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Genetics of resistance to ascochyta blight (*Ascochyta lentis*) of lentil and the identification of closely linked RAPD markers

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Abstract Foliar resistance to *Ascochyta lentis* is controlled at a single major locus by a dominant gene (AbR_l) in the lentil accession ILL5588 (cv 'Northfield'). Flanking RAPD markers that are closely linked to the resistance locus in coupling phase were identified by bulked segregant analysis. Out of 261 decanucleotide primers screened 7 produced a polymorphic marker that segregated with the resistance locus, and all markers were found to exist within a single linkage group. Five of the seven RAPD markers were within 30 cM of the resistance locus. Log likelihood analysis for detecting QTL associated with the foliar resistance revealed that a single narrow peak accounted for almost 90% of the variance of resistance between the bulks. Preliminary mapping in an F_3 population revealed that the closest flanking markers were approximately 6 and 14 centiMorgans (cM) away from the resistance locus. These markers should be useful for the discrimination of resistant germplasm through marker-assisted selection in future breeding programmes and represent the first essential step towards the map-based cloning of this resistance gene.

Key words *Ascochyta lentis* · *Lens culinaris* ssp. *culinaris* · Bulked segregant analysis · Resistance genes · RAPD · QTL analysis

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Introduction

Ascochyta blight, caused by *Ascochyta lentis* Kaiser et al. (1997) (syn. *A. fabae* Speg. f. sp. *lentis* Gossen et al. 1986), is a serious disease of lentil and may limit lentil production in Australia (Nasir and Bretag 1997 a). The pathogen favours cool, moist growth conditions and is prevalent in most lentil growing areas of the world (Nene et al. 1988). The disease, characterised by leaf, stem and pod lesions, results in reduced seed quality (Gossen and Morrall 1983, 1984) and consequent yield loss (Kaiser 1992; Brouwer et al. 1995). It may be controlled with fungicides (Beauchamp et al. 1986; Bretag 1989) and to a limited extent with heat treatment (Ahmed and Beniwal 1991); however, the most efficient method of control is through the use of resistant germplasm.

Cultivated lentil has a narrow genetic base thought to be caused by intensive selection and subsequent exclusion of much genetic variation from germplasm stocks (Ford et al. 1997). This has probably lead to the exclusion of many important traits such as disease resistance. Currently, programmes are underway to identify and introgress resistance gene(s) from other accessions and wild-related species of lentil into breeding germplasm through wide hybridisation (Cromey et al. 1987; Bayaa et al. 1994). Thus, successful monitoring of the introgression of disease resistance genes into superior breeding lines depends largely on the ability to identify them. Closely linked molecular markers would be useful tools for tagging these genes in glasshouse backcrossing programmes and during field testing for the selection of disease-resistant material, particularly in seasons of low-level natural infection.

First described by Michelmore et al. (1991), bulked segregant analysis (BSA) relies on the availability of two bulked DNA samples collected from individuals that segregate for an extreme phenotype within a single population. Together with random amplified

polymorphic DNA (RAPD) analysis, this method has been used to identify markers that are closely linked to disease resistance genes, such as downy mildew resistance in lettuce (Paran and Michelmore 1993), leaf rust resistance in barley (Poulsen et al. 1995) and rhizomania resistance in sugar beet (Pelsy and Merdinoglu 1996).

The level of seed infection by *A. lentis* on several cultivated lentil lines has been assessed in both field and glasshouse trials and varying levels of resistance have been observed (Ahmed et al. 1996b; Andrahennadi et al. 1996). More recently, the level of resistance to foliar infection has been assessed in several Australian accessions (Nasir and Bretag 1997a,c). Results from these studies indicate that the accession ILL5588 (cv 'Northfield') has a high level of resistance to seed and foliar infection to a range of Canadian and Australian isolates of the pathogen.

There have been conflicting reports of the genetic basis of resistance to seed infection by *A. lentis* due perhaps to different screening methods, sources of pathogen, environmental conditions and the population sizes assessed. Tay (1989) proposed a three-gene model for seed resistance, two dominant genes together with a single recessive gene. Sakr (1994) however, reported that two genes were involved, one dominant and one recessive. Andrahennadi (1994, 1997) showed that seed resistance was controlled by a single major dominant gene, a result that has recently been confirmed by Vakulabharanam et al. (1997). Resistance to foliar infection recently reported in wild species of lentil is thought to be controlled by two dominant genes in *Lens ervoides* and *Lens odemensis* and a single dominant gene in *Lens culinaris* ssp. *orientalis* (Ahmad et al. 1997). However to date, little is known about the genetic mechanism that controls the high level of resistance to foliar infection found in accession ILL5588.

