothesize that there is another QTL affecting AUDPC on chromosome 2, at a map position of about 95 cM. Therefore, the QTL at position of approximately 115 cM (*Rphq11*) not only has an effect in the seedling stage (Table 3, Fig. 2), but also in the adult plant stage. In addition, at a map position of 95 cM there may be a minor-effect QTL contributing to the partial resistance of adult plants. However, to designate these minor-effect QTLs further experiments are required.

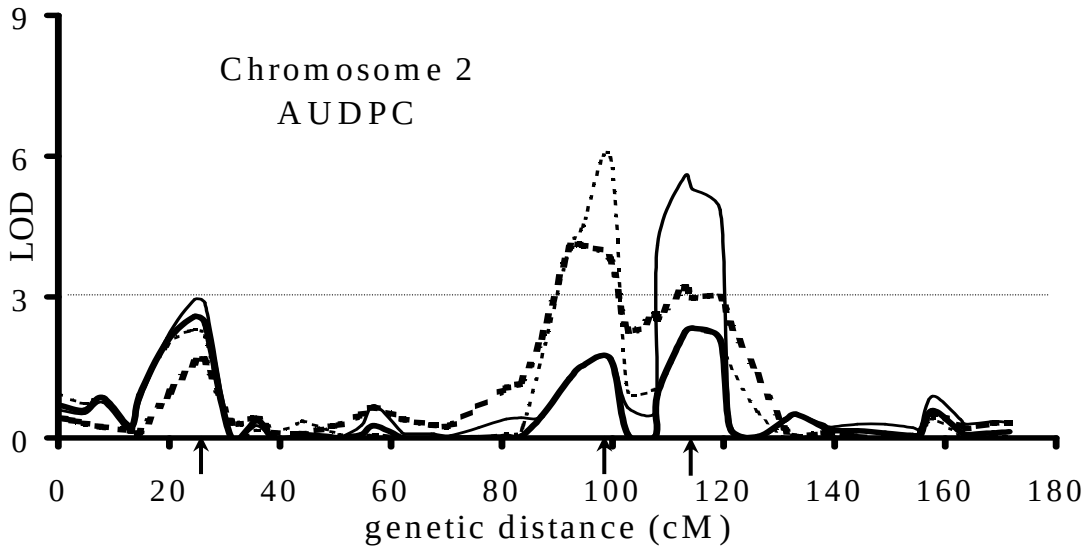


Fig. 4. LOD profiles of two linked QTLs on chromosomes 2. The *arrows* indicate the positions of the markers taken as cofactors for the MQM analysis. The thick dotted line is based on interval mapping and the thick solid line is based on MQM with seven cofactors, including the two at both peak positions (at 95 and 115 cM) simultaneously. The thin dotted and the thin solid lines were from MQM with taking six cofactors, including the one either at the peak position of about 95 cM or at the peak position of about 115 cM. The chromosome is oriented with the short arm to the left and corresponds to the map shown in Figure 2.

Discussion

Alignment and comparison of two AFLP linkage maps

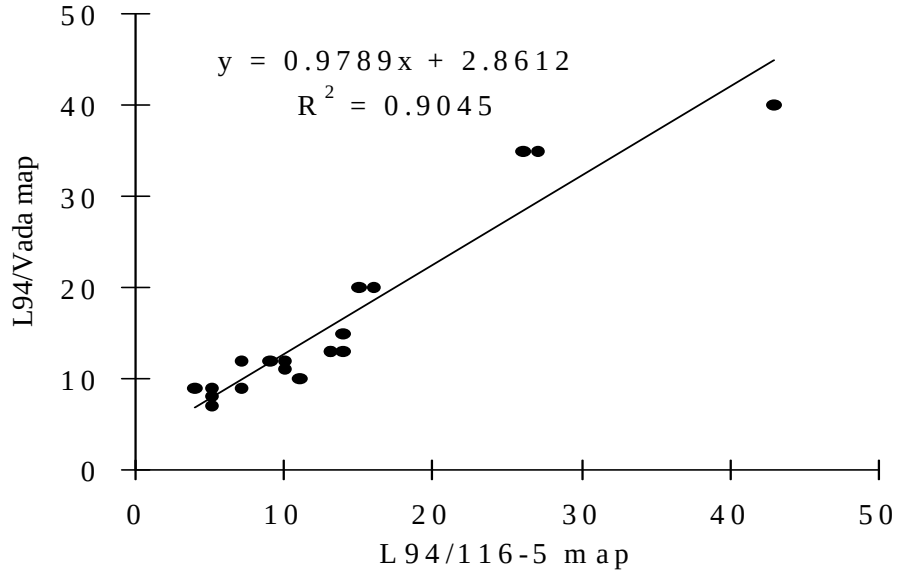
Since L94 was a parent for two mapping populations, L94 x 'Vada' and L94 x 116-5, the two corresponding linkage maps have a large number of markers in common. These common markers enabled the alignment of the present map with the L94 x 'Vada' AFLP linkage map (Qi et al. 1998c). However, the L94 x 116-5 map contained large gaps and even some missing chromosome regions (Fig. 2, dotted lines). The line 116-5 was derived from a cross of L94 x 'Cebada Capa' (Fig. 1, M&M for details), and any L94 derived locus or chromosome segment in 116-5 will not segregate in progeny of the cross L94 x 116-5. Consequently, these regions cannot be identified by markers. In this way, seven large segments from the line 116-5 remained unidentified. These seven segments covered approximately 210 cM, compared to 857 cM of identified regions.

