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ORIGINAL PAPER

Marker assisted separation of resistance genes *Rph22* and *Rym16^{Hb}* from an associated yield penalty in a barley: *Hordeum bulbosum* introgression line

Paul A. Johnston[1] · Vijitha Meiyalaghan[1] · Merle E. Forbes[1] · Antje Habekuß[2] · Ruth C. Butler[1] · Richard Pickering[1]

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Abstract
Key message **The resistance genes *Rph22* and *Rym16^{Hb}* transferred into barley from *Hordeum bulbosum* have been separated from a large yield penalty locus that was present in the original introgression line '182Q20'.**
Abstract The *Hordeum bulbosum* introgression line '182Q20' possesses resistance to barley leaf rust (*Rph22*) and *Barley mild mosaic virus* (*Rym16^{Hb}*) located on chromosome 2HL. Unfortunately, this line also carries a considerable yield penalty compared with its barley genetic background 'Golden Promise'. Quantitative trait locus (QTL) mapping of the components of yield (total yield, thousand grain weight, hectolitre weight, percentage screenings and screened yield) was performed using 75 recombinant lines derived from the original '182Q20' introgression line. A QTL for the yield penalty was located in the proximal region of the introgressed segment. Marker assisted selection targeting intraspecific recombination events between overlapping *H. bulbosum* introgression segments was used to develop the lines '372E' and '372H' which feature genetically small introgressions around *Rph22*. Further yield trials validated the separation of both *Rph22* and *Rym16^{Hb}* from the proximal yield penalty. These results, combined with molecular markers closely linked to *Rph22* and *Rym16^{Hb}*, make these resistance genes more attractive for barley breeding.

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R. Pickering: retired, formerly of The New Zealand Institute for Plant and Food Research Limited.

✉ Paul A. Johnston
paul.johnston@plantandfood.co.nz

[1] The New Zealand Institute for Plant and Food Research Limited, Lincoln 7608, New Zealand

[2] Julius Kühn-Institute, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

Introduction

The use of wild relatives for the genetic improvement of cultivated species is an important source of novel alleles and traits. However, the introgression of these target traits into commercial germplasm can often take many years to accomplish. *Hordeum bulbosum* L. is a member of the secondary gene pool of cultivated barley (*Hordeum vulgare* L.) and has been used mostly in barley improvement as a means to produce doubled haploids through chromosome elimination (Kasha and Kao 1970). In addition, *H. bulbosum* is considered a non-host to many pathogens which are virulent upon barley, and several resistance genes have been successfully introgressed from *H. bulbosum* into cultivated barley (Fetch et al. 2009; Johnston et al. 2013; Pickering et al. 1995, 1998, 2000, 2006; Ruge-Wehling et al. 2006; Ruge et al. 2003; Scholz et al. 2009; Shtaya et al. 2007; Toubia-Rahme et al. 2003; Walther et al. 2000; Xu and Kasha 1992). The development of crops which are resistant to diseases is a major goal of plant breeding in almost all commercial crop species. To provide a tangible benefit, crop resistance not only needs to provide yield stability under biotic stress, but also be durable. The test for durability of disease resistance requires that a given resistance has remained effective despite being challenged by the disease over a long period of time and over a large geographical area (Johnson 1984). Major resistance (R) genes have been used for many decades by plant breeders to protect cultivars from particular disease pathotypes in a gene-for-gene

manner (Flor 1956). Unfortunately, pathogens often overcome newly deployed R genes within a few years of widespread cultivation in regions conducive to disease (Clifford 1985). Once a resistance gene has been overcome, virulent pathotypes can spread rapidly across or between continents via long distance air dispersal of fungal spores, thus compromising previously resistant crops in distant areas (Brown and Hovmøller 2002). With limited sources of new disease resistance genes, there is a need to develop more durable solutions. Partial, quantitative or adult plant resistance (APR) genes fall into a second category of plant disease resistance that is currently receiving a resurgence of interest especially against the cereal rusts (Case et al. 2014; Derevnina et al. 2013; Herrera-Foessel et al. 2014; Hulbert and Pumphrey 2014; Singh et al. 2013a; Ziems et al. 2014). For the purposes of this paper, we will use the term partial resistance as inclusive of APR. This type of resistance is often conferred by many genes of small effect, which together act to reduce disease severity despite a susceptible infection type (Parlevliet 1975, 1976, 1978). For barley leaf rust (*Puccinia hordei* Otth.), there have been twenty quantitative trait loci (QTL) uncovered which contribute to this 'slow rusting' type resistance (Marcel et al. 2007, 2008; Qi et al. 1998, 2000). The best understood 'slow rusting' system is from the cultivar 'Vada', which when crossed with the susceptible line 'L94' resulted in the identification and genetic mapping of six QTL conditioning this response (Qi et al. 1998). In contrast to R genes against barley leaf rust, which result in a hypersensitive response, the 'slow rusting' resistance reduces disease severity by limiting the pre-haustorial establishment of some fungal infection units (reduced infection frequency) and by delaying the development of sporification in those infection units which are able to establish (increased latency period) (Niks 1986). The 'slow rusting' resistance found in 'Vada' has proven durable despite widespread cultivation for several decades (Parlevliet 2002). However, partial resistance can be very difficult to manipulate in a breeding program, as many small effect genes need to be maintained for the resistance to be effective. The use of partial resistance in breeding has been made easier by the cloning of genes such as *Lr34* (Krattinger et al. 2009) and *Yr36* (Fu et al. 2009) in wheat, thus providing perfect markers for marker assisted selection (MAS). These efforts have also revealed that the genes underlying partial resistance are likely to be diverse in type and function (Fu et al. 2009). However, most of the genes/QTL for partial resistance have not been closely linked to genetic markers to aid their incorporation into breeding lines. Even if markers were available, the time and expense involved is likely to be prohibitive for the degree of resistance gain from each of these small effect genes.

Rph22 is a large effect partial resistance gene that was introgressed into cultivated barley from the wild species *H. bulbosum*. *Rph22* is likely to be conferred by a single dominant gene located on the distal end of chromosome 2HL (Johnston et al. 2013). The presence of this single locus results in an increased latency period and reduced infection frequency that is superior to the degree of resistance found in the cultivar 'Vada', which is conditioned by at least six separate QTL (Qi et al. 1998). Partial resistance genes are also known as APR genes as their effect on extending fungal latency period increases over the course of plant development (Parlevliet 1975). The introgression line '182Q20', derived from the backcross of diploid barley cultivar 'Golden Promise', to a partially fertile triploid interspecific hybrid between 'Golden Promise' and the tetraploid *H. bulbosum* genotype A17-1 (Johnston et al. 2013) was first identified in the field because of its 'slow rusting' response to natural infections of barley leaf rust. This line was subsequently shown using genomic in situ hybridization (GISH) and molecular markers to possess a single introgression from *H. bulbosum* which covers approximately 6 % of the physical length (IBSC physical map, 2012) and 24 % of the genetic length (POPSEQ map, Mascher et al. 2013) of chromosome 2H (N. Wendler, pers. comm.). Because of the large effect on latency period and infection frequency of *Rph22*, the presence of this gene can also be readily detected at the seedling stage (Pickering et al. 2004a). Molecular mapping of *Rph22* has revealed a small overlapping genetic interval and the same phenotypic mechanism as *Rphq2* (Johnston et al. 2013), the largest effect and most stable QTL from 'Vada' (Marcel et al. 2007). It seems likely that *Rph22* and *Rphq2* are paralogs of the same ancestral gene or members of a gene cluster. *Rph22* is an attractive gene for barley breeding as it confers a large effect 'slow rusting' resistance which, because of its mechanism, may prove to be more durable than hypersensitive R genes currently available for the control of leaf rust. In addition, the incorporation of one large effect partial resistance gene is technically much simpler than tracking multiple genes of lesser effect.