This paper describes the genetics of foliar resistance to ascochyta blight of lentil in the resistant accession ILL5588 and identifies molecular markers closely linked to a major resistance locus.

Materials and methods

Plant materials and inoculation

F_2 and F_3 populations were produced from selfed F_1 and F_2 seed respectively, from a cross between two lentil accessions: ILL5588 (resistant to *A. lentis*) and ILL6002 (cv 'Palouse'; susceptible to *A. lentis*). Both accessions were pre-screened for disease reaction using a collection of *A. lentis* isolates (Nasir and Bretag 1997 c). There were insufficient F_2 seed produced to perform disease resistance trials, and therefore the F_2 plants were selfed in the field to provide a larger F_3 generation from which seed was bulked and sub-sampled. There was no visible evidence of ascochyta in the field at this time, and thus zero bias was assumed in the F_3 population. A second cross from the same parental lines produced a larger F_2 population which was subsequently used for segregation analysis. All parental and progeny seeds were sown in 10-cm diameter pots (5 plants per pot) in a climate-controlled growth chamber at 20–22°C with a 12-h photoperiod (320 $\mu E/m^2/s$) for 10 days. Four pots containing parental plants were distributed randomly within the progeny pots.

An isolate of *A. lentis* (ALH2-2) collected from Horsham, Australia was used to inoculate the seedlings. This isolate was chosen because it exhibited a moderate level of aggressiveness on the moderately susceptible line ILL6473 (data not shown) and thus would potentially allow a larger variation in the expression of the disease than an isolate that has either a high or low level of aggressiveness and therefore enable a better discrimination of the phenotype. Pycnidiospore inoculum (1×10^5/ml) of a single-spore isolate was produced from 14-day-old cultures that had been passaged through the susceptible host and grown on Coon's agar under UV light (Nasir and Bretag 1997 b). Pycnidia were scraped from flooded agar plates, suspended in sterile distilled water to release pycnidiospores and filtered through muslin to remove hyphae. Tween 20 (0.02% v/v) was added to the inoculum as a surfactant. Seedlings were inoculated by spraying with a fine pressurised spore suspension until run-off. They were then placed in the dark at 100% humidity for 48 h to allow for spore germination. Seedlings were then placed back into the growth chamber under conditions previously described until disease assessment.

Evaluation of disease reaction

Each F_3 plant was initially assessed at 11, 18 and 28 days post-inoculation during which time growth conditions were maintained at an optimum for infection. The three assessments were made so as to monitor the level of infection and to choose the best time for discriminating the host reaction. Ten seedlings of each parental line, 96 F_2 and 118 F_3 generations were assessed for severity to *A. lentis* with a 1-9 scoring system adapted from Nasir and Bretag (1997 c). Plants were assessed for disease symptoms and scored in the following mannar: 1 = no visible lesions; 3 = small flecks or small lesions on leaves only, chlorosis at leaf tip; 5 = many leaf lesions, leaf drop, stem flecks or small lesions; 7 = defoliation, extended stem lesions; 9 = stem girdling, plant death.

DNA extraction and polymerase chain reaction (PCR)

Total genomic DNA was extracted from the leaves of 10-day-old F_3 seedlings using the modified hexadecyltrimethylammonium bromide (CTAB) extraction method of Taylor et al. (1995). The concentration of DNA extracted from individuals was assessed spectrophotometrically. Equal amounts of DNA from seven individuals of each resistant or susceptible phenotype were pooled to form two bulks. Molecular markers were generated using arbitrary decanucleotide primers. Reaction mixtures (total volume of 25 µl) contained 30 ng of DNA template, 0.8 units of *Taq* DNA polymerase (Boehringer Mannheim Biochemica, Germany), 240 µ*M* each of dATP, dCTP, dGTP and dTTP, 0.4 µ*M* of primer (Operon Technologies, USA) and PCR buffer with a final concentration of 0.01 *M* TRIS-HCl/3 m*M* $MgCl_2$/0.05 *M* KCl/0.1 mg/ml gelatin, pH 8.3 (Boehringer Mannheim Biochemica, Germany). PCR was performed with a PTC-200 thermocycler (MJ Research, USA) programmed as follows: initial denaturation step at 94°C for 1 min followed by 35 cycles of 94°C for 10 s, 40°C for 30 s and 72°C for 1 min with a final extension at 72°C for 5 min. Amplification products were resolved by electrophoresis on a 1.2% agarose gel in TAE buffer, stained with ethidium bromide and visualised with UV illumination. Where bands of interest were observed, replicate PCR reactions were performed to confirm amplification patterns.

