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Identification of QTL Involved in Resistance of Barley Seedling to Scald (Rhynchosporium secalis)

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ABSTRACT:

A population of 103 recombinant inbred lines (RILs) of barley, that were developed from the cross L94x'Vada' was evaluated at seedling stage for resistance against scald (Rhynchosporium secalis) in a sporeproof air-conditioned glasshouse. Two isolates of R. secalis were used: B9 and 13-13. Both isolates were collected in fields at SCRI (Scottish Crop Research Institute) near Dundee, Scotland, UK. Under glasshouse conditions, two quantitative trait loci (QTLs) for partial resistance (PR) to scald were identified. One QTL (Rrsq1) was detected on chromosome 3 (3H) for latency period and lesion length only against B9 isolate, whereas the other QTL (Rrsq2, on chromosome 7H) was identified for lesion length of both isolates. Rrsq1 was mapped at a region where a QTL was detected, using the same population, for partial resistance to scald under field conditions whereas Rrsq2 was mapped at a similar position as the locus Rrs2 for scald resistance. These cases of co-location of QTL with major genes, not very common, may suggest that some QTL are actually alleles of known major resistance genes.

Key words: Barley, partial resistance, QTL mapping, Rhynchosporium secalis.

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الملخص:

أجريت دراسة على 103 RIL من الشعير والتي تم الحصول عليها من تلقيح L94x'Vada' وذلك لدراسة و تحديد مقاومتها لمرض Rhynchosporium secalis وذلك في داخل الحاضنة النباتية في ظروف مثالية. تم استخدام سلالتين من المرض والتي تم الحصول عليها من معهد أبحاث المحاصيل الاسكتلندي، دندي، المملكة المتحدة. تم تحديد جينتين تؤثر على الصفات الكمية (QTL) لمقاومة المرض. الأولى وهي Rrsq1 تم تحديدها على الكروموسوم رقم H3 والمسئولة عن إطالة فترة الحضانة وتقليل طول بثرة المرض للسلالة B2 فقط بينما كانت الجينة الثانية (Rrsq2) على الكروموسوم رقم H7 مسئولة عن تقليل طول بثرة المرض للسلالتين المستخدمتين. تم تحديد الجينة Rrsq1 في نفس الموقع الذي حددت به جينة مسئولة عن زيادة المقاومة الجزئية لنفس المرضوفي نفس المجتمع وتحت ظروف الحقل المفتوح بينما تم تحديد ألجين الثانية Rrsq2 في موقع مشابه لموقع ألجين Rrs2. إن تحديد الجينات الكمية في نفس مواقع الجينات النوعية، والذي لا يعتبر بالأمر الشائع، يمكن أن يدل على أن هذه الجينات الكمية عبارة عن أليلات للجينات الكبرى.

INTRODUCTION:

Scald, caused by the fungus Rhynchosporium secalis [Oudem.] J.J. Davis, is one of the major leaf-spotting diseases of barley, especially in areas of the world where the climate is temperate and humid. Average annual yield losses to scald have been estimated to range from 5 to 10% (Turkington et al., 1998), but can be as high as 20 to 36% in experimental plots (Orr and Turkington, 1997). Scald can be controlled by the use of fungicides, but the use of genetic resistance is the most effective, economic and environmentally-friendly way to control this disease. Sixteen major gene alleles across nine loci for resistance against scald have been reported so far (Zhan et al., 2008). However, nearly all the R-genes deployed in commercially grown cultivars have had a short effective life (Xi et al., 2002). This ephemeral effectiveness of resistance has caused breeders to look for other types of resistance such as partial resistance which appears to be quantitative, race-non-specific, more durable (Parlevliet, 1975) and is often inherited polygenically (Kari and Griffith, 1997; Zhan et al., 2008). Such resistance is expressed as prolonged incubation period (Xue and Hall, 1991) of the fungus and a reduced diseased leaf area (Robinson et al., 1997).