The complex of *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV) is transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* and has been a problem for winter grown barley crops in Asia and Europe since the late 1970s and 1980s (Huth and Lesemann 1978; Hill and Evans 1980; Lapierre 1980; Kobayashi et al. 1987; Ruan and Jin 1983; Rubies-Autonell et al. 1995; Katis et al. 1997). To date, 18 resistance loci have been identified against these viruses, with 15 recessive genes and one dominant gene located in *H. vulgare* (Ordon et al. 2005; Kai et al. 2012), and two dominant genes ($Rym14^{Hb}$ and $Rym16^{Hb}$) from *H. bulbosum* (Ruge et al. 2003; Ruge-Wehling et al. 2006). Development of resistant cultivars is the only effective tool for controlling the effect of these viruses. In addition to *Rph22,* the *H. bulbosum*

introgression line '182Q20' also carries the resistance gene *Rym16*^{Hb} which confers resistance to all known isolates of this virus complex in Germany (Habekuß et al. 2008). The resistance gene *Rym16*^{Hb} has been previously mapped to chromosome 2HL (Ruge-Wehling et al. 2006) in different *H. bulbosum* introgression mapping populations. Mapping of *Rym16*^{Hb} using the "182Q20_F4_Popn" would provide a greater mapping resolution and thus is seen as a useful population for examining both these resistance genes.

Some sources of durable disease resistance are known to be pleiotropic with traits that have a negative effect on yield, such as the leaf tip necrosis (*Ltn*) with *Lr34*. Indeed, the incorporation of *Lr34* (and *Ltn*) was shown to give a 5 % reduction in yield under disease-controlled conditions (Singh and Huerta-Espino 1997). Sourcing novel resistance genes from wild relatives can also be associated with problems of linkage drag, resulting in the transfer of additional deleterious (or undomesticated) alleles/genes along with the target trait. *Rph22* and *Rym16*^{Hb} are no exception, as the original *H. bulbosum* introgression line '182Q20' has an approximately 25 % lower yield than its genetic background 'Golden Promise' under fungicide control (Pickering et al. 2004b). Interestingly, there was no appreciable difference in yield between '182Q20' and 'Golden Promise' in the presence of natural leaf rust infection (Pickering et al. 2004b). This paper describes the experiments to locate *Rym16*^{Hb} genetically in the mapping population established for *Rph22* (Johnston et al. 2013) and to determine whether the yield penalty is a consequence of (or pleiotropic to) these resistance genes or whether it is the result of linkage drag of other alleles/genes from the wild species *H. bulbosum*.

Materials and methods

Mapping of *Barley mild mosaic virus* resistance (BaMMV)

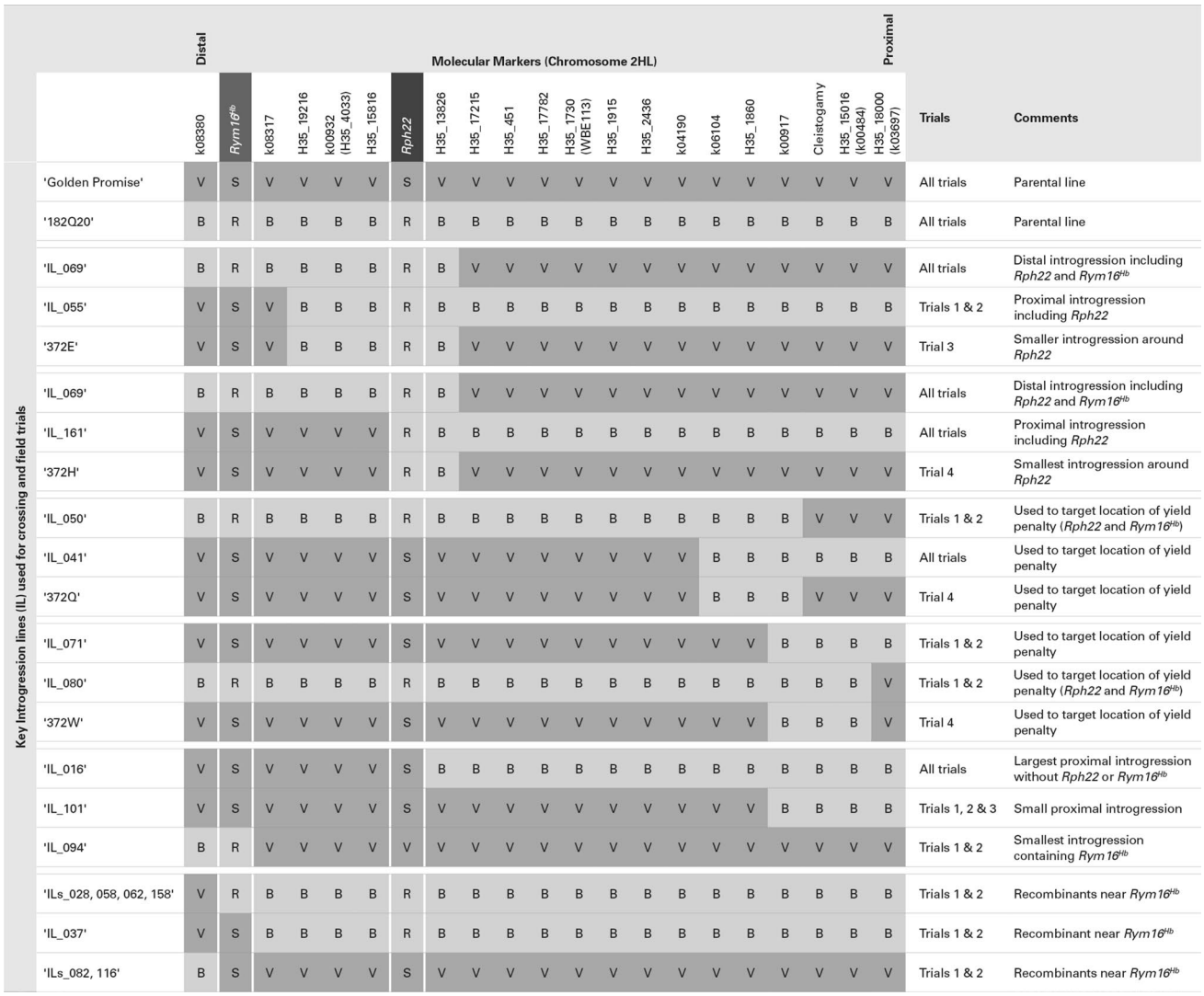
The "182Q20_F4_Popn", previously developed for mapping *Rph22* (Johnston et al. 2013) was also used to genetically map *Rym16*^{Hb}. This population consists of 176 lines, with between one and five independent F_4 homozygous sister lines derived from each of the original 76 F_2 recombinant lines as described in Johnston et al. (2013).

