Theor Appl Genet (2003) 106:428–434
DOI 10.1007/s00122-002-1042-x

A. Tullu · L. Buchwaldt · T. Warkentin · B. Taran
A. Vandenberg

Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus)

Received: 15 April 2002 / Accepted: 21 May 2002 / Published online: 19 September 2002
© Springer-Verlag 2002

Abstract Anthracnose, caused by *Colletotrichum truncatum*, is a major disease problem and production constraint of lentil in North America. The research was conducted to examine the resistance to anthracnose in PI 320937 lentil and to identify molecular markers linked to the resistance gene in a recombinant inbred line (RIL) population developed from a cross of Eston lentil, the susceptible parent, and PI 320937, the resistant parent. A total of 147 $F_{5:6}$ RILs were evaluated for resistance to anthracnose in the greenhouse using isolate 95B36 of *C. truncatum*. Bulked segregant analysis (BSA) strategy was employed and two contrasting DNA bulks were constructed based on greenhouse inoculation of F_5-derived F_6 RILs. DNA from the parents and bulks were screened with 700 RAPD primers and seven AFLP primer combinations. Analysis of segregation data indicated that a major dominant gene was responsible for resistance to anthracnose while variations in the resistance level among RILs could be the influences of minor genes. We designate the major gene as *LCt-2*. MapMaker analysis produced two flanking RAPD markers $OPEO6_{1250}$ and UBC-704_{700} linked to *LCt-2* locus in repulsion (6.4 cM) and in coupling (10.5 cM), respectively. Also, three AFLP markers, $EMCTTACA_{350}$ and $EMCTTAGG_{375}$ in coupling, and $EMCTAAAG_{175}$ in repulsion, were linked to the *LCt-2* locus. These markers could be used to tag the *LCt-2* locus and facilitate marker-assisted selection for resistance to anthracnose in segregating populations of lentil in which PI 320937 was used as the source of resistance. Also, a broader application of the linked RAPD markers was also demonstrated in Indianhead lentil, widely used as a source of resistance to anthracnose in the breeding program at the Crop Development Centre, University of Saskatchewan. Further selection within the few $F_{5:6}$ lines should be effective in pyramiding one or several of the minor genes into the working germplasm of lentil, resulting in a more durable and higher level of resistance.

Keywords Lentil · Disease resistance · *Colletotrichum truncatum* · RAPD and AFLP markers

Communicated by H.F. Linskens

A. Tullu (✉) · T. Warkentin · B. Taran · A. Vandenberg
Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
e-mail: tullu@sask.usask.ca
Tel.: +1-306-966-8682, Fax: +1-306-966-5015

L. Buchwaldt
Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada

Introduction

Lentil (*Lens culinaris* Medikus) seeds are a rich source of protein in many developing countries and they are becoming increasingly popular in developed countries as they are perceived as a healthy component of the diet.

Currently, Canada is leading the world in lentil production and export. However, lentil production is threatened by two major diseases, ascochyta blight (*Ascochyta fabae* f. sp. *lentis* Vassil.) and anthracnose (*Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore). Lentil anthracnose is a relatively new foliar disease in North America and it was first discovered in Manitoba in 1987 (Morrall 1988) and in Saskatchewan in 1990 (Morrall and Pedersen 1991). Lentil anthracnose spread southward and was first reported in North Dakota in 1992 (Venette et al. 1994). Currently, the disease is widespread in western Canada and the lentil-growing areas of the United States. In USA, widely grown cultivars, such as 'Brewer' and 'Crimson', are susceptible to anthracnose. Most of the Canadian cultivars such as 'Eston', 'CDC Milestone', 'Laird', 'CDC Richlea', 'CDC Redwing', 'CDC Glamis' and 'CDC Grandora', are susceptible to this disease. The fungus overwinters in infested plant debris as microsclerotia that serve as a primary inoculum in the field. During the growing season, anthracnose infection initially induces greenish water-soaked lesions on the lower stems. The leaves become necrotic by the early flowering stage, and are shed. By the pod-fill-

ing stage, severe infection may cause shoot dieback, reducing seed yield and quality (Buchwaldt et al. 1996; Chongo and Bernier 1999). Periodic disease epidemics result in drastic yield losses (20–100%) in isolated fields (Gibson et al. 1991; Buchwaldt et al. 1992).

Anthracnose is controlled by the use of fungicides and crop rotations with at least 3 years between lentil crops. Repeated use of fungicides is not recommended as a long-term solution and often discourages production as experienced with other anthracnose diseases in pulse crops, e.g., *Colletotrichum lindemuthianum* in common bean (Tu and McNaughton 1980; Tu 1982). A 4-year rotation is only partially effective, as wind-blown inoculum from infected fields can start new infections. A combination of partial resistance and fungicide application was suggested for the control of lentil anthracnose (Chongo et al. 1999).