The use of molecular markers has facilitated the construction of extensive linkage maps in barley (Graner et al., 1991; Qi et al., 1998a). These maps became denser by the integration of new marker types (Marcel et al., 2008). By the availability of such dense linkage maps. Quantitative Trait Loci (QTL) for scald resistance have been identified and mapped in different genetic backgrounds (Shtaya et al., 2006), many of which cluster around the major gene loci (Zhan et al., 2008). QTL mapping is an effective mean for studying genetically complex characters such as quantitatively inherited resistance. Not only we can determine and compare the loci involved in resistance expression, but

also the relative size of their effect on the epidemic, the parent contributing the allele for resistance, and the effectiveness of each locus at various plant development stages or against various pathogen isolates (Qi et al., 1998b, 1999).

In a previous work, Shtaya et al., (2006) found four QTL (Rrsq1, Rrsq2, Rrsq3 and Rrsq4) for resistance against scald in a population of 103 recombinant inbred lines (RILs) derived from a cross between L94 x 'Vada' in field trials.

The objective of this study was to map QTL for seedling resistance to scald under glasshouse conditions with two pure isolates using the same population used by Shtaya et al., (2006), and to compare the results with those obtained by Shtaya et al., (2006).

MATERIALS AND METHODS

PLANT MATERIAL

The resistance tests were performed on a population of 103 recombinant inbred lines (RILs, F9-derived lines) developed from the two-row spring barley cross L94 × Vada. L94 is an Ethiopian landrace with black and naked seeds which, is very susceptible to leaf rust and resistant to scald (Shtaya et al., 2006). 'Vada' is an old Dutch variety with white and covered seeds which, carries a high level of partial resistance to leaf rust (Parlevliet, 1975), and appears to be quite susceptible to scald (Rowling and Jones, 1976).

FUNGAL MATERIAL

Two isolates of R. secalis were used: B9 and 13-13. Both were collected in fields at SCRI (Scottish Crop Research Institute) near Dundee, Scotland, UK in 2005. Isolate B9 was virulent on the United Kingdom Cereal Pathogen Virulence Survey (UKCPVS) differential cultivars (Jones and Newton, 2005): Pirate (BRR7), la Mesita (BRR5), Igri (BRR4) and Athene (BRR3) (octal race 134), and isolate 13-13 was virulent on Pirate (BRR7), Igri (BRR4), Athene (BRR3), Astrix (BRR2) (octal race 116) (Gilmour, 1973; Goodwin et. al, 1990).

Sporulating cultures were obtained by inoculating Petri dishes of CzV8CM medium (Newton et al., 2001) and incubated at 17 ºC in the dark. Spores were harvested by scraping conidia from CzV8CM dishes with a spatula, diluting them in distilled water and macerating the solution for 1 minute at high speed to separate mycelial debris from conidiospores. These were washed by diluting them in sterile distilled water (to remove germination inhibitors), centrifuged at 3,000 g for 2 minutes, resuspended in sterile distilled water and the spore concentration adjusted to 105 spores/ml using a haemocytometer.

INOCULATION ON DETACHED LEAVES

The mapping population were sown in a sporeproof, air-conditioned glasshouse in individual 50 ml pots filled with John Innes no. 2 peat-based compost. The temperature in the glasshouse ranged between 15 ºC and 20 ºC. Natural lighting was supplemented with sodium lamps to extend daylength to 16 hours. Two inoculations were performed: one 14 days after sowing, on the primary leaf with isolate B9 and the other 21 days after sowing on the third leaf with the isolate 13-13.

The inoculation was performed on 30 mm detached leaves placed on 1% (w/v) water-agar containing 120 ppm benzimidazole, with seven randomised leaves per sealed Perspex box (Stewart Plastics, Croydon, UK 80x45x20 mm). Three leaves per RIL, including the parental lines L94 and Vada, were inoculated per isolate. Prior to inoculation, the centre of leaves were gently abraded by brushing with a sable hair paintbrush with the bristles cut to 3 mm long. Then, aliquots of 10 µl spore suspension were placed in the centre of each leaf segment. The sealed boxes were randomised and placed in an incubator at 17 °C under continuous light. After eight days, inoculated leaves were observed daily and the time to appearance of a lesion (latency period), and the lesion length, measured when all lesions showed up, were recorded. Leaves displaying no symptoms were given a lesion length zero.

STATISTICAL ANALYSIS AND MAPPING

A dense morphological and molecular markers map (709 AFLPs and 139 microsatellites) of L94 x Vada was constructed with JoinMap 3.0 by Marcel et al., (2007) was used to identify QTL in the present study. A skeletal map with uniformly distributed markers (approximately 5 cM per marker interval) was extracted (Fig. 1). This skeletal map was used for QTL analyses in a previous study by Shtaya et al. (2006b) and in the present study.