Bulked segregant analysis

"Resistant" and "susceptible" DNA bulks were screened with 261 10-mer primers, and ″resistance″ markers were identified.

Polymorphisms were confirmed between parental lines and between individuals within each bulk. Eighty-eight F_3 individuals were chosen that gave reproducible banding patterns, and these were screened with primers that produced polymorphic bands in the "resistant" bulked individuals and parents only.

Genetic analysis

Since the number of genes governing foliar resistance is unknown the data were analysed by two methods.

Resistance as a Mendelian trait

A phenotype cut-off (to distinguish resistant from susceptible individuals) was established within the segregating F_2 and F_3 populations. A score of 1 or 3 was chosen to indicate a resistant reaction, and a score of 5 and above was susceptible. The observed segregation ratio was compared to the expected ratio in order to establish the appropriate model for genetic control (chi-square test).

Resistance as a metric (quantitative) trait

A log likelihood analysis was performed on the F_3 data in order to identify any quantitative trait loci (QTLs) that may account for the majority of the variation caused by the phenotype. Disease scores (1-9) of individuals were treated as metric characters. Then, using MAPMAKER/QTL version 1.1 (Lincoln et al. 1993 b) with a LOD threshold of 2.00, we could identify potential gene effects as either dominant, recessive or additive, and the relative sizes and locations of any QTLs were observed on a LOD score curve.

Linkage and mapping

Linkage analysis was performed to estimate the relative genetic distances between the phenotype locus and the RAPD markers. The presence or absence of each polymorphic marker was compared with the phenotype of each F_3 individual. MAPMAKER/EXP version 3.0 (Lincoln et al. 1993 a) was used to construct a simple map that included the seven RAPD markers and the resistance locus (loci). Metric data of the phenotype and presence/absence data from the five most closely linked markers were used with MAPMAKER/QTL version 1.1 (Lincoln et al. 1993 b) to perform "Three-point" analysis with a LOD threshold of 3.0 and with a maximum distance of 50 cM between loci as default linkage criteria. A QTL map of best order was constructed from maximum likelihood analysis, and the resistance locus was placed manually. Distances between the resistance locus (loci) and linked RAPD markers were estimated in centimorgans (cM).

Results

Genetic analysis of resistance to *A. lentis* in ILL5588

The F_2 and F_3 distributions for disease reaction were heavily skewed towards the mean of the resistant parent. This indicated dominance at the locus (loci) controlling resistance. The distribution curve did not appear to alter significantly during the assessment period. Therefore, the 11-day scores were used to produce the phenotype ratio. The means of the parental

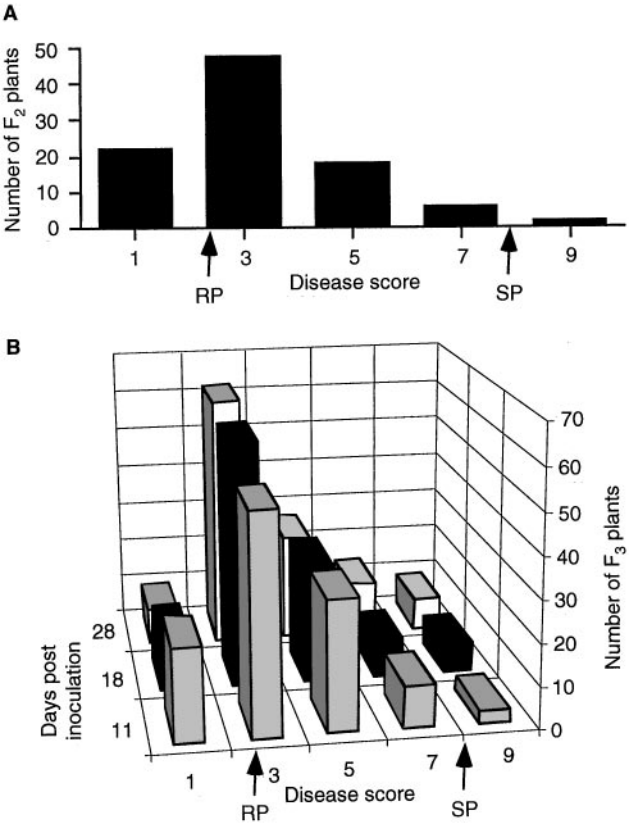

Fig. 1A, B Segregation of the F_2 population at 11 days (**A**) and F_3 population at 11, 18 and 28 days post-inoculation (**B**). *Arrows* indicate mean scores for parental accessions, ILL5588 (*RP*) and ILL6002 (*SP*)

