TWO MAJOR GENES CONFER ASCOCHYTA BLIGHT RESISTANCE IN *LENS ORIENTALIS*

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ABSTRACT

Genetic mechanisms of resistance to Ascochyta blight from wild lentil (*Lens orientalis*) were studied using F₃ families of a cross between the cultivar 'Titore' of the cultivated lentil (*Lens culinaris*) and accession 'W 6 3261' of the wild lentil. The simplest genetic model to adequately fit the data involved two genes, one partially dominant gene with a larger effect (A) and one dominant gene with a less effect (B), which acted additively to confer resistance. One copy of the resistance increasing allele of the A gene is not sufficient for a genotype to be resistant nor is one or two copies of the resistance increasing allele of the B gene alone. When fitted to other published data this model was as good as or better than alternative models that have been proposed.

Keywords: *Ascochyta* blight, disease resistance, genetic model, *Lens orientalis*.

INTRODUCTION

Resistance sources to Ascochyta blight in wild lentils were identified by Bayaa *et al.* (1994). The genetics of resistance to Ascochyta blight coming from *Lens orientalis* was first reported by Ahmad *et al.* (1997). Using F₂ generations, they concluded that the resistance in crosses between resistant *L. orientalis* collections and susceptible *L. culinaris* ssp. *culinaris* cultivars was controlled by a single dominant gene. Because the sample sizes were small and only one type of segregating population was used by Ahmad *et al.* (1997), the inheritance model suggested needs to be confirmed. Using the same cross, Parh (1998) found that the one-dominant gene model could be used to interpret his results if F₃ families were classified into non-segregating resistant, non-segregating susceptible and segregating groups. However, this model could not interpret the results from individual plants of segregating F₃ families because the number of susceptible plants were much more than expected. Parh (1998) did not give a satisfactory explanation of these results. The objectives of this study were to revisit the genetics of Ascochyta blight resistance coming from *L. orientalis* using F₃ families and to investigate the effect of sample size on selecting genetic models.

METHODS

Plant material

The segregating F₂ population derived from a single F₁ individual of the cross between a cultivated lentil, *L. culinaris* ssp. *culinaris* cv. Titore, and an accession of wild lentil, *L. orientalis*, W 6 3261 was used in this study. Eighty F₃ families were obtained by selfing randomly selected F₂ individuals. Each F₃ family plus the parents in the resistance test comprised 10 plants grown in two 2-litre pots. Bark and sand at a 60:40 ratio were used as the potting mixture. The pots were arranged randomly on a bench under greenhouse conditions where maximum and minimum temperatures were 25°C and 15°C respectively.

Isolation of the pathogen, inoculum preparation and pathogenicity test

A pathogenic isolate of *Ascochyta fabae* sp. *lentis* was obtained from infected seeds of cv. Invincible (isolate Rakaia) using the method of Parh (1998). Inoculum was prepared from 15 day old cultures using the method of Parh (1998). The concentration of the spore suspension was measured using a haemocytometer and adjusted to 7.5×10⁴ conidia/ml by dilution. Seedlings with 4-5 expanded leaves (15 days after

sowing) were inoculated with the suspension (1 ml/seedling) using an atomiser. After inoculation, pots were kept in a controlled environment cabinet (18°C and high humidity) for 24 h and then moved to a greenhouse bench for disease development to occur.

Data collection and analysis

Data on disease severity were recorded at four different dates (7, 11, 13 and 15 days after inoculation) according to a modified INCARD disease scoring procedure on a 1-9 scale as well as on both a 2 and 4 point susceptibility scale (Table 1). The data were analysed by χ^2 -statistic to test the goodness of fit of the observed ratio to expected ratios under various genetic models.

TABLE 1: Modified ICARDA disease rating scale (1-9) for *Ascochyta* blight.

Classifications		Scale	Disease severity
R ¹	HR	1	No visible lesions
R	HR	2	Very few lesions after careful examination
R	HR	3	Few scattered lesions after careful examination
R	MR	4	Lesions common & easily observed, no defoliation
R	MR	5	Lesions common & easily observed, little defoliation
S	MS	6	Lesions and defoliation common
S	MS	7	Lesions very common, defoliation moderate
S	HS	8	Lesions very common, defoliation high
S	HS	9	Extensive lesions on all plant parts with stem girdling

¹R=resistant, S=susceptible, H=highly, M=moderately.