A computer software package, MAPQTL version 5.0 modified from version 3.0 (Van Ooijen and Maliepaard, 1996), was used for interval mapping. In the region of the putative QTL, the markers with the highest LOD values (peak markers) were used as co-factors for running a multiple-QTL mapping programme, the MQM method (Jansen and Stam, 1994). The restricted MQM method (rMQM) was used to determine the values of the LOD, phenotypic variation, additive effect and the confidence interval for the detected QTL. A LOD value of 3 was chosen as threshold value for declaring a QTL. The non-parametric option of MAPQTL was used to test which markers contributed significantly to the severities observed.

RESULTS

Eighteen out of 103 RILs, including the parental line L94, showed immunity with isolate B9 (Figure 1 A & B). With isolate 13-13 only five RILs showed immunity and both parental lines, L94 and Vada displayed scald lesions. For both isolates the segregation for latency period and lesion size was continuous and quantitative, suggesting polygenic inheritance. The lesion size with isolate B9 ranged between 3.1 and 15.4 mm, the lesion size on Vada being 8.7 mm. The latency period of this isolate ranged between 11.8 and 27 days on the RILs and was 15.4 days on Vada.

The lesion size for isolate 13-13 ranged from 2.9 to 9.4 mm, L94 and Vada being 6.1 and 6.8 mm respectively (Figure 1 C & D), and the latency period ranged from 14.7 to 34 days, L94 and Vada being 31.5 and 19 days respectively.

The correlations between latency period and lesion size with the field assessments reported by Shtaya et al. (2006) are shown in Table 1. Lesion size and latency period with isolate 13-13 were negatively correlated (-0.21), but no correlation was found between these parameters with isolate B9. The correlation between the latency period of the two isolates was significant (0.38), but the correlation among lesion sizes was not significant (0.20). Field and seedling traits showed several correlations: disease severity and lesion size for isolate 13-13 (0.31), and disease severity and latency period for isolate B9 (-0.26).

The heritability of the lesion length was 0.52 and 0.18 with isolate B9 and 13-13 respectively, whereas the heritability of the latency period was 0.56 and 0.51. 100 RILs were used with isolate B9 and 101 with 13-13.

In total, two QTL for PR to scald were identified. They were tentatively named as Rrsq (quantitative genes for Resistance to Rhynchosporium secalis) (Table 2 and Fig. 1). One QTL (Rrsq1) was detected on chromosome 3 (3H) for latency period and lesion length only against isolate B9, whereas the other QTL (Rrsq2, on chromosome 7H) was identified for lesion length to both isolates. All alleles were contributed by L94. The total phenotypic variation explained by the QTL detected for lesion length was 34.5% and 14% for isolate B9 and 13-13 respectively and the total phenotypic variation explained by the QTL detected for latency period was 22.9% (to B9 isolate). The total additive effect explained by the QTL detected for lesion length was 39.6% and 12.2% for isolate B9 and 13-13 respectively and the total additive effect explained by the QTL detected for latency period was 2.7% to B9 isolate.

DISCUSSION

In the present work two QTL for partial resistance against Rhynchosporium secalis were detected, one on chromosome 3H and one on 7H. These two QTL act differently and may correspond to different families of genes.

Rrsq1 is a QTL that prolongs latency period and reduces lesion size, components of infection that, in this case,

are not correlated. This suggests a pleiotropic effect of this QTL or, what seems also likely, the presence of two closely linked genes (Wagner et al., 2008). But the most remarkable fact is that Rrsq1 is only effective to isolate B9, which is inconsistent with the race nonspecific nature of the horizontal resistance (Van der Plank, 1968) and, hence, of the QTL. However, cases of isolate specificity of QTL for resistance have been found (Qi et al., 1999; Marcel et al., 2008). This fact may lead to a minor-gene-for-minor-gene interaction between the QTL of the plant and a gene factor conditioning aggressivity or "partial virulence" on the pathogen.