(ILL6002 and ILL5588) scores were 2.6 ± 0.4 SE and 7.6 ± 0.7 SE, respectively (Fig. 1). When resistance was treated as a Mendelian character, the segregation ratio of both the F_2 and F_3 populations was consistent with that expected for a trait controlled at a single locus by a major dominant gene. The putative major gene for *A. lentis* resistance in accession ILL5588 was designated AbR_1 (Table 1).

Screening for RAPD markers linked to the resistant locus

F_3 individuals that maintained a score indicating an extreme phenotype over the assessment period were included in either the resistant or susceptible bulks which each comprised DNA of seven individuals. Of the 261 primers screened against the DNA bulks, 214 produced reproducible banding profiles during replicate amplifications. The presence of polymorphic bands was assessed in parental lines and individuals within each bulk (Fig. 2). This indicated whether bands were inherited from ILL5588 and therefore, their linkage to AbR_1. Twelve primers produced 13 polymorphic bands between ILL5588 and ILL6002. Seven of these

Table 1 Segregation and χ^2 tests of the loci for ascochyta blight resistance (AbR_1) and coupled RAPD markers in F_2 and F_3 populations

Population	Locus	Expected ratio	Observed frequency	Chi-square (χ^2)	Probability (P)
F_2	AbR_1	3:1	70:26	0.17	0.50–0.75
F_3	AbR_1	5:3	74:44	0.002	>0.95
	RAD11	5:3	50:38	1.21	0.25–0.50
	RB18	5:3	52:36	0.44	0.50–0.75
	RO09	5:3	37:35	3.79	0.05–0.10
	RP04	5:3	58:30	0.44	0.50–0.75
	RV01	5:3	57:28	0.80	0.25–0.50
	RV15	5:3	48:35	0.83	0.25–0.50
	RW19	5:3	57:31	0.19	0.50–0.75

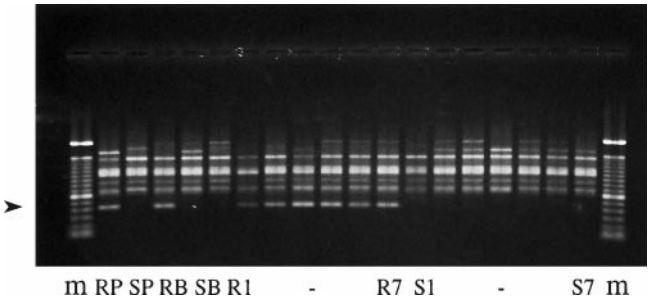

Fig. 2 Amplification pattern obtained with primer OPP-04 on DNA of the resistant and susceptible parents (*RP* and *SP*) and bulks (*RB* and *SB*) and individuals within each bulk (*R1-7 and S1-7*) during the initial screening. The *arrow* indicates the polymorphic band. Molecular weight maker (*m*) is a 100-bp ladder from Gibco-BRL (Bethesda, Md., USA)

primers produced single polymorphic bands common to only ILL5588 and the "resistant" bulked DNA which were linked in coupling phase to AbR_1. None of the remaining polymorphic bands were linked to susceptibility and were therefore unlinked to the resistance locus (Table 2).

The polymorphic markers ranged between approximately 400 bp and 1.7 kb in size. In the F_2 and F_3 populations from the cross ILL5588 × ILL6002, six of the seven polymorphic RAPD markers segregated in Mendelian fashion as dominant loci and with a similar ratio to the phenotype. The marker RO09 showed a segregation ratio distorted from the phenotype ratio, which may have been due to heterozygosity within the donor parent (Table 1). No markers were found that were linked in repulsion to the putative resistance gene (Table 2).