'Cebada Capa' possesses a gene for hypersensitive resistance, *Rph7* (Niks and Kuiper 1983; Parlevliet and Kuiper 1985; Parlevliet et al. 1985), on the short arm of chromosome 3 (Tan 1978; Tuleen and McDaniel 1971). 116-5 does not have *Rph7*, and indeed, a segment of about 35 cM on the short arm of chromosome 3 of 116-5 is derived from L94. In the absence of any selection, one would expect an equal proportion of the two parent (L94 and 'Cebada Capa') genomes in 116-5. The larger proportion (80%) of the 'Cebada Capa' genome in 116-5 is most likely due to the presence of at least five genes for partial resistance in 116-5 and the associated linkage drags.

Markers were not evenly distributed over the genetic map. Similar to the L94 x 'Vada' map, some gaps and a strong clustering of markers were found. The marker distribution along the seven chromosomes on the two maps was very similar (Fig. 5). Clear clustering of markers around the centromeres and a low-density in certain distal regions were observed on both the integrated RFLP linkage map (Qi et al. 1996) and the L94 x 'Vada' AFLP linkage map (Qi et al. 1998c). These results indicate that the distribution pattern of molecular markers is not specific for a certain type of markers (RFLP vs AFLP) nor depending on the mapping population, but rather reflects the distribution of recombination over the barley chromosome. The clustering of markers is possibly due to the

centromeric suppression of recombination (Tanksley et al. 1992; Frary et al. 1996), whereas the gaps in certain chromosome regions could correspond to recombination 'hotspots' (Lichten and Goldman 1995) in the barley genome.

Fig. 5. Regression of the number of markers in the corresponding



segments (bins) of the L94 x 'Vada' and the L94 x 116-5 maps. Number of markers were based on 21 corresponding segments according to the AFLP markers in common between the two mapping populations.

Comparison of QTLs for partial resistance in two populations

Comparison of QTLs for partial resistance showed that a QTL on chromosome 6 which was identified in the L94 x 116-5 mapping population coincided with *Rphq3* which was previously detected on the L94 x 'Vada' map (Qi et al. 1998a, 1998b). The exactly same position of the QTL on the two maps and the similar sizes in effect to the same rust isolate in both the seedling and the adult plant stages provided strong evidence for the same QTL on both maps. Consequently, we named the gene on this locus *Rphq3*, as we did on our earlier paper (Qi et al. 1998b). However, the other four QTLs mapped to different regions, and hence were assigned with different names. Interestingly, a QTL, *Rphq10*, on the distal part of the short arm of chromosome 4 of the L94 x 116-5 map, which was effective to isolate 1.2.1., has also been mapped on the same chromosome region of the L94 x 'Vada' map, but was effective to isolate 24, but not to isolate 1.2.1. (Qi et al. 1998a, 1998b). In both populations, this QTL

was effective only in the adult plant stage. We hypothesize that this is one locus with different alleles, i.e., an allele from 'Vada' being effective to isolate 24, but not to isolate 1.2.1., whereas another allele from 116-5 is effective to isolate 1.2.1.

Parlevliet and his colleagues (Parlevliet et al. 1980; Alemayehu and Parlevliet 1996) have shown that partial resistance to leaf rust occurs very frequently in West-European spring barley cultivars and Ethiopian barley landraces. The present research clearly demonstrates that several genes are involved in partial resistance in each barley line, and between lines different loci are involved. Although our results are based on only two resistant lines, we assume that these results can be extrapolated, and that many loci for partial resistance are present on the barley genome.

Development of durable resistant cultivars by MAS

Partial resistance in barley to barley leaf rust is likely based on a minor gene-for-minor gene interaction as proposed by Parlevliet and Zadoks (1977). Such a gene-for-gene interaction for partial resistance not necessary results in low durability, but even may enhance durability (Qi et al. 1998a; Parlevliet and Zadoks 1977). Accumulation of genes for partial resistance in breeding programs is probably the most durable way to protect crops in modern agriculture. Most genes for partial resistance in two partially resistant lines, 'Vada' and 116-5, mapped to different chromosome regions, supporting a strategy for accumulating many resistance genes in a single cultivar (Parlevliet and Kuiper 1985; Parlevliet et al. 1985). In a phenotypic selection experiment, Parlevliet et al. (1980) demonstrated that selection for high level of partial resistance could be effectively carried out in the seedling stage and in the adult plant stage. Still, the polygenic nature of the resistance and the relative small effects of individual genes have hampered an effective accumulation of genes in commercial breeding programs. Our results obtained from the current and the previous (Qi et al. 1998a, 1998b) studies have demonstrated that some genes for partial resistance were expressed in different plant development stages. Therefore, in the breeding program, phenotypic selection for resistance should take place in the adult plant stage. However, accumulation of genes for partial resistance that are effective in the adult plant stage can be achieved by marker

assisted selection in the seedling stage. By conversion of the AFLP markers flanking the mapped QTL region in to simple PCR markers, the resistance-enhancing QTL alleles can easily be introgressed into elite breeding lines.

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8

General discussion

Barley has been extensively studied as a favourite genetic experimental plant species. Although its large genome size (1C = 5.1 pg, Bennett and Leitch 1995) has slowed down the development of RFLP molecular marker maps, its diploid nature, self-fertility, the techniques for developing doubled haploid lines, and the availability of cytogenetic stocks have facilitated genetic mapping. In the beginning of the present research, at least five extensive RFLP marker linkage maps, covering the entire genome, were available (see chapter 2). Moreover, several recombinant inbred (RI) populations had been derived from crosses between line L94, susceptible to barley leaf rust, and partially resistant cultivars or lines ('Vada' and 116-5), providing excellent genetic material for the research presented in this thesis. The previous chapters describe results that shed new light upon the organisation of plant genomes and upon quantitative resistance of plants to pathogens.