Genetic resistance is a viable option for reducing losses from anthracnose infection of lentil. Field and indoor screening of 1,500 germplasm accessions of lentil demonstrated that the cultivar 'Lenka' and a few other accessions (PI 320937, PI 320952, PI 129331, PI 468899, PI 468900, and PI 468901) had partial resistance to anthracnose (Buchwaldt, unpublished). However, Bernier et al. (1992) reported that no lentil was immune to anthracnose. Based on disease severity, as measured by lesion formation, sporulation, and longer incubation and latent periods, high levels of partial resistance were reported in PI 320937, PI 345629, breeding line 458-57 and cultivar 'Indianhead' (Chongo and Bernier 1999). These sources of resistance have been used as parents in the Crop Development Centre (CDC), University of Saskatchewan pulse breeding program, and, consequently, hybridization and selection for increased resistance resulted in the development of cultivars, such as 'CDC Robin', with partial resistance. However, genetic information on the mode of inheritance of resistance to anthracnose is limited. In one such study, we reported two dominant genes and one recessive gene for resistance to anthracnose based on F_3 family segregation in three crosses of which PI 320937 was one of the resistant parents (Buchwaldt et al. 2001).

The genetic basis of resistance to anthracnose has been studied in several other crops and different genetic systems have been proposed. Monogenic resistance to *C. lindemuthianum* has been reported in common bean (Young and Kelly 1997), *Colletotrichum trifolii* in alfalfa (Elgin and Ostazeski 1985) and *Colletotrichum orbiculare* in cucumber (Abul-Hayja et al. 1978). Oligogenic resistance was also reported for races of *Colletotrichum lagenarium* in cucumber (Linde et al. 1990). Seven resistance genes for resistance to anthracnose have been identified and gene symbols *Co-1* to *Co-7* were assigned in common bean (Kelly and Young 1996). Carlson and Hooker (1981) reported that additive genetic effects accounted for more than 90% of the variation in resistance to anthracnose in corn.

Molecular markers, linked to genes for anthracnose resistance, can facilitate indirect selection of resistant plants and reduce the time required to identify resistant breeding lines or segregating lines carrying the desirable alleles. Molecular markers linked to different genes for resistance can also be used to pyramid resistance genes into an otherwise acceptable variety (Miklas et al. 1993; Johnson et al. 1995). Marker technology, coupled with the use of bulked segregant analysis (BSA, Michelmore et al. 1991) and recombinant inbred lines (RILs), provides the opportunity to rapidly identify tightly linked markers and map them in the *Lens* genome. Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) are linked to genes for resistance to various diseases in common bean (Miklas et al. 1993; Johnson et al. 1995; Young et al. 1998), chickpea (Mayer et al. 1997; Ratnaparkhe et al. 1998; Tullu et al. 1998, 1999; Winter et al. 2000), lentil (Ford et al. 1999) and in grapevine (Pauquet et al. 2001).

In this paper we report the mode of inheritance of resistance to anthracnose in PI 320937 lentil, and the identification of AFLP and RAPD markers closely linked to the gene for resistance in RILs developed from the cross between 'Eston' (susceptible) and PI 320937 (resistant).

Materials and methods

Plant material

The genetics of resistance to anthracnose in lentil was studied using 147 F_5-derived F_6 RILs developed by single-seed descent from a cross of the resistant PI 320937 and the susceptible cultivar 'Eston'. Other susceptible cultivars, such as 'Pardina', 'Chilean', 'CDC Milestone', and resistant cultivar, such as, 'Indianhead' and PI 345629 were used as controls. PI 320937 is a late-maturing accession with yellow cotyledons and a black seed coat color. 'Eston' is a small-seeded early maturing cultivar released in 1980 in Canada (Slinkard and Bhatty 1981). It has yellow cotyledons, a green seed coat and is widely grown in North America. PI 320937 was introduced from Germany. It is taller and produces more biomass and residue compared to the Eston lentil.

The isolate

Anthracnose-infected plant samples and seeds of lentil collected from the provinces of Manitoba and Saskatchewan were used to isolate and prepare single-spore cultures. Isolate 95B36 consistently differentiated disease reactions between the resistant PI 320937 and the susceptible Eston lentil. The fungus was grown on oat meal agar (OMA) at room temperature on a laboratory bench under continuous fluorescent light. The inoculum was produced by flooding 7-day old colonies with sterile distilled water and dislodging conidia with a sterile glass rod. The conidial suspension was filtered through Mira-cloth and adjusted to a final concentration of 1×10^5 conidia/ml. Tween 20 (Polyoxyethylene sorbitan monolaurate) was added as a wetting agent at a rate of one drop per 100 ml of the suspension.