Six seeds of each of 155 lines from the "182Q20_F4_Popn" (21 lines were not included because of low amounts of seed from genotyped seed lots) plus cv. 'Golden Promise', introgression lines '182Q20' and '372E' were sown in the greenhouse. In addition, 17 seeds from the susceptible standard cv. 'Maris Otter' were also included in the experiment. At the 2–3 leaf stage, the plants were transferred to a climatic chamber with 12 °C and 16 h photoperiod (16 kLx). Between four and six plants (depending on germination), from each line were screened for their response to mechanical inoculation with the isolate BaMMV-ASL1 as described by Habekuß et al. (2008). Five weeks after inoculation, the number of plants with mosaic symptoms was scored and DAS-ELISA using polyclonal antibodies prepared by Frank Rabenstein (JKI, Institute for Epidemiology and Pathogen Diagnostics, Quedlinburg) was carried out. The infection rate (%) was calculated as the number of infected plants/number of inoculated plants.

Plant materials and field trial designs

A total of four field trials were carried out, trial 1 (2010–11) and trial 2 (2011–12) were designed to map the QTL responsible for the yield penalty whilst trials 3 (2012–13) and 4 (2013–14) were used to examine the potential to separate *Rph22* and *Rym16*^{Hb} from the yield penalty QTL. All field trials were conducted near Lincoln, Canterbury, New Zealand. Field trials 1 and 2 featured 13 plots of each of two parent lines (high yielding *H. vulg*are cv. 'Golden Promise', low yielding *H. bulbosum* introgression line '182Q20') along with two plots each of 75 recombinant lines (one homozygous F_4 representative derived from each of the original 76 F_2 recombinants as previously described in Johnston et al. (2013), with one left out because of insufficient seed). The layout of trial 1 was derived from four 11 × 11 Latin squares. The same trial design was employed for field trial 2, but with a new randomization.

A crossing strategy was developed to reduce the size of the *H. bulbosum* introgression around *Rph22* and the yield penalty locus. Crosses were made between a line which had the proximal region of the original introgression including the locus of interest and a line which had the distal region of the original introgression including the locus of interest. These combinations are shown in Fig. 1 with grouped triplets featuring the two parental lines and the resulting line selected from the progeny of that cross. For example, the line '372H' (targeting *Rph22*) was derived from a cross between 'IL_161' and 'IL_069'. By selecting lines with recombination events close to the target locus, it was possible to minimize extraneous regions of the introgression. Previously developed PCR markers (Johnston et al. 2009, 2013) were used to identify intraspecific recombination events (within the overlapping introgressions) in the F_2 lines. These F_2 recombinants of interest would be homozygous for the *H. bulbosum* genotype at the target region, heterozygous for one end of the introgression and homozygous for the barley genotype at the opposite end of the introgression. Individual F_3 plants which possessed homozygous introgression genotypes of reduced size were then selected from the selfed progeny and coded '372E', '372H', '372Q' and '372W'. All lines discussed in this paper are available for distribution under MTA agreement.



Key Introgression lines (IL) used for crossing and field trials	Distal		Molecular Markers (Chromosome 2HL)																			Proximal	Trials	Comments
	k08380	*Rym16^{Hb}*	k08317	H35_19216	k00932 (H35_4033)	H35_15816	*Rph22*	H35_13826	H35_17215	H35_451	H35_17762	H35_1730 (WBE113)	H35_1915	H35_2436	k04190	k06104	H35_1860	k00917	Cleistogamy	H35_15016 (k00484)	H35_18000 (k03697)		
'Golden Promise'	V	S	V	V	V	V	S	V	V	V	V	V	V	V	V	V	V	V	V	V	V	All trials	Parental line
'182Q20'	B	R	B	B	B	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B	All trials	Parental line
'IL_069'	B	R	B	B	B	B	R	B	V	V	V	V	V	V	V	V	V	V	V	V	V	All trials	Distal introgression including *Rph22* and *Rym16^{Hb}*
'IL_055'	V	S	V	B	B	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B	Trials 1 & 2	Proximal introgression including *Rph22*
'372E'	V	S	V	B	B	B	R	B	V	V	V	V	V	V	V	V	V	V	V	V	V	Trial 3	Smaller introgression around *Rph22*
'IL_069'	B	R	B	B	B	B	R	B	V	V	V	V	V	V	V	V	V	V	V	V	V	All trials	Distal introgression including *Rph22* and *Rym16^{Hb}*
'IL_161'	V	S	V	V	V	V	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B	All trials	Proximal introgression including *Rph22*
'372H'	V	S	V	V	V	V	R	B	V	V	V	V	V	V	V	V	V	V	V	V	V	Trial 4	Smallest introgression around *Rph22*
'IL_050'	B	R	B	B	B	B	R	B	B	B	B	B	B	B	B	B	B	B	V	V	V	Trials 1 & 2	Used to target location of yield penalty (*Rph22* and *Rym16^{Hb}*)
'IL_041'	V	S	V	V	V	V	S	V	V	V	V	V	V	V	V	B	B	B	B	B	B	All trials	Used to target location of yield penalty
'372Q'	V	S	V	V	V	V	S	V	V	V	V	V	V	V	V	B	B	B	V	V	V	Trial 4	Used to target location of yield penalty
'IL_071'	V	S	V	V	V	V	S	V	V	V	V	V	V	V	V	V	V	B	B	B	B	Trials 1 & 2	Used to target location of yield penalty
'IL_080'	B	R	B	B	B	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	V	Trials 1 & 2	Used to target location of yield penalty (*Rph22* and *Rym16^{Hb}*)
'372W'	V	S	V	V	V	V	S	V	V	V	V	V	V	V	V	V	V	B	B	B	V	Trial 4	Used to target location of yield penalty
'IL_016'	V	S	V	V	V	V	S	B	B	B	B	B	B	B	B	B	B	B	B	B	B	All trials	Largest proximal introgression without *Rph22* or *Rym16^{Hb}*
'IL_101'	V	S	V	V	V	V	S	V	V	V	V	V	V	V	V	V	V	B	B	B	B	Trials 1, 2 & 3	Small proximal introgression
'IL_094'	B	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	Trials 1 & 2	Smallest introgression containing *Rym16^{Hb}*
'ILs_028, 058, 062, 158'	V	R	B	B	B	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B	Trials 1 & 2	Recombinants near *Rym16^{Hb}*
'IL_037'	V	S	B	B	B	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B	Trials 1 & 2	Recombinant near *Rym16^{Hb}*
'ILs_082, 116'	B	S	V	V	V	V	S	V	V	V	V	V	V	V	V	V	V	V	V	V	V	Trials 1 & 2	Recombinants near *Rym16^{Hb}*

Fig. 1 Genotypes of key introgression lines (ILs) and parents (from Johnston et al. 2013), that were used in field trials and for crossing to develop barley lines '372E', '372H', '372Q' and '372W'. Lines are shown in crossing groups, i.e., '372E' was developed from a cross between 'IL_069' with 'IL_055'. Only a single marker from each unique genetic locus is shown in the order of genetic linkage from the distal end of chromosome 2HL (*left*) to the proximal end of the original introgression from '182Q20' (*right*). Genotypes are displayed as V for the homozygous *Hordeum vulgare* allele and B for the homozygous *Hordeum bulbosum* allele. For *Rph22* and *Rym16^{Hb}*, phenotypes are shown as R for resistant and S for susceptible

Trial 3 included the newly developed '372E', five key introgression lines ('IL_016', 'IL_041', 'IL_069', 'IL_101', and 'IL_161') selected based on their genotype for containing different regions of the original introgression (Fig. 1) and the two parental lines '182Q20' and 'Golden Promise'. This trial was sown using a design modified from an 8 × 8 Latin square, with four of the eight plots of '372E' replaced by two additional plots of each of the two parents (because of insufficient seed from the glasshouse increase of '372E').