Locus specificity of AFLP markers. The AFLP technique (Vos et al. 1995) is now well-known and widely used in plant and animal genome studies (PAG-VI, 1998). By the use of AFLP markers, two molecular marker linkage maps, L94/Vada and L94/116-5, were constructed (chapters 4 and 7, respectively). A great advantage of the AFLP technique is the simultaneous generation of a large number of markers. Locus specificity of AFLP markers was assumed in chapter 3 and confirmed in chapters 4 and 7. An *Eco*RI/*Mse*I AFLP fragment is specified by 16 selective nucleotides (six nucleotides of the *Eco*RI restriction site, four for the *Mse*I site, plus six for the selective bases). Due to this high selectivity, co-migrating AFLP bands are likely to be very homologous and

locus-specific. When the DNA fragment sizes are identical or nearly so, probably the same locus is involved (Table 1, possibilities 1, 2 and 3). AFLP products with a larger deletion or insertion will have different sizes and, consequently, will not be detected as allelic, but may be at the same locus (possibility 4 and 5). Of course, non-homologous AFLP products of different sizes will not be considered as allelic (possibilities 7 and 8). However, identical sized AFLP fragments, that are not homologous may by chance have identical size (possibility 6), and therefore cannot be distinguished from possibilities 1 – 3. Considering the latter case, caution should be taken when AFLP markers are applied in genetic and evolution studies.

Table 1. Correspondence of possible AFLP products, generated by using the same restriction enzyme and primer combination.

	Homology	**Size**		**Locus**	
				Real[a]	**Interp**[b]
1	Identical	Identical		same	same
2	High (point mutation)	identical		same	same
3	High (inversion)	identical		same	same
4	High (deletion)	shorter		same	different
5	High (insertion)	longer		same	different
6	Low	identical		different	same
7	Low	shorter		different	different
8	Low	longer		different	different

[a] Real situation. [b] The interpretation based on the migration of AFLP products.
Note: The black bars on the two distal parts of fragments indicate identical sequences on the restriction sites and the selective bases. The different possibilities of number 1 to 8 are compared to the control at the top of this diagram.

The locus-specificity of AFLP markers was proven to be nearly always valid by comparing the sequences of co-migrating bands in potato (Rouppe van der Voort et al. 1997) and by genetic linkage analysis in barley (chapters 4 and 7, Waugh et al. 1997). Thirty-eight co-migrating bands, segregating in 'Proctor' x 'Nudinka' (Becker et al. 1995) and L94 x 'Vada' (chapter 4) populations, were mapped to similar positions on the barley genome. Of the 105 co-migrating AFLP bands (markers) in the two mapping populations, L94 x 'Vada' and L94 x 116-5, 104 markers were mapped to the same loci (this thesis, chapters 4 and 7). Only one marker, E39M61-360, formerly assigned to chromosome 7 of the L94 x 'Vada' map (chapter 4), but mapped to the centromeric region of chromosome 2 on the L94 x 116-5 map (chapter 7). This kind of exception was also found by Waugh et al. (1997) and Rouppe van der Voort et al. (1997), and it is most likely due to chance (possibility 6, Table 1). The proportion of co-migrating but locus-non-specific AFLP markers was too low to hamper construction of linkage maps by using locus-specific AFLP markers.

Comparing the AFLP patterns of barley with those of three *Triticum* species demonstrated that the genetic distance between these species is too large to identify common markers (chapter 4). Consequently, the use of the locus-specificity of AFLP markers is limited to populations within a species or to very closely related species.

Distribution of molecular markers on the barley genome. In chapter 2, more than 1000 RFLP markers which had been mapped to four individual maps (Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993; Kasha et al. 1994) were integrated into one composite map. A striking clustering of markers at centromeric regions was observed on this integrated RFLP map. This was even much more pronounced on both the L94 x 'Vada' (chapter 4) and L94 x 116-5 map (chapter 7). The much stronger clustering of AFLP markers relative to RFLP markers is probably due to the higher sensitivity of the AFLP technique in sampling DNA variation than of the RFLP technique (chapter 4). This clustering of markers reduces the efficiency of the AFLP markers in mapping the distal parts of the genome (s).

Comparison of the integrated RFLP map with the L94 x 'Vada' AFLP map and the L94 x 116-5 AFLP map not only revealed that clustering of markers

occurs at centromeric regions in all three maps, but also demonstrates that the overall distribution pattern of markers on the three maps is very similar. A low density of markers in certain distal regions was observed on the integrated map as well as on the two AFLP maps. These results indicate that the density of molecular markers on the genetic map is more likely related to the frequency of recombinations than to the types of markers (RFLP vs AFLP). The clustering of markers is probably due to the centromeric suppression of recombination (Tanksley et al. 1992; Frary et al. 1996) and the gaps at certain chromosome regions may correspond to recombination 'hotspots' in the barley genome.

Development stage specific expression of partial resistance genes. In total, 13 QTLs for partial resistance to barley leaf rust were identified and were mapped to the barley genome. The resistance alleles of the QTLs detected in the present study are not sensitive to environmental variation, e.g., different experiments in different years, either in the greenhouse or in the field. However, the development stage specific expression of resistance genes is clearly demonstrated in chapters 5, 6 and 7. Just a few QTLs, e.g., *Rphq3*, were effective in both the seedling and the adult plant stage, explaining why only moderate correlations have been observed between the resistance in the two development stages. The development stage specific effects of QTLs were also observed in the studies on quantitative resistance in barley to net blotch and spot blotch (Steffenson et al. 1996) and others (see Table 1 in chapter 1).