Rrsq1, on chromosome 3H, was mapped at a region where a QTL was detected in the same population for partial resistance to scald under field condition (Shtaya et al., 2006) and in three other mapping populations (Thomas et al., 1995; Spaner et al., 1998; Grønnerød et al., 2002, Hayes et al., 2001). Rrsq1 may be the same as the QTL reported by Shtaya et al., (2006) and the one by Grønnerød et al., (2002) since both Abyssinian and L94, also known as Abyssinian 1102, are of Ethiopian origin (Jørgensen, 1992).

Rrsq2 behaves quite differently. It was effective only for one component of infection, lesion length, but, interestingly, again both isolates. Rrsq2 may correspond to the QTL for scald resistance, at seedling stage, detected in the 'Ingrid' x 'Steudelli' cross (Bjørnstad et al., 2004).

Rrsq1 was mapped at a similar position as the complex locus, Rh(Rrs1)-Rrs3-Rrs4, for scald resistance (Graner and Tekauz, 1996) whereas Rrsq2 was mapped at a similar position as the locus Rrs2 for scald resistance (Chelkowski et al., 2003). These cases of co-location with major genes, not very common, may suggest that some QTL are actually alleles of known major R genes (Bjørnstad et al., 2004), although a complex locus where R genes and QTL cluster may be feasible as well.

The present study demonstrated that this population segregated for at least two QTL for scald resistance under controlled glasshouse conditions. It would be interesting to determine their mode of action of the pathogen in order to decide whether they may constitute a separate class of genes for partial resistance.