Host inoculation

The isolate 95B36 was first evaluated for virulence on the susceptible cultivar 'Eston'. Once the virulence was confirmed, eight seeds of each RIL and controls were planted in single 10 cm-diameter plastic pots filled with soil-less mix. Two seeds of the susceptible cultivar 'Eston' were sown in the middle of every pot to ensure that resistant plants were scored correctly. Two weeks after

sowing, the pots were thinned to five plants per pot. The parental and control lines were each planted in eight pots. Each of these was placed at different locations at random within the experimental bench in the greenhouse to determine if changes in greenhouse temperature affected the reaction of the parental and control lines. The experiment was repeated once. Plants were grown for 4 weeks in a growth room. Then, each pot with its plantlets was encased in a translucent plastic sheet extending above the height of the plants, and each pot was sprayed with 1.5 ml of aqueous conidial suspension of isolate 95B36 until run-off using an atomizer (DeVilbis, Somerset, Pa.,U.S.A.). Immediately after inoculation, the pots were incubated in a humidity chamber (enclosed within a translucent plastic sheet that provided 100% relative humidity during incubation). After 24 h of incubation, the pots were transferred to greenhouse benches. The temperature in the greenhouse was maintained at 18–20 °C/14–16 °C (day/ night) and a 16-h photoperiod with fluorescent and incandescent lighting providing approximately 200–300 µE m^{-2} s^{-1} of light intensity. Host plant reactions were then scored visually when the susceptible parent plants started wilting (10–14 days after inoculation). Disease rating was continued twice each week until maturity, to record the reaction of plants based on disease incidence, total lesion formation on the stem and wilting. Individual plants were scored on a 1–9 rating scale, where 1 = immune and 9 = severely diseased or wilted as described by Buchwaldt et al. (2001) with modifications. The RILs that remained green, produced flowers and pods, irrespective of lesion formation on the stem, were considered resistant, whereas those that were uniformly infected and then wilted similar to the susceptible parent ‘Eston’ were considered susceptible. The individual RILs were then grouped into either resistant, susceptible or segregating categories based on infection level and total wilting. For parental lines, a total of 12 pots per replication were rated. This rating system was also used to evaluate the disease reaction of the 12 individual F_1 plants and the RILs.

DNA extraction

Young leaf tissue from single plants of each RIL was harvested, lyophilized using liquid nitrogen and stored at –70 °C. DNA was extracted by the modified hexadecyltrimethylammonium bromide (CTAB) method of Saghai-Maroof et al. (1984), using leaf tissue (0.35 g) ground in liquid nitrogen. Before the tissue thawed, 0.9 ml of 2 × CTAB buffer containing 1% Na-bisulphate was added and ground again after thawing. The mixture was transferred to a 2-ml tube and incubated for 30 min at 60 °C with occasional mixing. Extraction was done by adding the same volume of 24:1 chloroform/isoamyl alcohol into the tubes, and continuously and gently shaking the tubes back and forth for 5 min. The tubes were centrifuged for 10 min at 13,000 rpm. The aqueous solution was transferred to a new tube and extracted one more time with chloroform/isoamyl alcohol. The extraction was isopropanol-precipitated. DNA was washed with 70% ethanol/0.2 M Na-acetate and 70% ethanol/10 mM NH4-acetate, spun for 3 min at 13,000 rpm and the NH_4 acetate was decanted. The DNA was dissolved in about 300 µl of TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) and quantified with spectrometry. DNA was then diluted to 25 ng/µl and used for PCR. For AFLP work, we used 10 µl of 50 ng/µl DNA concentration, digested with a combination of 2 µl of *Eco*RI and *Mse*I restriction enzymes at 37 °C for 2 h, and ligated at 20 °C for 2 h, to generate template DNA for amplification (AFLP Analysis System 1 Kit, GIBCO-BRL). The pre-amplification step was performed with primers specific for the *Eco*RI and *Mse*I adaptors, and one base pair extension, A and C, respectively, and a selective amplification step with the above primers for pre-amplification plus two additional selective nucleotides. Amplification products were electrophoresed in 6% denaturing polyacrylamide gel (Vos et al. 1995) at 80 W for 2.5 h and the DNA bands were observed by silver staining. The size of the amplification products was determined using a 100 base-pair ladder. The 3-digit number after the selective sequences represent the size of the amplified product in base pairs.

Two DNA bulks were constructed one from eight resistant, and the other from eight susceptible F_5 plants after the DNA volume of each RIL was spectrometer-standardized. Resistant and susceptible F_5 plants were inferred from the disease reaction of their $F_{5:6}$ RILs. The two DNA bulks and the two parental DNAs were simultaneously screened with AFLP and RAPD primers. Polymorphic bands between the contrasting DNA bulks and/or parents were further analyzed on the DNA of the individual plants constituting the bulks and of the remaining RILs.

DNA samples from 95 single F_5 plants were screened with 15 putative markers, and the segregation of marker loci was tested for goodness of fit to the expected Mendelian segregation ratio of 1:1 using a χ^2 test ($P < 0.05$). Linkage analysis was done using the Mapmaker/Exp program version 3.0 (Lander et al. 1987). A linkage group was established using the group command on the two-point data, with a maximum recombination value of 0.30 and a minimum LOD score of 4.0. The Kosambi mapping recombination function was used to determine the distance in centiMorgans (cM) between two markers (Kosambi 1944).