RESULTS

No plants developed any symptoms in the negative control, indicating that disease development in the sprayed plots occurred due to inoculation. Disease symptoms were similar to those reported by Ahmad *et al.* (1997). The average disease scores of the resistant and susceptible parents at day 15 were 3.33 and 8.00, respectively (Table 2). On day 7, disease was found sporadically on only a few lines. Thereafter within any group of plants the variability in disease development among the remaining three dates was not significant (Table 2) and thus results from day 15 were used in all the later analyses.

TABLE 2: *Ascochyta* blight disease ratings for parents and F₃ families from the lentil cross Titore × W6 3261.

Days	Parental mean		NSR	Family mean	
	W 63261	Titore		SEG	NSS ¹
7	2.00±0.73	3.33±2.15	1.71±0.78	2.44±1.41	2.76±1.81
11	2.67±0.76	6.92±1.18	2.49±0.81	4.10±1.66	6.22±1.17
13	3.17±0.94	7.75±1.14	2.73±0.92	4.78±1.71	6.86±1.13
15	3.33±0.90	8.00±1.14	2.93±0.93	4.92±1.67	6.98±1.25

¹NSR: non-segregating resistant; SEG: segregating; NSS: non-segregating susceptible.

Out of the 80 F₃ families, 17 were non-segregating and resistant (all plants were resistant), 38 were segregating (some plants were resistant and some were susceptible) and 25 were non-segregating and susceptible (all plants were susceptible). This ratio can be fitted by 1:2:1 ($P = 0.407$), which would be the expected segregation ratio of one dominant gene. If the analysis ended here, the "one (dominant) gene model" fitted the data well. However, instead of using families as the unit of measurement, an analysis was done using the individual plant disease scores. The result was far from

the expected 5:3 segregation ratio under the “one gene model” and consequently this model was rejected (Table 4).

To fit the observed segregation pattern properly, a “two gene model” was proposed. This assumed the existence of one partially dominant gene with a large effect (A) and one dominant gene with less effect (B) which conferred resistance additively. Using this model possession of one increasing allele of gene A would not be sufficient for genotypes to be resistant. The effect of gene B would be so small that the genotypes with one or two increasing alleles of gene B only would be susceptible. However, one increasing allele of gene B would be sufficient to make genotypes with one increasing allele of gene A resistant. Under this “two gene model”, the expected segregation ratio of resistant and susceptible F₂ plants is 5:3 (Table 3). The expected ratio of non-segregating resistant, segregating and non-segregating susceptible F₃ families is 1:2:1. The expected ratio of resistant and susceptible F₃ plants is 17:15. The observed segregation pattern fitted these ratios very well (Table 4). When plants were further classified into HR, MR, MS and HS, the segregation ratio was also very close to the expected ratio 17:17:21:9 of the “two-gene model” (Table 4).

TABLE 3: The segregation of F₂ plants under the proposed “two gene model”.

Parents		
W6 3261 (AA/BB)		Titore (aa/bb)
F ₁		
Aa/Bb(MR)		
F ₂		
1AA/BB (HR) ¹	2Aa/BB (MR)	1aa/BB (MS)
2AA/Bb (HR)	4Aa/Bb (MR)	2aa/Bb (MS)
1AA/bb(MR)	2Aa/bb (MS)	1aa/bb (HS)
Segregation ratio:		
5 resistant : 3 susceptible		
3 HR: 7 MR: 5 MS: 1 HS		

¹See Table 1 for explanation of abbreviations.

TABLE 4: Observed numbers of F₃ plants in four resistance classes tested against predictions from two genetic models for resistance to *Ascochyta* blight.

	Resistant		Susceptible		P for χ^2 (reject if <0.05)
	HR	MR	MS	HS	
Observed	201	212	288	99	
Expected ¹	212.5(17)	212.5(17)	262.5(21)	112.5(9)	0.193
Expected ¹		425(17)		375(15)	0.604
Expected ²		500(5)		300(3)	<0.000

¹ “two gene model”.
² “one gene model”.

When only plants from the segregating families were used, the observed segregation pattern fitted to the “two gene model” very well, no matter whether resistant and susceptible categories were further subdivided. Again the “one gene model” was not supported (Table 5).

To cross-validate the two-gene model, data obtained by Parh (1998) using the same cross was analysed. The goodness of fit was excellent as indicated by the P values (Table 6). Therefore, the proposed “two gene model” for *Ascochyta* blight resistance is appropriate.

TABLE 5: Observed numbers of F₃ plants from segregating F₃ families in four resistance classes tested against predictions from two genetic models for resistance to *Ascochyta* blight.