Trial 4 included the smallest introgression line around *Rph22* coded '372H' (which had superseded the previous line '372E') and additional lines '372Q' and '372W' which were also developed using the same system of overlapping crosses in an attempt to better resolve the genetic location of the yield penalty QTL. This trial featured two blocks, each containing three replicates of eight lines (six introgression lines '372H', '372Q', '372W', 'IL_016', 'IL_041', 'IL_069' and the two parents). The two blocks featured different fungicide regimes (treated and untreated), each laid out using a Latinized resolvable block design generated with CycDesigN (CycSoftware 2009). As there was insufficient seed of the line '372Q' for six plots, this line was replaced by 'Golden Promise' in the untreated block.

All trials were managed to maximize yield potential. Each trial received two applications of fungicides,

Proline® (prothioconazole, Bayer CropScience) and Proline® + Amistar® (Azoxystrobin, Syngenta) at the recommended rates and two applications of urea at the rate of 150 kg per hectare (a total of 138 units nitrogen per hectare). However, trial 4 featured a block which was not treated with fungicide, to examine the effect of *Rph22* in the presence of natural leaf rust infection. Plot sizes for all trials were 5.2 × 1.3 m (6.75 m^2) and agronomic data were collected from all field trials including total plot yield, thousand grain weight (TGW), hectolitre weight, percentage screenings (percentage of a grain sub-sample which fell through a 2.4-mm slotted screen) and screened yield (total plot yield less the weight due to screenings). Lodging was observed in all four trials but was only formally recorded in trial 2 (2011–12). Lodging score (proportion of each plot that was still standing, i.e., 10 = all standing, 0 = completely lodged) was recorded for the 2011–12 field trial only on two separate dates.

Statistical analysis

Spatial patterns in each trial were explored using the methods described by Verbyla et al. (1999). These analyses showed that there were trends across each trial. The trends were accounted for within a mixed model analysis fitted with restricted maximum likelihood (REML) (Payne et al. 2012). Lines were included in these analyses as fixed effects, and factors to adjust for spatial patterning as random effects. Adjusted means for each line and associated standard errors were obtained from the results of these analyses.

Genotypic data from 21 marker loci (a single marker from each unique recombination interval) were combined with the agronomic data from trials 1 and 2. Separate analyses were carried out for each marker locus. The REML analysis was extended by partitioning the line effects into B (allele derived from *H. bulbosum* introgression line parent '182Q20') versus V (allele derived from *H. vulgare* parent 'Golden Promise') for the marker, and lines within each of these groups. These analyses gave exactly the same adjusted line means, but allowed estimation of the mean for lines with B or V and a comparison of the B mean with the V mean. The approximate F-probabilities for these comparisons were obtained with denominator degrees of freedom for the F-statistic calculated using the method of Kenward and Roger (1997). The $-\log_{10}$ of the F probability is comparable with the logarithm of odds (LOD) score commonly used in QTL analyses.

Results

The resistance reactions of 155 lines from the "182Q20_F4_Popn" were evaluated in comparison to susceptible and resistant control lines after mechanical BaMMV inoculation. All tested plants of the susceptible control lines were infected; cv. 'Golden Promise' (5 infected plants/5 inoculated plants), '372E' (5/5) and cv. 'Maris Otter' (17/17). The infection rate of the resistant control '182Q20′ was 0 % (0/6). Of the 155 tested lines from the "182Q20_F4_Popn", 72 lines displayed a resistant reaction type with no visible leaf symptoms and no serological detection of virus. The remaining 83 lines showed a susceptible reaction type (infection rates of 33–100 %). The 155 lines tested here were homozygous lines derived from 74 unique F_2 recombinants (Johnston et al. 2013). This resulted in a total of 36 resistant families and 38 susceptible families and consistent with *Rym16*^{Hb} being conditioned by a single locus. Only two lines ('IL_021' and 'IL_141') gave inconsistent results within their families. Both these lines were initially classified as resistant whilst sister lines ('ILs_019', '020', '022' and 'IL_142', respectively) were all susceptible. Assuming that 'IL_021' and 'IL_141' were escapes (actually susceptible), the best fit of these data was for the resistance gene *Rym16*^{Hb} to co-segregate with the marker H35_17700 (k03475) near the distal end of the introgression (Figs. 1, 2).

For the agronomic yield traits measured, the introduction of the *H. bulbosum* introgression in '182Q20' led to a less desirable mean when compared to the genetic background, cv. 'Golden Promise' (Table 1), i.e., lower total yield, TGW, screened yield, lodging score and higher percentage screenings. However, hectolitre weight was variable between the parental lines in trials 1 and 2. Spatial adjustments were applied to the means of the agronomic data to account for trends across the field trials, however, in most cases these adjustments made only minor changes to those means. Some lines had yield components superior to those of cv. 'Golden Promise' and poorer than those of '182Q20', although none was significantly different ($p > 0.05$) (data not shown). As would be expected, total (raw) yield was correlated with screened yield for field trials 1 and 2 (Table 2 and 3), but it was not well correlated with the other variables. Screened yields were well correlated with TGW, and with percentage screenings, but not with hectolitre weights. In all field trials, there was a considerable degree of post-anthesis lodging observed because of relatively high yields combined with strong winds and the poor lodging resistance of the parental cultivars 'Golden Promise' and '182Q20'. In each trial, all plots were harvested using a small plot combine and manual lifting of stems to ensure that all the seed was harvested despite the crop lodging.

The marker analyses for the components of yield in field trials 1 and 2 were carried out using the adjusted means, as the differences between the *H. bulbosum* and barley marker alleles were enhanced in the adjusted analysis and thus the associated p values were smaller. In both trials 1 and 2, a QTL affecting several components of yield was detected