Parlevliet et al. (1980) demonstrated that selection for partial resistance was effective in the adult plant stage, i.e., single adult plant and adult plants in small plots, but less effective in the seedling stage. Studies in this thesis clearly revealed that different genes were effective in the different development stages. This explains why phenotypic selection for partial resistance in the seedling stage does not result in a high level of partial resistance in the adult plant stage. Therefore, selection indeed should be carried out in the adult plant stage, either in the greenhouse or in the field.

Isolate-specific QTLs for partial resistance. Isolate-specificity of QTLs for partial resistance to barley leaf rust was clearly demonstrated in chapter 6. Besides the three QTLs effective to both isolates (1.2.1 and 24), an additional QTL for partial resistance of seedlings to isolate 24 was detected. Of the eight QTLs effective in the adult plant stage, five were effective to only one of the

two isolates. Isolate-specific QTLs were also identified in plant-bacterial systems (potato late blight, Leonards-Schippers et al. 1994; tomato bacterial wilt, Danesh and Young 1994), a plant-nematode system (soybean cyst nematode, Concibido et al. 1997) and a plant-virus system (pepper potyviruses, Caranta et al. 1997). The isolate or race specificity of QTLs for quantitative resistance implies that minor gene-for-minor gene interactions (Parlevliet and Zadoks 1977) do occur in plant-pathogen systems.

However, in all examples cited above, some QTLs were effective to all isolates (races) tested, not showing isolate or race specificity. As described in chapter 6, three major-effect QTLs were effective to two rust isolates tested. The question whether these QTLs are isolate-specific as well can only be answered when a large number of isolates are tested. Furthermore, full proof of the minor gene-for-minor gene hypothesis requires a genetic analysis of avirulence genes in the pathogen (Flor 1956, 1971). To this end, identification of the corresponding genes (QTLs) for aggressiveness in the pathogen should be conducted.

Map locations of resistance genes. Many examples (chapter 1) are known of resistance genes that occur in clusters on plant genomes. These are either heterospecific gene clusters, i.e., genes controlling resistance against different pathogens, or homospecific gene clusters, i.e., genes controlling resistance to a single pathogen. Also, some QTLs for quantitative resistance were mapped to the same chromosome regions as qualitative resistance genes, supporting the hypothesis that QTLs are actually allelic versions of qualitative resistance genes with intermediate phenotypes.

In the barley-barley leaf rust system, 14 genes (*Rph* genes) conferring hypersensitive resistance have been identified. Comparison of the map positions of the *Rph* genes with those of the mapped QTLs did not indicate that QTLs are located on the same region where the genes for qualitative resistance are located (chapters 5, 6 and 7). This was also confirmed by Thomas et al. (1995). These results suggest that different genes are controlling these resistance. Also, histological studies showed that the qualitative resistance conferred by *Rph* genes acts post-haustorially with hypersensitivity, whereas partial resistance is based on pre-haustorial mechanisms associated with the formation of papillae (Niks 1986). However, to verify whether QTLs for partial resistance represent

functionally different genes or are allelic versions of qualitative resistance genes, more precise mapping, cloning and sequencing of genes for both qualitative and partial resistance are required.

Durability of partial resistance. It is widely accepted that polygenic resistance is more durable than monogenic resistance. The latter operates on a gene-for-gene basis and is based on hypersensitivity; any mutation in the avirulence gene could lead to a virulence on a host genotype with the corresponding resistance gene (Joosten et al. 1994, 1997). Consequently, this resistance is not durable (chapter 6). The map positions and mode of action of genes for partial resistance suggest that they differ from the *Rph* genes (see above). Breaking down the effectiveness of these resistance genes may require major gain mutations in the pathogen.

Furthermore, the isolate-specificity of QTLs for partial resistance implies that partial resistance can operate according to a minor gene-for-minor gene model. Genes operating on a minor gene-for-minor gene basis may also result in higher durability of resistance than genes with additive effects that are effective to all genotypes (races or isolates) of the pathogen (Parlevliet and Zadoks 1977, and chapter 6).

Utilisation of mapped QTLs. The research of this thesis has not only increased our understanding of partial resistance but also provides valuable information for practical plant breeding. The better understanding of the genetics of partial resistance and the host-pathogen interactions are helpful for breeders to apply partial resistance in the development of durably resistant cultivars. Most QTLs identified in two different populations were mapped to different locations on the barley genome (chapters 5, 6 and 7), and, predominantly, are effective in an additive fashion. This indicates that loci for partial resistance to barley leaf rust are probably scattered all over the barley genome, which makes accumulation of many minor genes for partial resistance in a single cultivar feasible.

As mentioned in the previous paragraph, the development stage-specific expression of QTLs for partial resistance requires that the phenotypic selection for partial resistance is carried out in adult plants rather than in seedlings. However, since several QTLs diagnostic molecular markers are now available,

accumulation of genes for partial resistance that are effective in the *adult* plant stage can be achieved by marker assisted selection in the *seedling* stage.