Mapping RAPD markers linked to the resistance gene AbR_1

Analysis with MAPMAKER software showed that all eight loci (AbR_1 and the seven RAPD markers) formed a single linkage group spanning a total area of 98 cM (data not shown). The RAPD markers flanked the resistance locus and, using MAPMAKER/QTL software, a narrow peak with a log likelihood of 16 represented 89% of the total genetic variation between the resistant and susceptible phenotypes. This occurred when the software was constrained to observe a "dominant" effect; the resulting LOD score curve is represented in Fig. 3. The peak was within 30 cM of five of the RAPD markers and was closely flanked by RV01 and RB18 at distances of 6 and 14 cM, respectively. A simple map, spanning 56 cM, was constructed from this information to show the approximate linkage distances between the five closest linked RAPD loci and the resistance locus (Fig. 3).

Discussion

Classical Mendelian genetics and QTL analysis have shown that a major dominant gene (AbR_1) is responsible for resistance to foliar infection by *A. lentis* in the accession ILL5588. This is the first conclusive report of a dominant gene controlling foliar resistance to ascochyta blight in cultivated lentil. However, it is not known if this gene is homologous or indeed identical to the gene(s) expressed during seed resistance to infection by this pathogen (Andrahennadi 1997; Vakulabharan et al. 1997). Also, further progeny testing is essential for the confirmation of genetic inheritance of resistance to *A. lentis* in this cultivar.

Other genetic models for seed resistance in the accession ILL5588 have previously been reported. A three-gene model was proposed by Tay (1989). However, this research was inconclusive because the F_2 populations surveyed comprised a maximum of 35 individuals. Sakr (1994) proposed a two-gene model which was also based on percentage seed infection for differentiation of plant phenotype. The endosperm is one of the most protected regions of the plant and therefore may not give a true representation of susceptibility towards an invading pathogen. A more recent study has shown that resistance found within wild species of lentil is controlled by major dominant genes. It has been

Table 2 RAPD polymorphism and linkage phase of markers to the resistance locus in the susceptible parent (SP) ILL6002, the resistance gene donor parent (RP) ILL5588 and the resistant-bulk (RB) and susceptible-bulk (SB)

Primer	Primer sequence (5′ to 3′)	Size of band (bp)	The RAPD locus	RP[a]	SP	RB	SB	Linkage phase to R-locus
OPAD-11	CAATCGGGTC	500	RAD11	+	−	+	−	Coupling
OPB-18	CCACAGCAGT	680	RB18	+	−	+	−	Coupling
OPO-09	TCCCACGCAA	450	RO09	+	−	+	−	Coupling
OPP-04	GTGTCTCAGG	400	RP04	+	−	+	−	Coupling
OPV-01	TGACGCATGG	1500	RV01	+	−	+	−	Coupling
OPV-15	CAGTGCCGGT	1300	RV15	+	−	+	−	Coupling
OPW-19	CAAAGCGCTC	700	RW19	+	−	+	−	Coupling
OPB-17	AGGGAACGAG	700	−	−	+	+	−	−
OPC-10	TGTCTGGGTG	300	−	−	−	+	−	−
OPN-14	TCGTGCGGGT	300	−	−	+	+	−	−
OPP-09	GTGGTCCGCA	600	−	−	−	+	−	−
OPU-01	ACGGACGTCA	1000	−	+	−	−	+	−
OPP-04	GTGTCTCAGG	1700	−	+	−	−	+	−

[a] +, Presence of RAPD marker; −, absence of RAPD marker

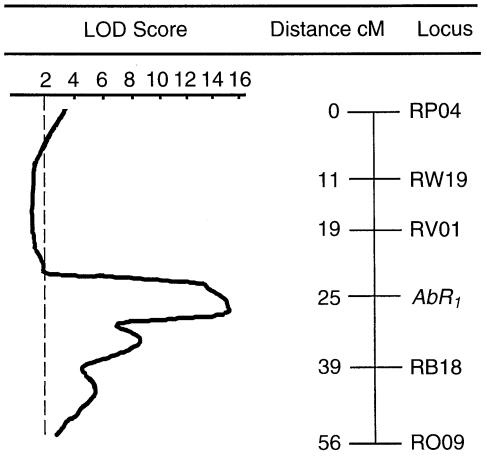

Fig. 3 LOD score variation along the linkage group where the QTL peak represents the probable location of the AbR_1 gene and the genetic map of the RAPD markers that are linked to this resistance locus