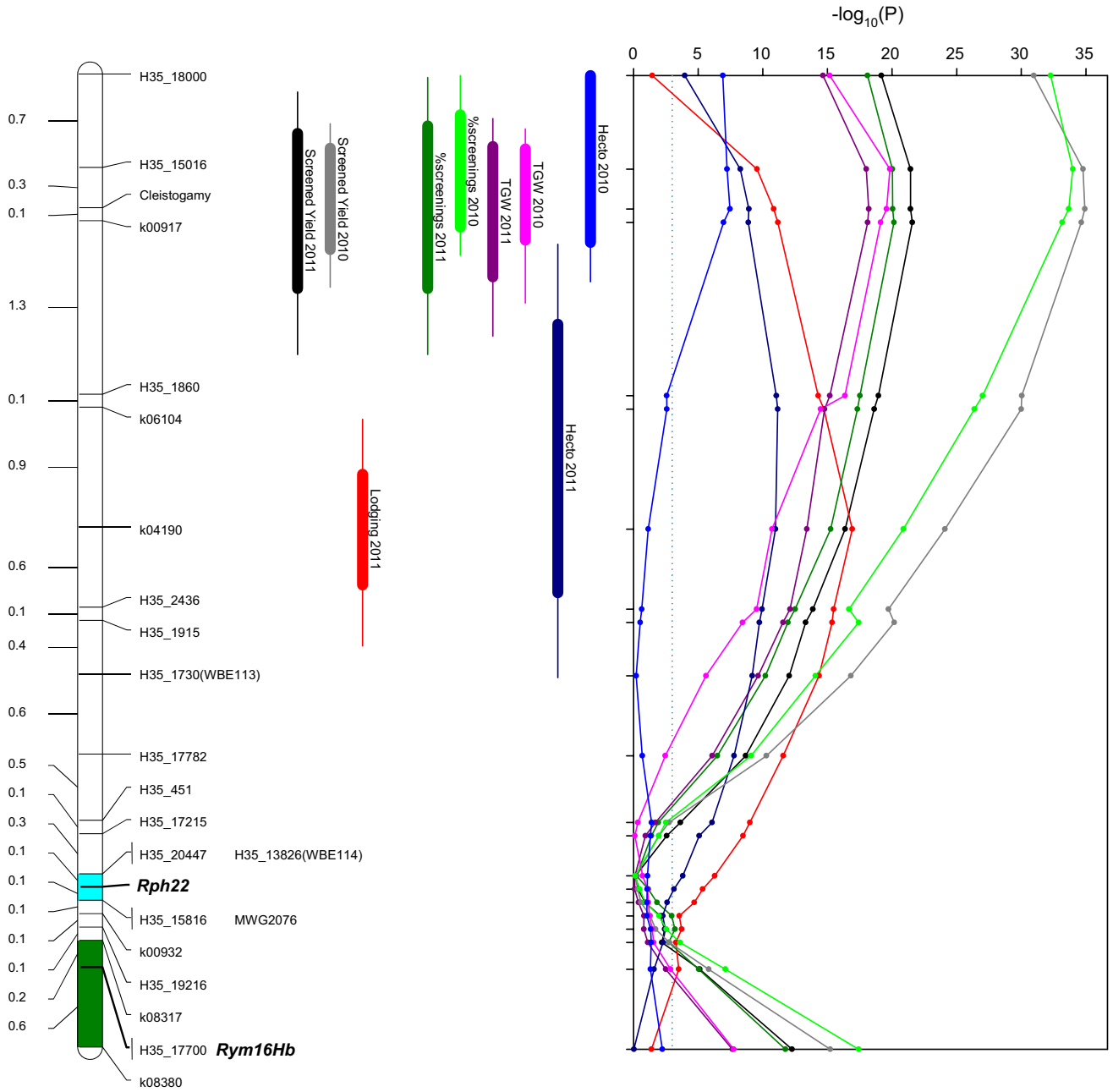


Fig. 2 Marker/trait associations from the 2010–11 (trial 1) and 2011–12 (trial 2) barley field trials (75 introgression lines). The *blue* and *green bars* on the linkage map indicate the position of the resistance loci *Rph22* and *Rym16*^{Hb}, respectively. The *thick bars* displaying the location of quantitative trait loci (QTL) on the linkage map show the area covered by a drop of one $-\log_{10}(P)$ (equivalent to LOD) from the peak and the *thin lines* show an additional drop of one $-\log_{10}(P)$. *Similar colours* have been used to group each component of yield trait for each field trial. Lodging was recorded in 2011–12 (trial 2) only. *Numbers* to the *left* of the linkage map indicate intervals between markers in cM (colour figure online)

in the proximal region of the introgression between markers H35_1860 and H35_18000 (Fig. 2). Lines with barley alleles in this region were strongly associated with an increase in total yield, TGW, hectolitre and screened yield, and a decrease in percentage screenings compared with lines having the *H. bulbosum* alleles. The most significant differences between the marker alleles were seen in the data for screened yield and percentage screenings (Fig. 2). The QTL peaks for lodging (which was only recorded in trial 2, 2011–12) and hectolitre weight (2011–12) were located more centrally within the introgressed segment, with a peak at marker k04109 (Fig. 2), compared with the other traits.

Table 1 Parental means with standard error of the means (sem) in brackets (13 plots of each line, after spatial adjustments) for the components of barley grain yield data from field trial 1 (2010–11) and field trial 2 (2011–12)

Year	Plant code	Total yield (kg per plot)	Hectolitre weight (g)	Percentage screenings	Thousand grain weight (g)	Screened yield (kg per plot)	Lodging score
2010-11	Golden Promise	5.57 (0.07)	61.01 (0.29)	16.31 (1.22)	41.86 (0.39)	4.67 (0.08)	n/a (n/a)
	182Q20	5.16 (0.07)	62.62 (0.29)	36.90 (1.20)	38.92 (0.39)	3.27 (0.08)	n/a (n/a)
2011-12	Golden Promise	7.62 (0.10)	65.63 (0.39)	10.73 (1.50)	44.08 (0.43)	6.82 (0.13)	6.79 (0.49)
	182Q20	7.17 (0.10)	64.08 (0.38)	32.79 (1.49)	39.17 (0.43)	4.75 (0.13)	5.58 (0.49)

Lodging score was not measured in 2010–11

n/a not available

Table 2 Correlations between raw means for five barley yield variables for trial 1 (2010–11)

	Yield	Hectolitre	% Screenings	TGW	Screened yield
Yield	1.00				
Hectolitre	−0.18	1.00			
% Screenings	−0.34	0.24	1.00		
TGW	0.27	−0.11	−0.73	1.00	
Screened yield	0.68	−0.25	−0.92	0.68	1.00

Both, the *Rph22* and *Rym16*^{Hb} resistance loci are located near the distal end of the introgression and hence were not closely associated with any of the proximal QTL regions affecting the yield parameters in either of the field trials (Fig. 2).

To validate the separation of *Rph22* and *Rym16*^{Hb} from the yield penalty QTL near *Cly1*, five key introgression lines and the newly developed line '372E' were included in trial 3 with a higher rate of plot replication. Analysis of data from this third field trial revealed a clear grouping of the lines with one or other of the two parents. Yield component data for lines '372E' and 'IL_069' both clustered with the parent cv. 'Golden Promise', whilst the yield component data for the remaining lines ('IL_016', 'IL_041', 'IL_101', 'IL_161') clustered with the parent line '182Q20′ (Fig. 3). This confirmed that the smaller distal introgression of 'IL_069' (containing both *Rph22* and *Rym16*^{Hb}) and the reduced introgression around *Rph22* in line '372E' resulted in the same yield characteristics as those of cv. 'Golden promise' (Fig. 3). There were significant differences between the lines for all five variables ($p < 0.001$ for an overall test for line differences for variables other than hectolitre weight, where $p = 0.024$). A glasshouse pathology screen confirmed that '372E' possessed the same 'slow rusting' response to leaf rust as '182Q20' (data not shown).

Trial 4 featured two blocks that differed in their fungicide treatment. In the fungicide treated block, there was a clear demarcation between lines with either 'Golden Promise' or '182Q20' yield component data. The line '372H' carrying the smallest introgression around *Rph22* plus lines '372Q' and 'IL_069' (containing both *Rph22* and *Rym16*^{Hb}) possessed the same yield parameters as cv. 'Golden Promise', whereas lines '372W' 'IL_016' and 'IL_041' possessed the yield parameters of '182Q20' (Fig. 4). In the block without fungicide treatment, there was a small amount of late leaf rust infection, which allowed confirmation of the same 'slow rusting' resistance in '372H' as in '182Q20'. Under this disease pressure from barley leaf rust, lines 'IL_069' (both *Rph22* and *Rym16*^{Hb}) and '372H' (*Rph22* only), which feature small distal introgressions, both outperformed cv. 'Golden Promise' for total yield, percentage screenings and screened yield (Fig. 4). The original introgression parent '182Q20' had very high percentage screenings in this untreated block and consequently the lowest screened yield.