Results and discussion

Segregation for resistance to anthracnose

The consistent reaction of resistant and susceptible controls confirmed the validity of the disease-rating technique. Inoculated plants of ‘Pardina’, ‘Chilean’ and ‘CDC Milestone’ lentil wilted uniformly, while inoculated plants of ‘Indianhead’ and PI 345629 lentil were rated as resistant with a few superficial lesions. The random placement of controls at different locations in the greenhouse showed no change in disease rating, but relatively higher infection levels were observed in pots near the glass wall which were exposed to higher temperatures. During the course of our study, we screened the two parents for resistance to anthracnose several times in the greenhouse. Out of 150 plants of each of the parental lines tested in the greenhouse, PI 320937 remained resistant to *C. truncatum* isolate 95B36 with slight superficial infection; whereas Eston was completely killed 2–3 weeks after inoculation. These differences in disease reactions were consistent in repeated tests in the greenhouse with minor differences in the disease rating attributable primarily to temperature differences within the greenhouse. The effect of temperature on the incidence and severity of anthracnose on lentil was reported earlier by Chongo and Bernier (1999, 2000). The variation in disease severity on PI 320937 lentil in their studies was far less compared to the variation observed in other resistant lines and in ‘Eston’ lentil (Chongo and Bernier 2000). Similar effects of temperature were observed in alfalfa to race 1 of *C. trifolii* (Welty and Rawlings 1985), and of white bean to *C. lindemuthianum* (Tu 1982).

A total of 147 $F_{5:6}$ RILs were inoculated with *C. truncatum* isolate 95B36 and rated for their disease reaction along with the parents and controls. The data for the RILs were used to infer the genotype of the individual F_5 plants from which DNA samples were collected. The F_5 population consisted of 70 resistant plants and 77 susceptible plants giving a good fit to the expected single gene (1:1) ratio ($\chi^2 = 0.43$, $P < 0.5$–0.75, 1 *df*). The con-

tribution from the major resistance allele of PI 320937 resulted in the RILs retaining their leaves with normal flowering and podding with little or no apparent loss of resistance, whereas susceptible RILs were completely and uniformly wilted similar to the susceptible parent 'Eston'. We designate the gene for resistance to anthracnose of the lentil as *LCt-2*. However, while resistance for anthracnose was associated with a major gene effect, a wide range of partial resistance was present among the RILs containing the same major allele. This was characterized by symptoms ranging from varying degrees of superficial lesions to a mixture of superficial and deep non-spreading lesions on the stem. Phenotypes similar to the parental resistance exhibited by PI 320937 were recoverable among the RILs with no apparent transgressive segregation. Similarly, disease severity varied among the susceptible RILs. Plants either became completely and uniformly wilted within 2–3 weeks after inoculation or, in a few cases, a few more days were required for full susceptibility.

This variation in both the resistance and susceptible reactions was interpreted as a minor gene effect. Based on our observations, minor genes contributed positively to resistance and had a continuous effect across the resistant RILs, suggesting the polygenic effect of minor genes. Rodier et al. (1995) made similar conclusions where minor genes with additive effects controlled the level of resistance, or partial resistance, in maize to the maize-streak virus. Chongo and Bernier (2000) described the reaction to *C. truncatum* in four resistant lentil lines, including PI 320937, as partial resistance based on components, such as latent periods, infection frequency and sporulation. The combined effect of the two genetic systems should result in a durable resistance to anthracnose in lentil. Singh and Reddy (1989) and Tekeoglu et al. (2000) also reported genetic resistance to ascochyta blight in chickpea caused by genes with minor effects, in addition to resistance due to a major gene. Other studies have reported a combination of a major gene plus several minor genes controlling resistance to southern corn rust (Holland et al. 1998) and to the P3 isolate of phytophtora blight of pigeon pea (Gupta et al. 1997).

A dominant gene for resistance to anthracnose in lentil PI 320937

Twelve F_1 plants and 11 of the RILs that were segregating for resistance to anthracnose were analyzed to determine whether the gene for resistance to anthracnose was dominant or recessive. The F_1 plants were all resistant, suggesting a dominant gene. In addition, a heterogeneity χ^2 test on the segregating RILs, based on individual and pooled chi-square values of the resistant vs susceptible plants in those RILs was non-significant (χ^2 = 0.76, 1 *df* and χ^2 = 5.02, 10 *df* pooled and heterogeneity, respectively), further indicating that the gene for resistance to anthracnose in PI 320937 lentil was dominant.