Prospects for future research. The present study has provided a solid basis for further research towards a better understanding of the mechanisms and genetics of partial resistance of barley to barley leaf rust. The constructed L94 x 'Vada' and L94 x 116-5 maps contain a large number of AFLP markers (chapters 4 and 7). The constructed integrated RFLP map (chapter 2) has facilitated the merging of molecular marker data and other genetic data into one composite genetic map of barley. Screening representative barley germplasm of common ancestry with a large number of mapped molecular markers may identify QTLs directly from the germplasm without the use of segregating populations.

Thirteen QTLs for partial resistance have been identified and mapped to the seven barley chromosomes based on two crosses, L94 x 'Vada' and L94 x 116-5. Using marker assisted selection with these mapped AFLP markers, sets of backcross inbred lines (BILs) can be developed by repeated backcrossing to the susceptible parent, L94. Each BIL will harbour one chromosome segment of the resistant parent in an otherwise uniform L94 genetic background, while in the complete set of BILs the entire genome of the resistant parent is present. This set of BILs can be used to confirm the identified QTLs, to discover possible unmapped minor-effect QTLs and possible epistatic QTLs that have remained unnoticed in this study. In addition to the BILs, a set of near-isogenic line (NILs) can be developed by marker assisted selection with the markers flanking the mapped QTL regions. The generated NIL-QTLs can be used to more accurately study the effects of each QTL on the histology of the development of the rust fungus and on the epidemic components of resistance, and to more accurately study the expression of resistance alleles in different plant development stages. Further, NIL-QTLs can be applied to evaluate the isolate specificity of each QTL with a large number of isolates. Moreover, NIL-QTLs are very helpful to map QTLs for aggressiveness in the rust pathogen, creating the opportunity of more fully testing the minor gene-for-minor gene hypothesis.

The identified major-effect QTLs, e.g., *Rphq3* and *Rphq4*, explained a large part of the total variance. Sets of NIL-QTL of these major-effect QTLs will provide excellent starting materials for cloning genes for partial resistance. To

this end, the strategies that have been successfully used for cloning of many qualitative resistance genes, e.g., map-based cloning (Büschge et al. 1997), can be applied to isolate genes for partial resistance.

The prospects of gain more detailed knowledge about the functioning of genes for partial resistance are flourishing. We expect that this will eventually lead to a better acceptance that breeding for partial resistance is a must for sustainable agriculture.

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Summary

In plant-pathogen systems, qualitative resistance with hypersensitivity has been extensively studied. This resistance can be explained with the gene-for-gene model which has been confirmed at the molecular level. This hypersensitive resistance is widely used in plant breeding programmes. However, this resistance is often not durable because the resistance genes can easily be overcome by new variants of the pathogen. Alternatively, quantitative resistance is widely considered to be more durable. However, the polygenic nature of the resistance in the host and the large experimental error in disease tests hamper its application in plant breeding programmes. These same drawbacks also hampered the study of the genetics and of the mechanism of quantitative resistance.

Recently, various types of DNA markers have been developed that open a new gateway towards further study of quantitative traits, including quantitative resistance. In this thesis, barley (*Hordeum vulgate* L.)-barley leaf rust (*Puccinia hordei* Otth) is chosen as a model system to study the quantitative resistance. This plant-pathosystem has been extensively studied by Parlevliet and his colleagues at the Department of Plant Breeding of the Wageningen Agricultural University. Several recombinant inbred populations had been developed from crosses between partially resistant cultivars or lines, e.g., 'Vada' and 116-5, and an extremely susceptible line, viz., L94. Two populations, L94 × 'Vada' and L94×l16-5, were used to generate molecular linkage maps and, consecutively, genes for partial resistance in these populations were identified and mapped to the barley genome.

In chapter 2, a compilation of publicly available RFLP marker linkage maps of barley is presented. The data from four maps were used to produce an integrated map The overall order of markers on the individual maps was similar, enabling the construction of this integrated map. The integrated map contained 880 markers, covering 1060 cM. Marker clustering was observed in the centromeric regions of the seven chromosomes.

The AFLP fingerprint technique was used to generate molecular markers in barley as described in chapter 3. With 24 primer combinations a total of 2188 different amplification products were generated from 16 selected barley lines. The size of these

amplification products ranged from 80 to 510 bp. Of these barley lines, L94 versus 'Vada' showed the highest polymorphism rate (29%), and 'Proctor' versus 'Nudinka' showed the lowest (12%). The efficiency of primer combinations for identifying genetic markers was similar for any set of barley lines. By using 24 AFLP primer combinations more than 100 markers could be generated that segregated in at least two of six crossing combinations, and therefore could be used as common markers to compare linkage maps.

A high-density AFLP marker linkage map which was constructed using recombinant inbred population (103 RILs, F_9) derived from a cross between L94 and 'Vada' is presented in chapter 4. The constructed map contained 56l AFLP markers, three morphological markers, one disease resistance gene and one STS marker covering a genetic distance of 1062 cM. Uneven distributions of AFLP markers over the chromosomes and strong clustering of markers around the centromeres were found. A skeletal map with a uniform distribution of markers was extracted from the high-density map, and was applied to detect and map loci underlying partial resistance. The same set of 103 RILs was evaluated in the seedling and in the adult plant stages in the greenhouse and in the field for resistance to 1eaf rust isolates1.2.1 and 24, and quantitative trait loci (QTLs) for partial resistance to these two isolates were identified and mapped on the L94×'Vada' map (chapters 5 and 6, respectively). Six QTLs were identified for partial resistance to isolate 1.2.1. Three QTLs were effective in the seedling stage and contributed approximately 55% to the phenotypic variance. Five QTLs were effective in the adult plant stage and contributed approximately 60% to the phenotypic variance. In addition to the three QTLs that were also effective against isolate 1.2.1. in the seedling stage and additional QTL for resistance of seedlings to isolate 24 was identified. These four QTLs for resistance to isolate 24 jointly explained more than 45% of total phenotypic variance. Also, six QTLs collectively explained approximately 59% of the phenotypic variance of resistance to isolate 24 in the adult plant stage. Of the eight QTLs detected to be effective in the adult plant stage, three were effective to both isolates and five were effective to only one of the two isolates. The isolate specificity of the QTLs supports the hypothesis of Parlevliet and Zadoks that a minor gene-for-minor gene interaction can occur in partial

resistance. Of the ten identified QTLs for resistance to the two isolates in this population, QTLs *Rphq2* and *Rphq3* were the only two effective in both the seedling and the adult plant stages. The remaining QTLs were effective in either of the two developmental stages.