The lines '372E', '372H', '372Q' and '372W', with reduced introgressions around loci of interest were successfully developed from crosses between ILs that possessed proximal and distal introgressions which overlapped around the locus of interest. The use of markers across the entire introgression segment allowed lines resulting from only intraspecific recombination events within this overlapping region to be identified in the F_2 progeny. The number of these recombinants detected was dependent on the genetic size of the overlap based on the interspecific genetic map (Fig. 2). For instance; three intraspecific recombinants were detected from 184 '372H' F_2 seedlings (~0.5 cM overlap, Figs. 1, 2), whilst eight intraspecific recombinants were detected from only 92 '372Q' F_2 seedlings (~2.4 cM overlap, Figs. 1, 2). Subsequent selection of F_3 selfed seed from these intraspecific recombinants resulted in the identification of lines that were homozygous for these introgressions of reduced size. These lines possessed homozygous introgressions which spanned the marker intervals H35_19216 to H35_13826 ('372E'), *Rph22* to H35_13826 ('372H'), k06104 to k00917 ('372Q') and k00917 to H35_15016 ('372W') (Fig. 1). The cleistogamy locus was phenotyped

Table 3 Correlations between raw means for six barley yield variables for trial 2 (2011–12)

	Yield	Hectolitre	% Screenings	TGW	Screened yield	Lodging
Yield	1.00					
Hectolitre	0.33	1.00				
% Screenings	−0.53	−0.49	1.00			
TGW	0.62	0.49	−0.82	1.00		
Screened yield	0.72	0.49	−0.97	0.85	1.00	
Lodging	0.37	0.72	−0.44	0.55	0.46	1.00

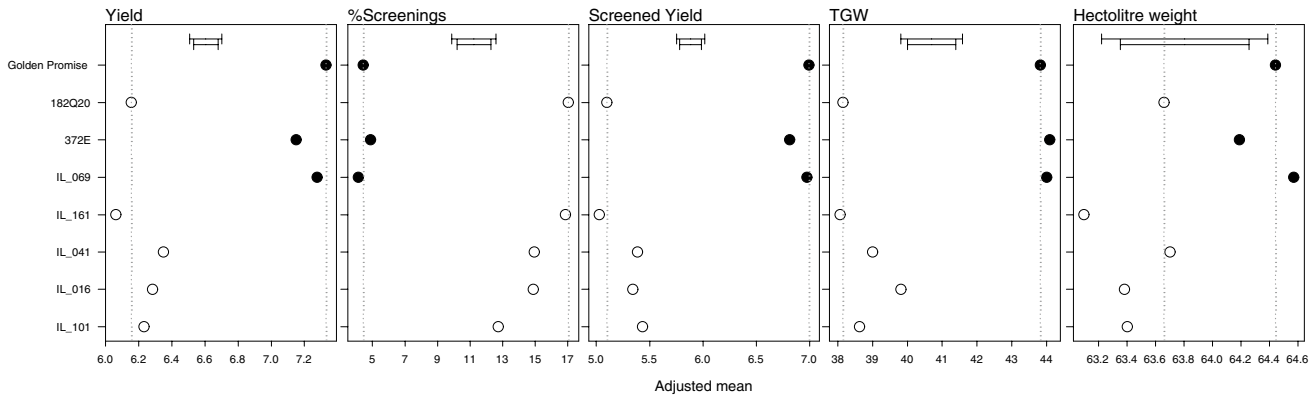


Fig. 3 Adjusted means for eight barley lines across five yield components, Yield, % screenings, screened yield (kg per plot), thousand grain weight (TGW, g per 1000 seed) and hectolitre weight (g) from field trial 3 (2012–13). *Closed symbols* indicate *lines* share similar yield parameters to 'Golden Promise' and *open symbols* to '182Q20'. *Error bars* are LSD 5 % to compare a *line* with a parent, with the *shorter* (*lower*) *bar* for comparisons of most *lines* with the parents, and the *longer* (*upper*) *bar* to compare line '372E' with the parent (because of a lower number of replications in the trial)

as a morphological marker as described previously (Johnston et al. 2013).

Discussion

Disease resistance is a key target trait for plant breeding and a major contributing factor to yield stability. Conventional breeding has historically focused on the selection of breeding lines displaying immunity or hypersensitive response under disease pressure. This type of selection favours the incorporation of major R genes, which give a clean crop in the field. However, these single dominant, major resistance genes are often rapidly overcome upon widespread cultivation. For example, from 235 worldwide isolates of wheat stripe rust, virulence was detected for all but two R genes tested (*Yr5* and *Yr15*) (Sharma-Poudyal et al. 2012). In contrast, partial resistance genes, as a group, are generally considered to be a more durable mechanism of disease resistance because of their polygenic nature and non-race specificity (Brown 2002). For instance, there are several examples of partial resistance genes which have remained effective over long time periods, such as *Sr2* derived from *Triticum turgidum* in the wheat variety 'Hope' (McFadden 1930), *Lr13* from the wheat variety 'Frontana' (Dyck et al. 1966), *Lr34/Yr18/Pm38* from the wheat variety 'Terenzio' (Dyck 1987) and *mlo* from mutants and Ethiopian landraces of barley (Jørgensen 1992). The classic example of partial resistance, *Lr34/Yr18/Pm38* in wheat, is effective against multiple pathogens such as leaf rust, stripe rust, powdery mildew and also stem rust in some genetic backgrounds (Dyck 1987). However, even amongst partial resistance genes there are examples which are race specific, for instance *Lr12* against wheat leaf rust (Park and McIntosh 1994). The two partial resistance genes cloned to date from wheat (*Yr36* and *Lr34/Yr18/Pm38*) have revealed different classes of genes with presumably different mechanisms of action (Fu et al. 2009; Krattinger et al. 2009). It also seems probable that if partial resistance genes are varied in form and function, their individual durability will be similarly heterogeneous.