suggested that this source may aid in improving the genetic resistance of cultivated lentil through the development of interspecific hybrids (Ahmad et al. 1997). However, these studies have been carried out with a mixed inoculum which may mask disease reactions to individual pathotypes. In Canada, separate mating types of *A. lentis* are known to exist (Ahmed et al. 1996 a). In Australia, six pathotypes of *A. lentis* have been identified (Nasir and Bretag 1997 d), and both mating types have also been found (WJ Kaiser, personal communication). Therefore, the conditions for disease assessment within the above studies may provide an unreliable infection system from which to score phenotypes. In our study these variables were eliminated by using a single-spore inoculum and controlled environmental conditions during disease development. By repeatedly assessing the level of infection on foliage over an extended period after inoculation we were able to show that the disease reaction was stable.

Due to the possibility of the existence of several different genes encoding resistance to different pathotypes, the identification and tagging of major resistance genes is important for gene pyramiding in superior breeding lines. This will aid in combating any potential break-down of resistance when more than a single pathotype is present in the infection complex.

Bulked segregant analysis was effective in identifying seven RAPD markers linked to the AbR_1 gene, five of which were within a genetic "window' of approximately 30 cM either side of the resistance locus. The two closest linked markers, RB18 and RV01, flank the resistance locus and will be useful in marker-assisted selection for lentil breeding programmes. By selecting for the presence of these two markers, breeders will be assured of selecting for a major locus that governs resistance to *A. lentis*. Therefore, these markers will greatly increase the speed and efficiency of conventional repeated backcrossing by reducing the need for subjective disease screening. These markers also offer the potential to detect resistance to foliar infection prior to seed germination.

Due to the dominant nature of RAPD markers, the complete genotype of the F_2 and F_3 progeny could not be assigned as it is not possible to determine between homozygotic and heterozygotic resistant plants. However, individuals of a susceptible phenotype may be considered homozygous for the recessive allele. This is a criticism of the use of RAPD markers, whereby the potential exists for a breeder to pick an individual that is heterozygotic for resistance. Therefore, in order to provide a co-dominant marker, we intend to clone and sequence both flanking RAPD markers and identify internal PCR primer sites. Sequence-characterised amplified regions (SCARs) will be produced which may show a marker length polymorphism between

a homozygotic and heterozygotic individual. Such SCARs will be used for assessing the presence of AbR_1 and the specific genotype in other lentil accessions and wild relatives.

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References

Ahmad M, Russell AC, McNeil DL (1997) Identification and genetic characterization of different resistance sources to ascochyta blight within the genus *Lens*. Euphytica 97 : 311–315

Ahmed S, Beniwal SPS (1991) Ascochyta blight of lentil and it's control in Ethiopia. Trop Pest Manage 37 : 368–373

Ahmed S, Morrall RAA, Kaiser WJ (1996 a) Distribution of mating types of *Ascochyta fabae* f. sp. *lentis*. Can J Plant Pathol 18 : 347–353

Ahmed S, Morrall RAA, Sheard JW (1996 b) Virulence of *Ascochyta fabae* f. sp. *lentis* on lentil. Can J Plant Pathol 18 : 354–361

Andrahennadi CP (1994) Genetics and linkages of isozyme markers and resistance to seed-borne ascochyta infection in lentil. MSc. thesis, University of Saskatchewan, Saskatoon, Sask., Canada

Andrahennadi CP (1997) RAPD markers for Ascochyta blight resistance, phylogenetic studies and cultivar identification in lentil. PhD thesis, University of Saskatchewan, Saskatoon, Sask., Canada

Andrahennadi CP, Slinkard AB, Vandenberg A (1996) Ascochyta resistance in lentil. LENS Newsl 23 : 5–7

Bayaa B, Erskine W, Hamdi A (1994) Response of wild lentil to *Ascochyta fabae* f. sp. *lentis* from Syria. Genet Resources Crop Evol 41 : 61–65

Beauchamp CJ, Morrall RAA, Slinkard AE (1986) The potential for control of ascochyta blight of lentil with foliar-applied fungicides. Can J Plant Pathol 8 : 254–259

Bretag TW (1989) Evaluation of fungicides for the control of Ascochyta blight in lentils. Test of agro-chemicals and cultivars. Ann Appl Biol 114 [suppl] : 44–45

Brouwer JB, Bretag TW, Materne MA (1995) Coordinated improvement program for Australian lentils. In: Proc 2nd Eur Conf Grain Legumes. Copenhagen. Denmark, p 25