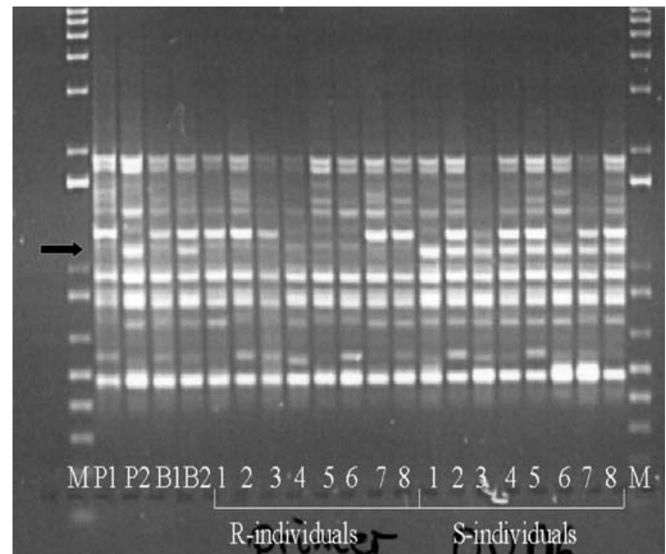

Fig. 1 Amplification of genomic DNA from F_5 RILs of Eston × PI 320937 lentil using the $OPEO6_{1250}$ RAPD marker. *Lane M* 1-kb ladder; *lane P1* resistant parent; *lane P2* susceptible parent; *lane B1* resistant bulk; *lane B2* susceptible bulk; *lanes 1–8* (left) individuals in the resistant bulk; and *lanes 1–8* (right) individuals in the susceptible bulk. *Arrow* indicates the $OPEO6_{1250}$ band

Linkage of resistance gene and markers

A total of 700 random oligonucleotide primers and 12 AFLP primer combinations were used to screen DNA from the two parental lines and the two DNA bulks. Primers that exhibited similar banding patterns for resistance or susceptibility in parental lines and the bulks were then analyzed in the eight individual lines constituting each of the resistant and susceptible bulks and the remaining RILs. Amplification of the individual DNA samples (used to construct the bulks) with operon primer OPEO6 and AFLP primer combinations (*Eco*RI+CTA/*Mse*I+AAG) revealed the presence of the band in susceptible plants, whereas primer UBC-704 and AFLP primer combinations, (*Eco*RI+CTT/*Mse*I+ACA and *Eco*RI+CTT/*Mse*I+AGG) produced bands in the resistant individuals. Figures 1 and 2 illustrate the BSA strategy, using RAPD primer, OPE06 and AFLP primer, *Eco*RI+CTT/*Mse*I+ACA, respectively, and amplification of the bands in PI 320937, Eston, resistant and susceptible bulks, and eight individual samples each constituting the two bulks. When a total of 95 DNA samples from F_5 RILs were screened with these primers, $OPE06_{1250}$, *Eco*R+CTA/*Mse*I+AAG_{175}, UBC-704_{700}, *Eco*R+CTT/*Mse*I+ACA_{350} and *Eco*R+CTT/*Mse*I+AGG_{375} bands segregated conforming to a 1:1 (present : absent) segregation ratio (Table 1). Linkage analysis using the Mapmaker program indicated that $OPE06_{1250}$ and *Eco*R+CTA/*Mse*I+AAG_{175} were linked to the major gene (*LCt-2*) for resistance to anthracnose in repulsion, while UBC-704_{700} and *Eco*R+CTT/*Mse*I+ACA_{350} were linked in the coupling phase. The closest markers $OPE06_{1250}$ and UBC-704_{700} flanked the *LCt-2* locus at 6.4 and 10.5 cM,

Table 1 Chi-square test for segregation ratios of AFLP and RAPD markers in $F_{5:6}$ RILs from a cross of Eston and PI 320937 lentil

Primers	Sequences	Segregation ratio (1:1)		χ^2 (1:1)	*P*
UBC 704	GGAAGGAGGG	48(+)[a]	45(–)[b]	0.10	0.75–0.90
OPEO6b	CCACGGGAAC	46(–)	49(+)	0.10	0.75–0.90
OPEF4	GGTGATCAGG	48(+)	47(–)	0.01	0.9–0.95
UBC 229B	CCACCCAGAG	48(–)	47(+)	0.01	0.9–0.95
OPER4	CCCGTAGCAC	38(–)	56(+)	3.45	0.05–0.10
UBC 18b	GGGCCGTTTA	45(+)	48(–)	0.10	0.75–0.90
EM[c]$CTTAGG_{375}$[d]	CTT/AAG[e]	47(+)	45(–)	0.04	0.75–0.90
$EMCTTACA_{350}$	CTT/ACA[e]	50(+)	42(–)	0.70	0.25–0.50
$EMCTAAAG_{175}$	CTA/AAG[e]	45(–)	48(+)	0.10	0.75–0.90

[a] + = presence of the band
[b] – = absence of the band
[c] EM = corresponds to *Eco*R1 and *Mse*1 primers while
[d] = the numbers indicate the size of the AFLP band
[e] = indicates the three selective sequences for the primers, respectively