While most partial resistance genes characterized to date have only small effects on the phenotypic variance of disease resistance, *Rph22* is a single locus which contributes a large effect partial resistance response. The genetic mapping of *Rph22* and the identification of molecular markers closely linked to this resistance gene (Johnston et al. 2013) will allow it to be incorporated into modern barley

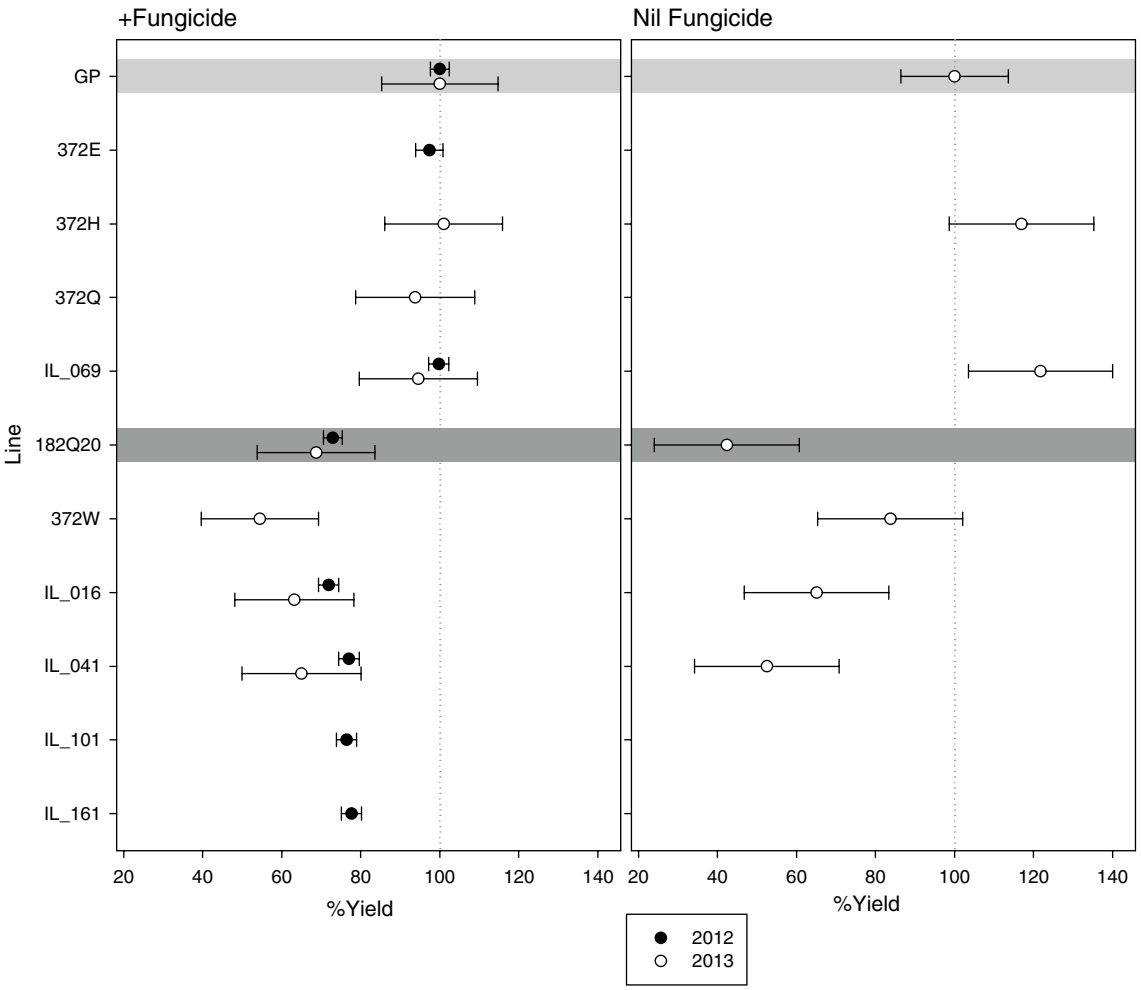


Fig. 4 Relative adjusted mean screened grain yield (cv. 'Golden Promise' 100) from trial 3 (2012–13) and trial 4 (2013–14) featuring fungicide-treated (*left*, trials 3 and 4) and untreated (*right*, trial 4 only) blocks. Parental lines cv. 'Golden Promise' (GP *light grey*) and '182Q20' (Q *darker grey*) are highlighted for reference purposes. *Bars* indicate 95 % confidence intervals. The confidence intervals differ between the trials due to differences in underlying random variation and the number of replicates (8 plots per line in trial 3, except '372E' which had 4 plots; but only 3 plots per line in trial 4)

varieties. However, because of a considerable yield penalty associated with the *H. bulbosum* introgression containing *Rph22*, this gene has not been an attractive target for barley breeders. In this paper, we have used the "182Q20_F4_Popn" mapping population (Johnston et al. 2013) to QTL map (single marker analysis), the individual components of the yield penalty (total yield, TGW, hectolitre weight, percentage screenings and screened yield) to the proximal end of the introgression and genetically distinct from both *Rph22* and $Rym16^{Hb}$ (Fig. 2). To validate the results from the mapping population, two introgression lines, '372E' and '372H', were developed using targeted intraspecific recombination within overlapping introgression segments. This strategy successfully avoided the problem of highly suppressed recombination that has been previously seen in a physically small introgression on chromosome 2HL (Johnston et al. 2013). The use of overlapping introgression segments exploits intraspecific recombination to obtain the desired recombination events in areas where interspecific recombination is suppressed. The resulting lines have introgression boundaries that are defined by the original lines used in the cross, thus removing the need to screen a large number of lines to locate rare recombination events which are suitably close to the target gene. Instead, the position of the introgression boundaries used in the cross will determine the extent of the introgression in the newly developed line. The two lines developed in this manner, '372E' and '372H', were subsequently shown to have yield parameters comparable to those of the barley parent cultivar 'Golden Promise', with the additional benefit of the large effect partial resistance response conditioned by *Rph22*. The line '372H', retains only a very small introgression around

Rph22 (Fig. 1) and would be a suitable donor line for the incorporation of *Rph22* into advanced breeding lines using marker assisted selection. In a previous study (Pickering et al. 2004b), plots of the IL '182Q20' gave a similar yield to 'Golden Promise' under natural leaf rust infection. In trial 4, without fungicide treatment, the three replicates of '182Q20' gave unusually high percentage screenings (52–68 %) compared with means of 33 and 37 % in trials 1 and 2 (Table 1). As there was only a small amount of late leaf rust infection in this trial, it seems likely that some other unrecorded biotic stress may have resulted in the poor performance of '182Q20'. The newly developed '372H' and 'IL_069' had mean screened yields slightly higher than 'Golden Promise' (Fig. 4), indicating that they did not suffer from the problems associated with the full introgression of '182Q20' in trial 4. The yield comparisons performed in this study were against the parental genetic background 'Golden Promise', which is no longer a high-yielding cultivar by modern standards. Incorporation of *Rph22* into elite barley germplasm will be required to determine if there is an identifiable cost to the inclusion of this partial resistance gene itself. However, by reducing the overall size of the introgression around the target trait, it is possible to minimize the likelihood that other alleles/genes derived from *H. bulbosum* may contribute additional yield and/or quality issues.

Another partial resistance gene against barley leaf rust, called *Rph20*, was genetically mapped to chromosome 5HS (Hickey et al. 2011) and has been linked via pedigree/molecular marker analysis as having the same common origin as the cultivar 'Vada' from the barley landrace *H. laevigatum*. The partial resistance in 'Vada', including *Rphq2* (likely to be a paralog of *Rph22*) and *Rphq4* (likely to be the same gene as *Rph20*), has been durable over a considerable period of time (Parlevliet 2002). By extrapolation, we postulate that *Rph22* and *Rph20* may represent durable components of the resistance in 'Vada' against barley leaf rust. Further characterization of these partial resistance genes across a range of plant developmental stages, genetic backgrounds and temperatures, will lead to a greater understanding of the mechanisms involved and how effective these genes will be under field conditions for controlling barley leaf rust (Singh et al. 2013b). Wang et al. (2010) were able to show the different effects that *Rphq2*, *Rphq3* and *Rphq4* (and their susceptible alleles) conferred at different growth stages, with *Rphq2* more effective at seedling stages and *Rphq4* more effective at adult stages. Combinations of QTL/genes for partial resistance to barley leaf rust are known to improve the overall effectiveness of the disease resistance (Parlevliet 1976). *Rphq2* and *Rphq4* can clearly have synergistic roles in plant defense at different plant development stages and perhaps using different pathways (Wang et al. 2010). With molecular markers linked to genes such as *Rph22*/*Rphq2*, *Rph20*/*Rphq4*, it is now possible to combine, track and confirm the effectiveness of these combinations. Additional partial resistance genes against barley leaf rust have also been identified from *H. bulbosum* introgressions on chromosomes 1HL and 5HL (Pickering et al. 2004b). Combinations of these resistance genes would also be beneficial to explore but will need considerable work in marker development and genetic mapping.