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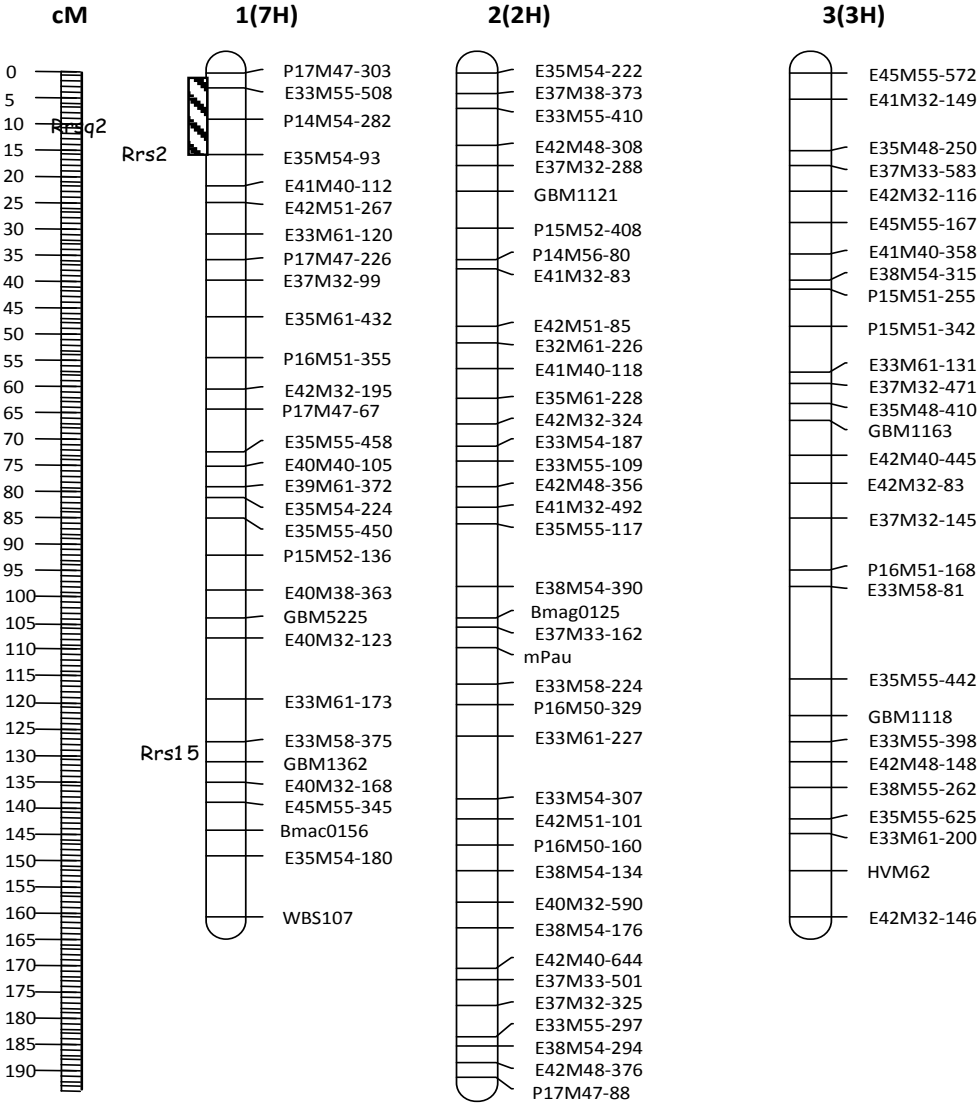
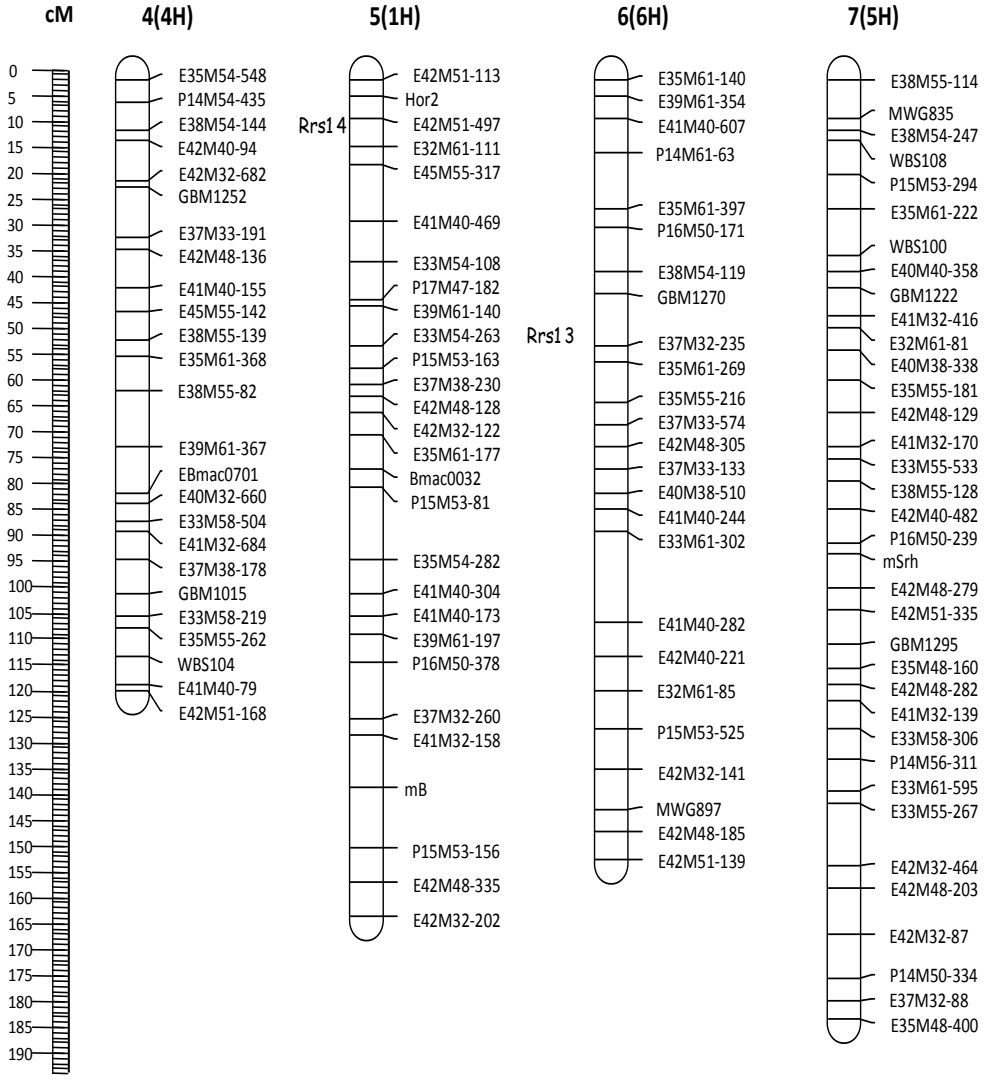
FIGURE 2.