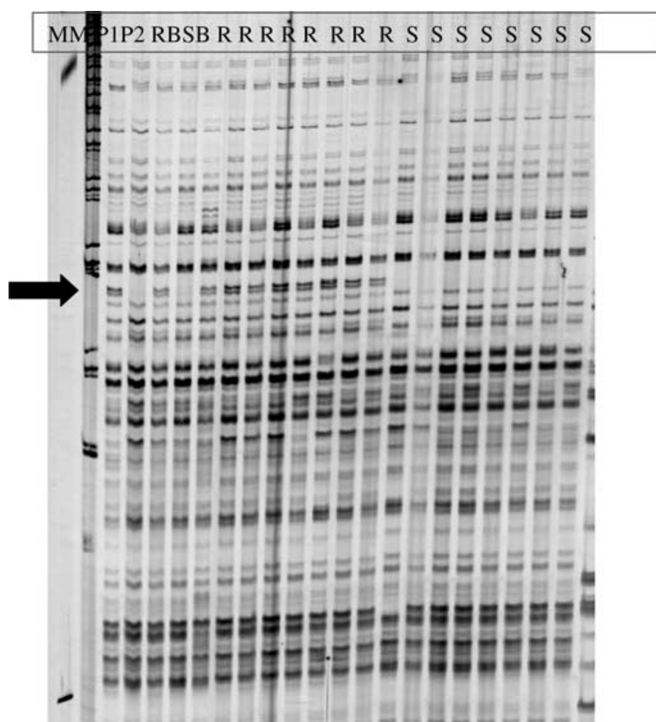

Fig. 2 Amplification of genomic lentil DNA from F_5 RILs of the Eston × PI 320937 lentil using the CTT/ACA primer combination. *Lane MM* I-kb and 100-bp ladders; *lane P1* resistant parent; *lane P2* susceptible parent; *lane RB*, resistant bulk; *lane SB*, susceptible bulk; *lanes R* individuals in the resistant bulk; and *Lanes S* individuals in the susceptible bulk. *Arrow* indicates $CTTACA_{350}$ band

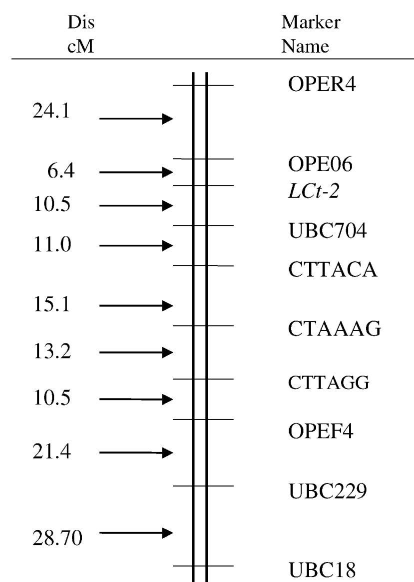

Fig. 3 A linkage group showing the linkage relationships of the domainant gene for resistance to anthracnose (*LCt-2*) in lentil with AFLP and RAPD

respectively (Fig. 3). Fifteen RAPD and AFLP markers were identified in the same linkage group with the gene for resistance, but three of them were discarded because they departed from the expected 1:1 ratio ($P < 0.05$). Among the 12 remaining markers, four were discarded because a stringent LOD score of 4.0 was used. The ten loci including the *LCt-2* locus cover the 140.9-cM region of the lentil genome. The sequence information and segregation ratios for the marker loci are given in Table 1.

One important goal of gene mapping in any crop is to identify molecular tags to genes for resistance. Flanking markers located near the *LCt-2* locus, either individually or in combination, could be used in marker-assisted selection (MAS). Young and Kelly (1997) reported the usefulness of MAS in breeding common bean. Haley et al. (1994) demonstrated a higher selection efficiency for a repulsion-phase marker over a coupling-phase marker in an F_2 population when the two markers flanked the gene for resistance. In our study, DNA samples from 58 F_2 plants from a cross of Eston and PI 320937 were screened for the presence or absence of markers with primers OPEO6 and UBC 704. These two markers, $OPE06_{1250}$ and UBC-704_{700}, and the gene for resistance segregated in a 3:1 ratio (data not presented) ($P > 0.049$, $P > 0.31$ and $P > 0.89$, respectively). In the F_2, the repulsion marker was 11.6 cM away from the *LCt-2* locus, while the coupling marker was 13.8 cM away from the repulsion-marker locus. However, the markers were on one side of the resistance gene and were not linked tightly enough to the *LCt-2* locus to effectively use them as

co-dominant markers for greater selection efficiency. The result of screening the F_2 lines, however, indicated that selection against the presence of the repulsion marker, $OPE06_{1250}$, was effective in rejecting the susceptible plants, while selection for the presence of the coupling marker, UBC-704_{700}, was efficient in identifying all homozygous and 61% of heterozygous resistant F_2 plants. Interestingly enough, these two markers consistently flanked the *LCt-2* locus even with the use of a stringent linkage criterion of the LOD score of 5.0 and a 'ripple' command in Mapmaker 3.0 in the RIL population.