Resistance to BaMMV/BaYMV in most winter barley cultivars is conditioned by the recessive genes *rym4* and *rym5*. However, pathotypes of the soil-borne barley mosaic virus complex have been identified which have overcome *rym4* (BaYMV-2, Huth 1989) and *rym5* (BaMMV-Sil, Hariri et al. 2003; Kanyuka et al. 2004 and BaMMV-Teik, Habekuß et al. 2008). Consequently, it is important to look for new, effective resistance genes from other sources including the secondary gene pool of barley. Two dominant resistant genes, namely $Rym14^{Hb}$ located on chromosome 6HS and $Rym16^{Hb}$ on chromosome 2HL, were found in *H. bulbosum* (Ruge et al. 2004; Ruge-Wehling et al. 2006). The "182Q20_F4_Popn" was used to map the resistance gene $Rym16^{Hb}$ against *Barley mild mosaic virus* with a greater number of 2HL markers, and for its position relative to *Rph22* to be determined. In this study, $Rym16^{Hb}$ was shown to co-segregate with the molecular marker H35_17700 (k03475) near the distal end of the introgression (Fig. 2). Previously, $Rym16^{Hb}$ was mapped as the most distal 2HL marker, 3.6 cM distal of the RFLP marker MWG949 and 5.5 cM distal of the STS marker MWG2076 (Ruge-Wehling et al. 2006). In this study, $Rym16^{Hb}$ was located 0.5 cM distal of MWG2076 (which co-segregates with H35_15816, Fig. 2). The presence of two resistance genes (*Rph22* and $Rym16^{Hb}$) against two different diseases located near the distal end of a single introgression shows the high value of *H. bulbosum* as a resource for barley improvement. As BaMMV is not a problem in New Zealand barley crops, neither '372H' nor '372E' were developed to carry the $Rym16^{Hb}$ resistance gene. However, the line 'IL_069' carries both *Rph22* and $Rym16^{Hb}$, possesses a minimal distal introgression, and was shown in trials 3 and 4 to have similar yield parameters to those of cv. 'Golden Promise'. Another line of interest, 'IL_094', was shown to have the genetically smallest distal introgression containing $Rym16^{Hb}$, spanning the genetic interval between markers k08380 and H35_17700 and thus should also not carry the yield penalty.

QTL for yield characteristics such as TGW have been previously mapped to chromosome 2HL for several barley mapping populations. Bezant et al. (1997) detected a QTL for TGW between markers bcd512b and bcd266 and three QTL for plant grain weight, ear grain weight and plot yield at the distal end of chromosome 2HL. Coventry et al.

(2003) have reviewed a range of QTL contributing to grain yield, many of which are pleiotropic effects of plant development genes such as *Ppd-H1*, *eps2*, *sgh1-3*, *sdw1/denso* and *Vrs1*. In addition, there were several QTL mapped in three different mapping populations between bins 12 and 15 on chromosome 2HL 'Blenheim'/'Kym' (bins 12–14, thousand grain weight), 'Igri'/'Danilo' (bin 15, TGW) and 'Sloop'/'Alexis' (bin 13, screenings) by Coventry et al. (2003). The inheritance of genes/paralogs from *H. bulbosum*, within the '182Q20' introgression on chromosome 2HL, resulted in a considerable deterioration in most of the agronomic traits measured (total yield, TGW, percentage screenings, screened yield and lodging). *H. bulbosum* is a wild species which possesses very slim seeds so these genes/paralogs on chromosome 2HL have obviously not been under the same degree of selective pressure as during the domestication and subsequent breeding of cultivated barley. The cleistogamy locus (*Cly1* or *HvAp2*), which controls open/closed flowering (Nair et al. 2010), has been previously linked to QTL for TGW and lodging (Hori et al. 2005; Korff et al. 2006). The data from this study also support an association between the yield penalty inherited from *H. bulbosum* and the *Cly1* locus. A broad QTL for reduction in yield attributes was initially mapped to the proximal end of the introgression using 75 lines from the "182Q20_F4_Popn" mapping population. This QTL location was then refined by the development of the additional introgression lines '372W' and '372Q' which possess introgressions that span different areas of this proximal region (Fig. 1). The line '372Q' had yield characteristics similar to those of the *H. vulgare* parent cv. 'Golden Promise' (Fig. 4). However, the line '372W', which carries an introgression spanning the marker interval k00917 to H35_15016 (including the *Cly1* locus), had the same poor yield parameters as the original introgression line '182Q20' (Fig. 4). This indicates that one locus or a cluster of tightly linked loci near *Cly1* is responsible for the entire yield penalty. An additional locus involved in lodging resistance appears to be located more distally, but this is based on one year's field trial only (2012–13). The initial concern was that lodging in the trials may contribute to measurable yield differences if there were appreciable differences in maturity between the lines. However, the mean lodging score was only correlated with hectolitre weight and less so with the other variables in 2011–12 (Table 3). Lodging can lead to 'pinched' grain resulting in smaller wrinkled seeds and thus have an effect on hectolitre weights. However, hectolitre weight was shown to be variable between seasons for the two parental lines and was not significantly different between 'Golden Promise' and '182Q20' in trial 3 (2012–13, Fig. 3). Despite the lodging, we have managed to validate 'Golden Promise' yield characteristics in both '372E' and '372H' and thus the separation of *Rph22* from the proximal yield penalty QTL. Even with only a small amount of late natural leaf rust infection, lines '372H' and 'IL_069' both outperformed 'Golden Promise' due to the presence of the large-effect, partial leaf rust resistance of *Rph22*.

The distal end of barley chromosome 2HL is known to have high rates of recombination and to be gene rich (Chen et al. 2009; Künzel et al. 2000) and the corresponding region of wheat (2L1.0) covers only 5 % of the physical length but 68 % of the genetic length of that chromosome (Dilbirligi et al. 2005). Chromosome 2HL has also been the most abundant introgression location detected between *H. vulgare* and *H. bulbosum* (Johnston et al. 2009), which is most likely to be because of good rates of interspecific recombination, a high degree of co-linearity and the absence of critical barley genes which may not be compensated for by the introgressed segment. Additional traits or paralogs of interest may await discovery in these introgression lines which cover a gene-rich region of the barley genome.

Author contribution statement Study was conceived by PAJ and RP, plants materials were initially developed by RP and MEF, marker genotyping was performed by VM and PAJ, linkage mapping by PAJ, plant crosses were performed by MEF, trial plans, statistical analysis and figures were done by RCB, and virus phenotyping was performed by AH. Manuscript was written by PAJ with contributions from AH and RCB. All authors contributed to editing.

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Conflict of interest The authors declare that they have no conflict of interest.

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