Cromey MG, Mulholland RI, Russell AC, Jermyn WA (1987) *Ascochyta fabae* f. sp. *lentis* on lentil in New Zealand. NZ J Exp Agric 15 : 235–238

Ford R, Pang ECK, Taylor PWJ (1997) Diversity analysis and species identification in *Lens* using PCR generated markers. Euphytica 96 : 247–255

Gossen BD, Morrall RAA (1983) Effects of ascochyta blight on seed yield and quality of lentils. Can J Plant Pathol 5 : 168–173

Gossen BD, Morrall RAA (1984) Seed quality loss at harvest due to ascochyta blight of lentil. Can J Plant Pathol 6 : 233–237

Gossen BD, Sheard JW, Beauchamp CJ, Morrall RAA (1986) *Ascochyta lentis* renamed *Ascochyta fabae* f. sp. *lentis*. Can J Plant Pathol 8 : 154–160

Kaiser WJ (1992) Fungi associated with the seeds of commercial lentils from the US Pacific Northwest. Plant Dis 76 : 605–610

Kaiser WJ, Wang BC, Rogers JD (1997) *Ascochyta fabae* and *A. lentis*: host specificity, teleomorphs (*Didymella*), hybrid analysis, and taxonomic status. Plant Dis 81 : 809–816

Lincoln SE, Daly MJ, Lander ES (1993 a) Constructing genetic linkage maps with MAPMAKER/EXP version 3.0. Whitehead Institute for Biomedical Research and the Massachusetts Institute of Technology, Centre for Genome Research, Cambridge, Mass., USA

Lincoln SE, Daly MJ, Lander ES (1993 b) Mapping genes controlling quantitative traits using Mapmaker/QTL version 1.1. Whitehead Institute for Biomedical Research and the Massachusetts Institute of Technology, Centre for Genome Research, Cambridge, Mass., USA

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88 : 9828–9832

Nasir M, Bretag TW (1997 a) Prevalence of *Ascochyta fabae* f. sp. *lentis* on lentil seed from Victoria, Australia. Australian Plant Pathol 26 : 117–120

Nasir M, Bretag TW (1997 b) Variability in the virulence of *Ascochyta fabae* f. sp. *lentis*. In Proc Int Food Legume Res Conf III. Adelaide, Australia, p 160

Nasir M, Bretag TW. (1997 c) Resistance to *Ascochyta fabae* f. sp. *lentis* in lentil accessions. In Proc Int Food Legume Res Conf III. Adelaide, Australia, p 161

Nasir M, Bretag TW (1997 d) Pathogenic variability in Australian isolates of *Ascochyta lentis*. Australasian Plant Pathol 26 : 217–220

Nene YL, Hanounik SH, Qureshi SH, Sen B (1988) Fungal and bacterial foliar diseases of pea, lentil, faba bean and chickpea. In: Summerfield RJ (ed). World crops: cool season food legumes. Kluwer Academic Publ, Dordrecht, The Netherlands, pp 577–589

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85 : 985–993

Pelsy F, Merdinoglu D (1996) Identification and mapping of random amplified polymorphic DNA markers linked to a rhizomania resistance gene in sugar beet (*Beta vulgaris* L.) by bulked segregant analysis. Plant Breed 115 : 371–377

Poulsen DME, Henry RJ, Johnston RP, Irwin JAG, Rees RG (1995) The use of bulk segregant analysis to identify a RAPD marker linked to leaf rust resistance in barley. Theor Appl Genet 91 : 270–273

Sakr B (1994) Inheritance and linkage of genetic markers and resistance to ascochyta blight in lentil. PhD thesis, Washington State University, Pullman, Wash., USA

Tay J (1989) Inheritance and linkage of morphological markers and isozymes in lentil. MSc Thesis, University of Saskatchewan, Saskatoon, Sask., Canada

Taylor PWJ, Geijskes JR, Ko H-L, Fraser TA, Henry RJ, Birch RG (1995) Sensitivity of random amplified polymorphic DNA analysis to detect genetic changes in sugarcane during tissue culture. Theor Appl Genet 90 : 1168–1173

Vakulabharanam VR, Slinkard AE, Vandenberg A (1997) Inheritance of resistance to Ascochyta blight in lentil and linkage between isozyme. In: Proc In Food Legume Res Conf III. Adelaide, Australia, p 162