Chapter 7 present results of mapping QTLs for partial resistance to leaf rust isolate l.2.1 on another AFLP linkage map which was constructed by using 117 RILs (F_8) derived from a cross between L94 and 116-5. Three QTLs were effective in the seedling stage, jointly contributing 42% to the total phenotypic variance. Also, three QTLs were effective in the adult plant stage, collectively explaining 35% of the phenotypic variance. *Rphq3*, with a major-effect, was the only QTL being effective in both developmental stages. This QTL was also found to be effective in the L94 × 'Vada'population. The remaining QTLs in the L94 × 116-5 population were mapped to different positions on the linkage map than those found in the L94 ×'Vada' population. This suggests that loci for partial resistance to leaf rust are scattered all over the barley genome. Consequently, a strategy to accumulate many resistance genes in a single cultivar is feasible, which would result in a very high 1evel of partial resistance.

Studies in chapters 5, 6 and 7 showed that map positions of QTLs for partial resistance do not coincide with those of the race specific resistance genes (*Rph* genes), supporting the theory that genes for partial resistance and genes for hypersensitive resistance are entirely different gene families.

Samenvatting

In plant-pathosystemen is kwalitatieve resistentie, gebaseerd op overgevoeligheid, uitgebreid bestudeerd. Deze resistentie kan worden verklaard met het gen-om-gen model, dat op moleculair niveau bevestigd is. Deze overgevoeligheidsresistentie wordt op grote schaal toegepast in plantenveredelingsprogranmma's. Deze vorm van resistentie is echter vaak niet duurzaam effectief, doordat de resistentiegenen gemakkelijk doorbroken kunnen worden door nieuwe varianten van het pathogeen. Als alternatief bestaat er ook een kwantitatieve resistentie, die algemeen als duurzamer wordt beschouwd. Het polygene karakter van deze resistentie in de waardsoort en de grote proeffout in ziektetoetsen belemmeren de toepassing van deze resistentie in de veredelingsprogramma's. Dezelfde nadelen belemmerden de bestudering van de overerving van de mechanismen van kwantitatieve resistentie.

Recent zijn verscheidene typen DNA merkers ontwikkeld die nieuwe uitzichten bieden op verdere studie van kwantitatieve eigenschappen, waaronder kwantitatieve resistentie. In dit proefschrift werd de gerst (*Hordeum vulgare* L.)-dwergroest (*Puccinia hordei* Otth) relatie gekozen als modelsysteem om kwantitatieve resistentie te bestuderen. Dit plant-pathosysteem is uitgebreid bestudeerd door Parlevliet en zijn collega's aan de vakgroep Plantenveredeling van de Landbouwuniversiteit Wageningen. Verscheidene recombinante inteeltpopulaties zijn ontwikkeld uit kruisingen tussen partieel resistente cultivars of lijnen, zoals 'Vada' en 116-5, en een extreem vatbare lijn, L94. Twee populaties, L94 × 'Vada' en L94 × 116-5, werden gebruikt om moleculaire koppelingskaarten te vervaardigen. Vervolgens werden in deze populaties genen voor partiële resistentie geïdentificeerd en gekarteerd op het genoom van gerst.

In hoofdstuk 2 wordt een samenvoeging van publiek beschikbare RFLP koppelingskaarten van gerst gepresenteerd. De gegevens van vier kaarten werden gebruikt om een geïntegreerde kaart te maken. Over het algemeen was de volgorde van de merkers op de individuele kaart dezelfde, wat de vervaardiging van de geïntegreerde kaart mogelijk maakte. De geïntegreerde kaart bevatte 880 merkers, welke in totaal 1060 cM dekten. Rond de centromeren trad een clustering van merkers op.

Met de AFLP techniek werden in gerst moleculaire merkers gegenereerd (hoofdstuk 3). Met 24 primercombinaties werden in 16 geselecteerde gerstlijnen in totaal 2188 verschillende amplificatieproducten verkregen die in grootte varieerden van 80 tot 510 bp. Van deze gerstlijnen vertoonden L94 versus 'Vada' het hoogste percentage polymorfisme (29%) en 'Proctor' versus 'Nudinka' het laagste percentage (12%). De efficiëntie waarmee primercombinaties genetische merkers opleverden was voor alle gerstlijn-combinaties ongeveer hetzelfde. Met 24 AFLP primercombinaties konden meer dan 100 merkers worden geïdentificeerd die in minstens twee van de zes kruisingscombinaties uitsplitsten en dus als gemeenschappelijke merkers voor het vergelijken van kaarten bruikbaar waren.