Location of QTL for resistance to scald on the skeletal map of barley cross L94 x 'Vada', based on 100 RILs for QTL mapping to scald with isolate B9 and 101 with 13-13. Chromosomes were oriented with the short arms to the top. Kosambi's mapping function was used. Names of QTL are on the left of each QTL box. Boxes left to the chromosome bars are the QTL for lesion length and boxes inside the chromosome bars are the QTL for latency period. On the left of the chromosome bars all reported loci of race specific resistance genes against scald (Rrs) are indicated.





Lesion length

Latency period

QTL detected by Shtaya *et al.,* 2006

Table 1. Correlation coefficients between field parameters and macroscopic components of the resistance to scald.

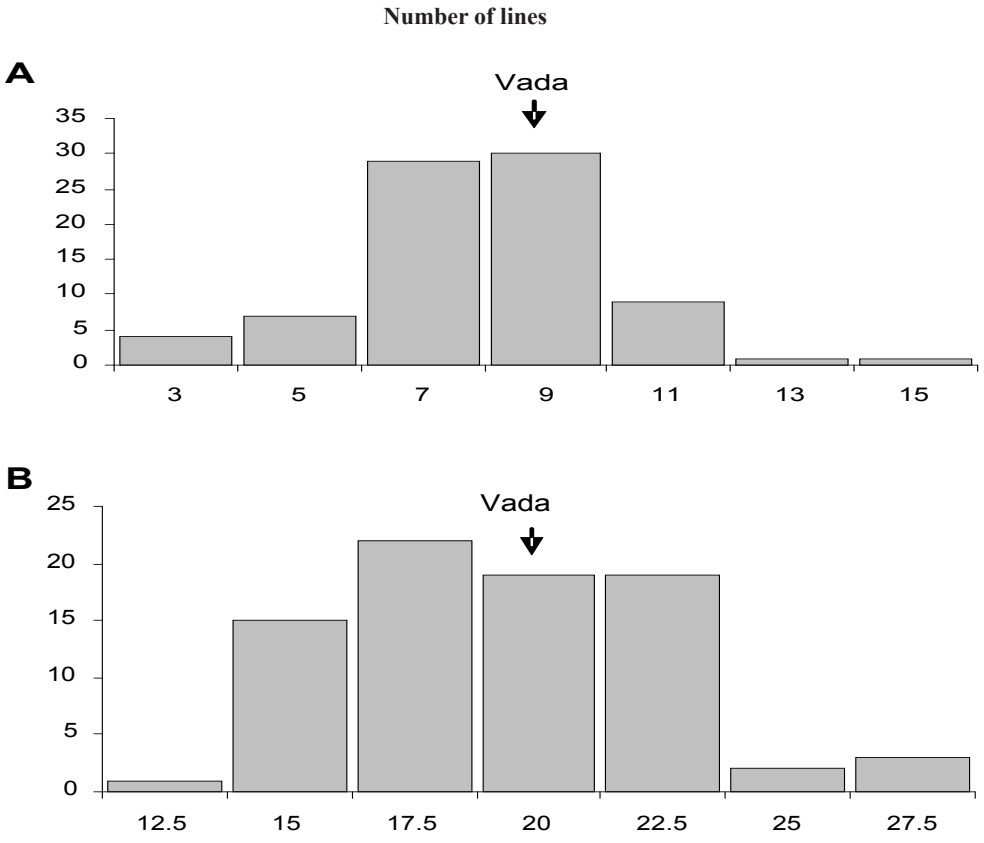
	DS02[1]	Lieson size B8	Lesion size 13–13	Latency Period B9	Latency Period 13–13
DS021	1				
Lieson Size B9	0.02	1			
Lesion size 13–13	0.31××	0.20	1		
Latency Period B9	–0.26××	0.04	–0.13	1	
Latency Period 13–3	–0.06	0.03	–0.21×	0.38×××	1