We screened seven lentil cultivars (one resistant and seven susceptible) developed at the CDC, University of Saskatchewan, and a resistant germplasm with the two closest RAPD markers, $OPE06_{1250}$ (6.4 cM) and UBC-704_{700} (10.5 cM), linked in repulsion and coupling to *LCt-2* locus, respectively, to demonstrate their applicability in identifying the disease reactions of these lines.Lentil cultivar, Indianhead (widely used as resistance source in the breeding program) showed an absence/presence pattern of these markers similar to the banding pattern observed for PI 320937. However, when tested with the remaining susceptible and resistant genotypes, these markers picked up susceptible plants as resistant (false positives) in 50% of the cases, and vice versa (data not shown), suggesting that their utility in different genetic backgrounds for MAS is limited. Therefore, the need to identify markers tightly linked (<5 cM, Tanksley 1983) to the *LCt-2* locus is necessary for successful MAS. In summary, the two markers could be utilized to increase the frequency of plants resistant to anthracnose in segregating populations where PI 320937 and Indianhead are used as one of the parents or as donor parents in backcross populations. Whether the resistant genes present in these two lines are the same has yet to be determined.

Seed-coat color and resistance gene

PI 320937 and 'Indianhead' lentil have been extensively used as sources of disease resistance in the lentil breeding program at the CDC. Both lines have black seed-coat color. We scored the $F_{5:6}$ RILs for seed-coat color (uniformly black vs other colors) and conducted linkage analysis, but failed to find any association between black seed-coat color and the dominant gene for resistance to anthracnose. These black-seeded breeding lines could be selected against during early generations of the breeding program, since the probability of losing genetic variability is low and black-seed coat is currently not a desired market class.

In the lentil breeding program at the CDC, hundreds of crosses are made each year, using the few known sources of partial resistance to anthracnose. The F_1s are usually grown in the greenhouse or field under disease-free conditions, followed by evaluation of F_2-derived F_3 irrigated families in disease nurseries in the field before yield testing begins in the F_4 to F_6. In drought years the disease severity may be very low reducing the opportunity to select for resistance. Even though this is a daunting task, screening and selection at later stages (F_5 or F_6) allows for more recombination, so enabling the breeder to select lines with a major gene and more of minor genes for better resistance to anthracnose. The availability of closely linked markers flanking the *LCt-2* locus provides the opportunity to increase selection efficiency and genetic gain when final selection is made within F_5 or F_6 lines that carry the major dominant gene for resistance to anthracnose. To our knowledge, this is the first report on the genetics of the genes for resistance and the development of AFLP and RAPD markers linked to the major gene conferring resistance to anthracnose in lentil.

Acknowledgements The authors acknowledge Drs. A. Slinkard, F. Ahmad, G. Chongo and S. Banniza, CDC, University of Saskatchewan, Saskatoon, for their comments and valuable suggestions. We are grateful to Mr. B. Barlow and Mrs. C. Breitkreutz for their assistance in the field and laboratory experiments. This project is funded by Agriculture Development Fund (ADF) and Agri-Food Innovative Fund (AFIF).

References

Abul-Hayja Z, Williams PH, Paterson CE (1978) Inheritance of resistance to anthracnose and target leaf spot in cucumbers. Plant Dis Rep 62:43–45

Bernier CC, Buchwaldt L, Morrall RAA (1992) Screening for resistance to anthracnose in lentil (*Lens culinaris*). In: AEP (ed) Proc 1st European Conf on Grain Legumes, June 1–3, Recevil de Communications, Angers, France, pp 37–38, Poster

Buchwaldt L, Bernier CC, Platford RG (1992) Diseases of lentil in Southern Manitoba in 1991. Can Plant Dis Surv 72:78–79

Buchwaldt L, Morrall RAA, Chongo G, Bernier CC (1996) Windborne dispersal of *Colletotrichum truncatum* and survival in infested lentil debris. Phytopathology 86:1193–1198

Buchwaldt L, Vandenberg A, Tullu A, Bernier CC (2001) Genetics of resistance to anthracnose (*Colletotrichum truncatum*) in lentil. In: AEP (ed) Proc 4th European Conf on Grain Legume Research, Cracow, Poland, pp 242, Poster

Carlson ML, Hooker AL (1981) Inheritance of resistance to stalk rot of corn caused by *Colletotrichum graminicola*. Phytopathology 71:1190–1196

Chongo G, Bernier CC (1999) Field and growth chamber evaluation of components of partial resistance to *Colletotrichum truncatum* in lentil. Can J Plant Pathol 21:58–63

Chongo G, Bernier CC (2000) Disease incidence, lesion size, and sporulation in *Colletotrichum truncatum* as influenced by lentil genotype and temperature. Can J Plant Pathol 22:236–240

Chongo G, Bernier CC, Buchwaldt L (1999) Control of anthracnose in lentil using partial resistance and fungicide applications. Can J Plant Pathol 21:16–22

Elgin JH Jr, Ostazeski SA (1985) Inheritance of resistance to race 1 and race 2 anthracnose in Arc and Sarenac AR alfalfa. Crop Sci 25:861–885