Een zeer dichte AFLP-merker koppelingskaart kon worden geconstrueerd op basis van een recombinante inteeltpopulatie (103 RILs, F_9), die was verkregen uit een kruising tussen L94 en 'Vada' (hoofdstuk 4). De kaart bevatte 561 AFLP-merkers, drie morfologische merkers, één ziekteresistentie-gen en één STS merker, met een totale lengte van 1062 cM. De verdeling van de AFLP-merkers over de chromosomen bleek onregelmatig te zijn door een sterke clustering van merkers rond de centromeren. Een basiskaart met een zo gelijkmatig mogelijke verdeling van merkers werd uit de oorspronkelijke dichte kaart afgeleid, en gebruikt om de loci die de partiële resistentie bepalen te identificeren en op de chromosomen te localiseren.

Dezelfde 103 RILs werden in het zaailingstadium en in het volwassen plant- stadium in de kas en op het veld getoetst op resistentie tegen dwergroestisolaten 1.2 .1. en 24. QTLs voor partiële resistentie tegen deze twee isolaten werden gevonden en gekarteerd op de L94 ×'Vada' kaart (respectievelijk hoofdstukken 5 en 6). Zes QTLs voor partiële resistentie werden gevonden die effectief waren tegen isolaat 1.2.1. Drie QTLs waren effectief in het zaailingstadium, en verklaarden ongeveer 55% van de fenotypische variatie. Vijf QTLs waren effectief in het volwassen plant stadium, en verklaarden ongeveer 60% van de fenotypische variatie. Behalve de drie QTLs die ook effectief waren tegen isolaat 1.2.1. in het zaailingstadium, werd nog een extra QTL ontdekt voor resistentie in het zaailingstadium tegen isolaat 24. Deze vier QTLs voor resistentie tegen isolaat 24 verklaarden samen meer dan 45%van de totale fenotypische variantie. Verder

werden zes QTLs gevonden die samen ongeveer 59% van de fenotypische variantie van de resistentie tegen isolaat 24 in het volwassen plant stadium verklaarden. Van de acht QTLs die effectief waren in het volwassen plant stadium waren er drie effectief tegen beide isolaten en vijf effectief tegen slechts een van beide isolaten. Deze isolaat-specificiteit van de QTLs ondersteunt de hypothese van Parlevliet en Zadoks, dat partiële resistentie mogelijk berust op een minor-gen-om-minor-gen interactie. Van de tien QTLs voor resistentie tegen de twee isolaten die in deze populatie werden gevonden, waren alleen de QTLs *Rphq2* en *Rphq3* zowel in het zaailingstadium als in het volwassen plant stadium effectief. De overige QTLs waren effectief in een van beide ontwikkelingsstadia.

In hoofdstuk 7 worden de resultaten beschreven van de kartering van QTLs voor partiële resistentie tegen dwergroestisolaat 1.2.1 op een andere AFLP-koppelingskaart, die was gebaseerd op 117 RILs (F_8) die voortkwamen uit een kruising tussen L94 en 116-5. Drie QTLs waren effectief in het zaailingstadium en verklaarden gezamenlijk 42 % van de totale fenotypische variantie. Verder bleken drie QTLs effectief in het volwassen plant stadium. Deze verklaarden samen 35% van de fenotypische variantie. *Rphq3*, met een relatief groot effect, was het enige QTL dat in beide ontwikkelingsstadia effectief was. Deze QTL werd ook in de L94 × 'Vada' populatie gevonden. De overige QTLs in de L94 × 116-5 populatie bleken op andere kaartposities te liggen dan die in de L94 × 'Vada' populatie. Dit suggereert dat loci voor partiële resistentie tegen dwergroest wijd verspreid op het gerstgenoom aanwezig zijn. Daardoor is het goed mogelijk om vele van deze resistentiegenen te accumuleren in één enkele cultivar, die daarmee een erg hoog niveau van partiële resistentie krijgt.

De resultaten die beschreven worden in hoofdstukken 5, 6 en 7 toonden aan dat QTLs voor partiële resistentie niet samenvallen met de loci van fysio-specifieke resistentiegenen *(Rph* genen). Dit ondersteunt de theorie dat genen voor partiele resistentie en genen voor overgevoeligheidsresistentie behoren tot geheel verschillende genfamilies.

概要

植物一病原体系统中， 过敏性质量抗性已得到了较为深入的研究，这种抗性符合基因对基因原理，在分子水平也已得到了证实；过敏性抗性也已广泛应用于植物育种。但是，由于过敏性抗性基因非常容易被新的病原体变异克服，通常这种抗性不会持久。相反，数量抗性一般更具持久性。然而，寄主抗性的多基因特性以及在抗病鉴定中较大的试验误差阻碍了它在植物育种上的应用，这同样的不利因素也阻碍了对数量抗性的遗传学和抗性机理的研究。

近些年来，已开发出的多种 DNA 分子标记开辟了一条进一步研究数量性状，包括数量抗性的新途径。本论文选用大麦 (*Hordeum vulgare* L.)一大麦叶锈 (*Puccinia hordei* Otth) 为模式系统研究数量抗性。此植物病原体系统已得到瓦根宁根农业大学植物育种系的 Parlevliet 及其同事们的广泛地研究。由部分抗病品种或株系，例如，‘Vada’和 116-5，与极端感病株系 L94 杂交之后，已培育出多个重组自交群体。L94 × ‘Vada’ 和 L94×116-5 用来构建分子连锁图谱，随之，这两个群体中的部分抗性基因被鉴别并定位于大麦基因组。

在第二章，已发表的大麦 RFLP 分子标记连锁图谱被编纂整理。用四个图谱的数据构建了一个整合图谱。在单独的图谱上，标记排列的顺序非常相似，这使得能够构建这个整合图谱。整合图谱含有 880 标记，覆盖 1060cM，发现标记集 (marker-clustering) 位于七条染色体的着丝点区域。