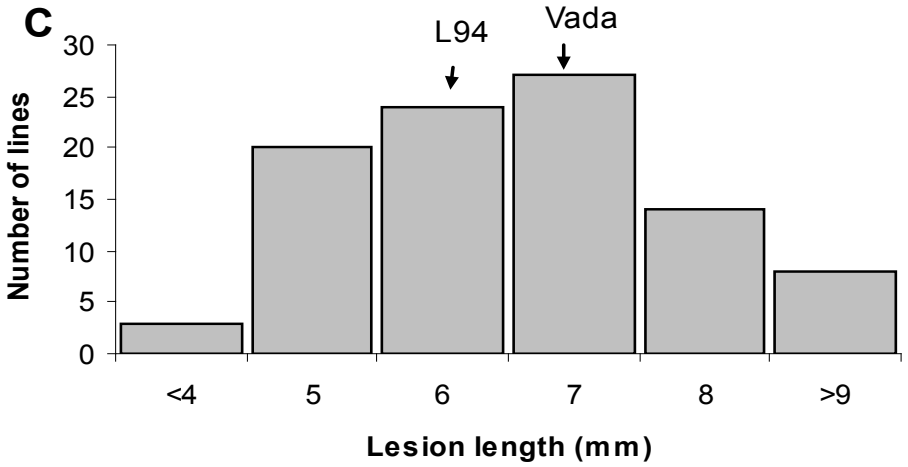
.(Data after Shtaya et al. 2006 (DS02=disease severity in 2002 field trial. and AUDPC=AUDPC in 2004 field trial 1
Correlation is significant at the 0.05 level. ×× at the 0.01 level. ××× at the 0.001 level 2

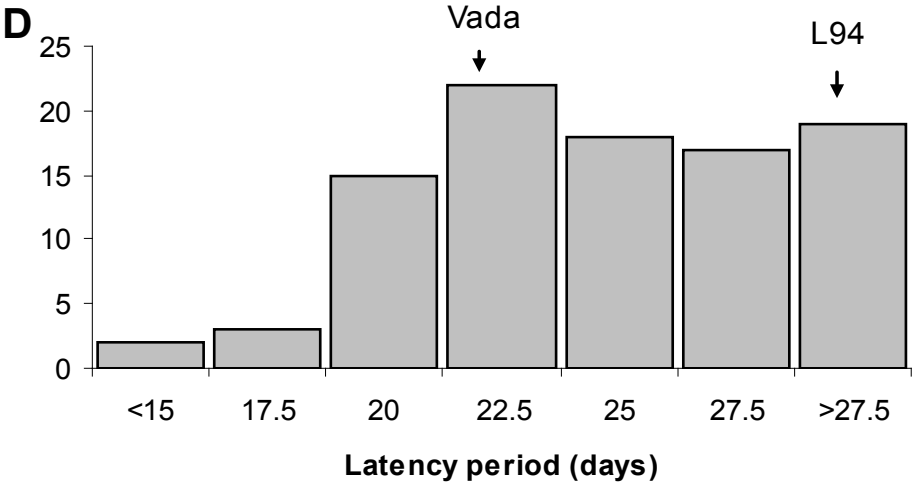
Table 2. Summary of QTL for resistance to scald identified in the cross L94 x Vada .(inoculated with two isolates (B9 and 13–13

			Lesion Length						Letancy Period					
			Isolate B9			Isolate 31–3			Isolate B9			Isolate 13–3		
QTL	Chrom	cM	LOD	Exp%	Add[b]	LOD	Exp%	Add[b]	LOD	Exp%	Add[b]	LOD	Exp%	Add[b]
Rrsq1	3(3H)	64.1	4.1	17.7	–19.7	0.1	0.5	–2.4	6.0	22.9	2.7	0.1	0.3	0.3
Rrsq2	1 (7H)	9.98	3.9	16.8	–19.9	3.1	14.0	–12.2	2.0	8.2	1.6	2.1	8.9	1.6
Total				43.5	–39.6		14.0	–12.2		22.9	2.7			

Figure 1. Frequency distribution of individuals derived from a cross L94×Vada for resistance to scald. A and B represent lesion length and latency period of isolate B9 (85 RILs F9, L94 excluded because of complete resistance). C and D represent lesion length and latency period and of isolate 13-13 (98 RILs F9)




C
L94
Vada
30
25
20
15
10
5
0
Number of lines
<4
5
6
7
8
>9
Lesion length (mm)


D
Vada
L94
25
20
15
10
5
0
<15
17.5
20
22.5
25
27.5
>27.5
Latency period (days)