	Resistant		Susceptible		P for χ^2 (reject if <0.05)
	HR	MR	MS	HS	
Observed	97	126	115	42	
Expected ¹	83.125(7)	130.625(11)	130.625(11)	35.625(3)	0.139
Expected ¹	83.125(7)	130.625(11)		166.25(14)	0.224
Expected ¹		213.75(9)		166.25(7)	0.339
Expected ²		285(3)		95(1)	0.000

¹“two gene model”.²The model of one dominant gene and one recessive gene.**TABLE 6: Fitness of two genetic models to the segregation patterns of the F₃ data reported by Parh (1998).**

	Plants from all F ₃ families				P for χ^2 (reject if <0.05)
	HR	MR	MS	HS	
Observed	76	88	112	49	
Expected ¹	86.3(17)	86.3(17)	106.6(21)	45.7(9)	0.620
Expected ¹		172.7(17)		152.3(15)	0.336
Expected ²		203.1(5)		121.9(3)	0.000

	Plants from segregating F ₃ families				
	HR	MR	MS	HS	
Observed	44	70	74	20	
Expected ¹	45.5(7)	71.5(11)	71.5(11)	19.5(3)	0.981
Expected ¹		117(9)		91(7)	0.675
Expected ³		156(3)		52(1)	0.000

¹“two gene model”.²“one gene model”.³the model of one dominant gene and one recessive gene.

DISCUSSION

For the F₃ families used in this study both the proposed “two gene model” and the “one gene model” suggested by Ahmad *et al.* (1997) had the same expected non-segregating resistant, segregating and non-segregating susceptible segregation ratios (1:2:1). If all the segregating F₃ families came from resistant F₂ plants, the one-gene model could be correct. However, if some of the segregating F₃ families came from the susceptible F₂ plants the “one gene model” would be rejected. Therefore, taking F₃ family as the observation unit does not provide enough information to produce a firm conclusion.

If the “two-gene model” is correct, it should also fit the resistance and susceptibility ratios in the F₁ and F₂ generations. Ahmad *et al.* (1997) found that their single F₁ plant did not have any disease symptoms whereas the “two gene model” would predict only moderate resistance. However, far more plants are needed to adequately test the F₁ generation. The F₂ data reported by Ahmad *et al.* (1997) was fitted to the “two gene model” using a Chi-square test. This model was rejected at a probability of 0.037 (Table 7). However, only 48 F₂ plants were used by Ahmad *et al.* (1997) and misclassification of a single susceptible plant as resistant would have lead to non

rejection of the “two gene model” (Table 7). Moreover, there is a data range (grey range) within which neither model can be rejected. Another two gene model (one dominant and one recessive gene) with a 13:3 resistance and susceptibility ratio in the F_2 was even more difficult to reject using the data of Ahmad *et al.* (1997), although this model was rejected by using individual plant disease scores (Table 4).

TABLE 7: Sensitivity analysis of F_2 data reported by Ahmad *et al.* (1997).

Resistant	Susceptible	P One-gene model (3:1)	P Two-gene model (5:3)	P Other model ¹ (13:3)
37 ²	11	0.739	0.037	0.460
36	12	1.000	0.074	0.267
35	13	0.739	0.136	0.139
34	14	0.505	0.233	0.064

¹ “one dominant gene and one recessive gene”.

² actual published data.

In order to distinguish among genetic models with close segregation ratios using one segregating generation, a large sample size is required. A simulation indicated that more than 200 F_2 plants were required to unequivocally distinguish the “one gene” and “two gene models”. The difficulty in getting F_1 seeds in lentil inter-specific crosses makes this sample size for the F_2 generation formidable. Micropropagation (e.g. by the method of Ye (2000)) of limited F_1 materials by *in vitro* culture could be used to enlarge the F_1 population and hence the F_2 population.

Alternatively other generations with different segregation ratios can be used to reveal the genetic model more accurately. Examples commonly used are backcross (difficult in this situation) and F_3 plants. Use of the F_3 generation has the advantage of: 1) being analysable in three different ways, 2) using the average disease scores to represent the performance of the source F_2 plants and 3) having the ability to easily increase sample size. However, the sample size of the F_3 family must also be large enough to avoid misclassifying segregating families as non-segregating ones. Hanson (1959) demonstrated that 11 is the minimum family size to detect segregation for a dominant gene. In this study, failure to detect segregation would imply that more F_3 families were classified as non-segregating resistant if the one-gene model were correct; this was the opposite to our result. Thus the small F_3 family sample size that we used ($n=10$) could have made it harder to reject the “one gene model” if some segregating families were misclassified. This enhances our confidence in the validity of the “two gene model”.

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