第三章描述利用 AFLP 指纹技术开发大麦分子标记。用 24 个引物对对 16 个大麦品系的 DNA 扩增，共产生出 2188 个不同的扩增产物。产物的大小从 80 到 510 碱基对。在这些大麦品系中 L94 和 ‘Vada’ 之间表现出最高多态率(29％)，‘Proctor’ 与‘Nudinka’之间最低(12％)。对任何一组大麦品系，这些引物对检测遗传标记的效率非常相似。用 24 个 AFLP 引物对，能从六个杂交组合的至少两个组合中检测出 100 多个分离标记，这些标记可用作通用标记 (common markers) 用于比较不同的连锁图谱。

第四章介绍了由 L94 与 ‘Vada’ 杂交产生的一个重组自交群体 (103 个重组自交系，F_9) 构建的一个高密度 AFLP 标记连锁图谱。构建的图谱含有 561 个 AFLP 标记，三个形态标记，一个抗病基因和一个 STS 标记，共覆盖 1062 cM 遗传距离。发现 AFLP 标记在染色体上分布不均匀，很强的标记集位于着丝点附近。一个标记分布均匀的基干图谱 (skeletal map) 从高密度图 157 概要谱抽提出来，用于检测和定位部分抗性基因。

在温室和大田对同样的103个重组自交系在苗期和成株期对叶锈病菌株l.2.1和 24 的抗性进行了检测，鉴别出抗这两个菌株的部分抗性的数量性状位点(QTL)，并将其定位到了 L94×‘Vada’ 图谱上 (第五章和第六章)。发现六个 QTL 对菌株 1.2.1.有部分抗性，三个 QTLs 在苗期起作用，引起大约 55％的表型变异；五个 QTL 在成株期发挥作用，引起大约 60％的表型变异。 在苗期除了对菌株 1.2.1.有抗性的三个 QTL 外，发现额外一个 QTL 只对菌株 24 起作用，这四个抗菌株 24 的 QTLs 共解释多于 45％的表型变异；同样，在成株期六个 QTL 共解释大约 59％的抗菌株 24 的表型变异。在成株期检测出八个抗病 QTL，其中三个对两个菌株都具抗性；五个只对两个菌诛中的一个起作用。这种 QTL 的菌株特异性支持 Parlevliet 和 Zadoks 的假设，即，在部分抗性中，存在微基因对微基因的相互作用。在此群体中共鉴别出十个对两个菌株有抗性的 QTL，其中只有 *Rphq2* 和 *Rhpq3* 两个 QTL 在苗期和成株期都起作用，其余 QTL 只在两个发育时期的一个时期发挥作用。

第七章介绍了将抗叶锈菌株 1.2.1 的部分抗性 QTL 定位到另外一个用 L94 和 1165 杂交产生的 117 个重组自交系 (F_8) 构建的 AFLP 连锁图的结果。三个 QTL 在苗期起作用，共引起 42％的总表型变异。同样，三个 QTLs 在成株期起作用，共解释 35％的表型变异。具较大效应的 *Rphq3* 是唯一一个在两个发育时期都发挥作用的 QTL。发现在 L94 ×‘Vada’ 群体中，此 QTL 也发挥作用。L94 × 116-5 群体中的其余 QTL 被定位在不同于那些在 L94×‘Vada’群体中发现的 QTL 位置。这一结果说明抗叶锈病的部分抗性基因位点分布在整个大麦基因组。于是，在一个品种中累积很多抗性基因的策略是可行的，以此可产生非常高的部分抗性。

第五，第六和第七章的研究表明部分抗性 QTL 同小种特异性抗性基因 (*Rph* 基因)在连锁图上位于不同的位置，这支持部分抗性基因同过敏性抗性基因是根本不同的基因家族。

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Xiaoquan Qi
Wageningen, June 1998

Curriculum Vitae

Xiaoquan Qi was born in Huanggang County, Hubei Province, the People'S Republic of China on December 15th, 1963. In 1980 he finished his high school education at the Third High School of Huanggang County in Zong-Lu-Zui and entered the Department of Horticulture, Huazhong (Central China) Agricultural University in Wuhan. He received his BSc degree in July l 984. From l 984 to1994, he worked, as a research assistant (from 1984 to 1989) and an assistant professor (from 1990) at the Institute of Vegetables and Flowers (IVF) of the Chinese Academy of Agricultural Sciences (CAAS) in Beijing. He was mainly involved in the conservation, evaluation and utilisation of vegetable genetic resources. From October to December 1987, he attended the International Cowpea and Soybean Production and Research Course conducted by the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria and UNDP. Supported by FAO fellowship, in l989 he entered the School of Biological Sciences, The University of Birmingham, and obtained his Master Degree in Science for "Conservation and Utilisation of Plant GeneticResources" with the accomplishment of the dissertation："A study of isozyme allele frequencies and the mating system in a wild *Brassica oleracea* L. population". Since 1991 he has been project leader for one of the national projects of conservation, evaluation and data management of national vegetable germplasms and a State Science and Technology Commission (SSTC, China) project on "Construction of molecular linkage map in *Brassica campestris* L.", 1994-1996. In September 1994, he started a four-year PhD. research project, which is described in this thesis, at the Department of Plant Breeding and Crop Protection of the Wageningen Agricultural. In April l998, he started a three-year post-doctoral position at the John Innes Centre, UK.

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