Ford R, Pang ECK, Taylor PWJ (1999) Genetics of resistance to ascochyta blight of lentil and the identification of closely linked markers. Theor Appl Genet 98:93–98

Gibson RJ, Bernier CC, Morrall RAA (1991) Anthracnose of lentil in Manitoba in 1990. Can Plant Dis Surv 71:104

Gupta AK, Singh IS, Reddy MV, Bajpai GC (1997) Genetics of resistance to *P3* isolate of Phytophthora blight in pigeonpea. Euphytica 95:73–76

Holland JB, Uhr DV, Jeffers D, Goodman MM (1998) Inheritance of resistance to southern corn rust in tropical-by-corn-belt maize populations. Theor Appl Genet 96:232–241

Haley SD, Afanador L, Kelly JD (1994) Selection for monogenic pest resistance traits with coupling- and repulsion-phase RAPD markers. Crop Sci 34:1061–1066

Johnson E, Miklas PN, Stavely JR, Martinez-Cruzado JC (1995) Coupling- and repulsion-phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean. Theor Appl Genet 90:659–664

Kelly JD, Young RA (1996) Proposed symbol for anthracnose resistance genes. Annu Rep Bean Improv Coop 39:20–24

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrhamson J, Barlow A, Daly MJ, Lincoln SE, Newburgh L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Linde DC, Bridges WC, Rhodes BB (1990) Inheritance of resistance in cucumber to race 2 of *Colletotrichum lagenarium*. Theor Appl Genet 79:13–16

Mayer MS, Tullu A, Simon CJ, Kumar J, Kaiser WJ, Kraft JM, Muehlbauer FJ (1997) Development of a DNA marker for fusarium wilt in chickpea. Crop Sci 37:1625–1629

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

Miklas PN, Stavely JR, Kelly JD (1993) Identification and potential use of a molecular marker for rust resistance in common bean. Theor Appl Genet 85:745–749

Morrall RAA (1988) A new disease of lentil induced by *Colletotrichum truncatum* in Manitoba. Plant Dis Rep 72:994

Morrall RAA, Pedersen EA (1991) Discovery of lentil anthracnose in Saskatchewan in 1990. Can Plant Dis Surv 71:105–106

Pauquet J, Bouquet A, This P, Adam-Blondon AF (2001) Establishment of a local map of AFLP markers around the powdery mildew resistance gene *Run1* in grapevine and assessment of their usefulness for the marker-assisted selection. Theor Appl Genet 103:1201–1210

Ratnaparkhe MR, Santra DK, Tullu A, Muehlbauer FJ (1998) Inheritance of inter-simple sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theor Appl Genet 96:348–353

Rodier A, Assie J, Marchand J-L, Herve Y (1995) Breeding maize lines for complete and partial resistance to maize streak virus (MSV). Euphytica 81:57–70

Saghai-Maroof MA, Soliman KM, Gorgenson RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8011–8018

Singh KB, Reddy MV (1989) Genetics of resistance to ascochyta blight in four chickpea lines. Crop Sci 29:657–659

Slinkard AE, Bhatty RS (1981) Eston lentil. Can J Plant Sci 61:733–734

Tanksley SD (1983) Molecular markers in plant breeding. Plant Mol Biol Rep 1:3–8

Tekeoglu M, Santra DK, Kaiser WJ, Muehlbauer FJ (2000) Ascochyta blight resistance inheritance in three chickpea recombinant inbred line populations. Crop Sci 40:1251–1256

Tu JC (1982) Effect of temperature on incidence and severity of anthracnose on white bean. Plant Dis 66:781–783

Tu JC, McNaughton ME (1980) Isolation and characterization of benomyl resistant biotypes of the Delta race of *Colletotrichum lindemuthianum*. Can J Plant Sci 60:585–589

Tullu A, Muehlbauer FJ, Simon CJ, Mayor MS, Kumar J, Kaiser WJ, Kraft JM (1998) Inheritance and linkage of a gene for resistance to race 4 of fusarium wilt and RAPD markers in chickpea. Euphytica 102:227–232

Tullu A, Kaiser WJ, Kraft JM, Muehlbauer FJ (1999) A second gene for resistance to race 4 of fusarium wilt in chickpea and linkage with a RAPD marker. Euphytica 109:43–50

Venette JR, Lamey HA, Lamppa L (1994) First report of lentil anthracnose (*Colletotrichum truncatum*) in the United States. Plant Dis 78:1216

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Welty RE, Rawlings JO (1985) Effects of inoculum concentration and temperature on anthracnose severity in alfalfa. Ecol Epidemiol 75:593–598

Winter P, Benko-Iseppon A-M, Huttel B, Ratnaparkhe M, Tullu A, Sonnante G, Pfaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum*) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163

Young RA, Kelly JD (1997) RAPD markers linked to three major anthracnose resistance genes in common bean. Crop Sci 37: 940–946

Young RA, Melotto M, Nodari RO, Kelly JD (1998) Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, "G2333" Theor Appl Genet 96: 87–94