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ORIGINAL PAPER

M. Iruela · J. Rubio · F. Barro · J. I. Cubero
T. Millán · J. Gil

Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance

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Abstract Two quantitative trait loci (QTLs), (QTL_{AR1} and QTL_{AR2}) associated with resistance to ascochyta blight, caused by *Ascochyta rabiei*, have been identified in a recombinant inbred line population derived from a cross of kabuli×desi chickpea. The population was evaluated in two cropping seasons under field conditions and the QTLs were found to be located in two different linkage groups (LG4a and LG4b). LG4b was saturated with RAPD markers and four of them associated with resistance were sequenced to give sequence characterized amplified regions (SCARs) that segregated with QTL_{AR2}. This QTL explained 21% of the total phenotypic variation. However, QTL_{AR1}, located in LG4a, explained around 34% of the total phenotypic variation in reaction to ascochyta blight when scored in the second cropping season. This LG4a region only includes a few markers, the flower colour locus (*B*/*b*), STMS GAA47, a RAPD marker and an inter-simple-sequence-repeat and corresponds with a previously reported QTL. From the four SCARs tagging QTL_{AR2}, SCAR ($SCY17_{590}$) was co-dominant, and the other three were dominant. All SCARs segregated in a 1:1 (presence:absence) ratio and the scoring co-segregated with their respective RAPD markers. QTL_{AR2} on LG4b was mapped in a highly saturated genomic region covering a genetic distance of 0.8 cM with a cluster of nine markers (three SCARs, two sequence-tagged microsatellite sites (STMS) and four RAPDs). Two of the four SCARs showed significant alignment with genes or proteins related to disease resistance in other species and one of them ($SCK13_{603}$) was sited in the highly saturated region linked to QTL_{AR2}. STMS TA72 and TA146 located in LG4b were described in previous maps where QTL for blight resistance were also localized in both inter and intraspecific crosses. These findings may improve the precision of molecular breeding for QTL_{AR2} as they will allow the choice of as much polymorphism as possible in any population and could be the starting point for finding a candidate resistant gene for ascochyta blight resistance in chickpea.

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M. Iruela · J. Rubio
Dpto. de Mejora y Agronomía, IFAPA, Córdoba,
14080 Córdoba, Spain

F. Barro
Dpto de Agronomia y Mejora Genetica Vegetal,
IAS-CSIC, Córdoba, 14080 Córdoba, Spain

J. I. Cubero · T. Millán · J. Gil (✉)
Dpto de Genética, ETSIAM, Córdoba, 14080 Córdoba, Spain
E-mail: ge1gilij@uco.es
Tel.: +34-957-218510
Fax: +34-957-218503

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse in terms of production, after dry beans and peas (FAOSTAT data 2004). For breeding purposes this species is frequently divided into two main types: desi and kabuli. Desi types mainly have small and dark coloured seeds whereas seeds of kabuli types are larger and cream coloured. The kabuli type is preferred for human consumption in the Mediterranean basin, the Near East, Central Asia and America (Gil et al. 1996) while desi types are widely sown in South Asia and East Africa. Both chickpea types are susceptible to ascochyta blight, caused by *Ascochyta rabiei* (Pass) Lab. This is the most devastating disease of chickpea and may cause total loss of the crop under conditions that are favourable for the pathogen (Singh and Reddy 1983).

Originally the fungus could have been confined to the primary and secondary centres of origin but has been spread worldwide by the movement of infected chickpea germplasm (Kaiser 1997). The disease affects all aerial parts of the plant causing necrotic lesions on stems, leaflets, pods and seeds. Breakage of stems and petioles is also a prominent symptom. In the field, infected plants are commonly seen as patches, reflecting foci from which

the pathogen has spread (Nene and Reddy 1987). Several pathotypes of the fungus have been described (Navas et al. 1998; Udupa et al. 1998) and pathogenicity or aggressiveness within the same isolate can vary according to environmental conditions (Porta-Puglia 1992).

An integrated management approach, including the use of cultural practices, monitoring field populations of the pathogen, seed treatments, foliar sprays of fungicide and the use of resistant or tolerant cultivars is often practised in order to control ascochyta blight of chickpea (Strange et al. 2004). Sources of resistance to ascochyta blight have been identified (Singh et al. 1983) and breeding chickpea for resistance to blight has become an important objective of chickpea improvement programmes (Muehlbauer and Singh 1987). Development of blight resistant germplasm has allowed the promotion of winter sowing of chickpea in the Mediterranean basin with the prospect of significantly increasing yields (Singh and Reddy 1996).

Marker-assisted selection (MAS) for blight resistance would greatly accelerate the development of new chickpea cultivars. Two major quantitative trait loci, QTL-1 and QTL-2, that confer resistance have been tagged with RAPD, inter-specific-sequence-repeat (ISSR) and isozyme markers in a recombinant inbred line (RIL) population from an interspecific cross of *C. arietinum* L. (resistant parent) and *Cicer reticulatum* Lad. (susceptible parent) (Santra et al. 2000). Later, six co-dominant sequence-tagged microsatellite sites (STMS) markers and two DNA amplification fingerprinting (DAF) were integrated into the map region where the two QTLs were located using the same RIL population (Tekeoglu et al. 2002; Rakshit et al. 2003). New sources of resistance have also been identified in *C. echinospermum* and have been used to detect QTLs associated with ascochyta blight resistance in an F_2 population derived from a wide cross of *C. arietinum*×*C. echinospermum* (Collard et al. 2003). It is relevant to consider that many of the polymorphisms detected in populations derived from interspecific crosses may be absent in cultivated varieties; therefore, it will be necessary to verify QTLs found in wide crosses within *C. arietinum* intraspecific crosses in order to determine if they can be used in MAS.

A major gene conferring resistance to pathotype I of *A. rabiei* and two independent QTLs conferring resistance to pathotype II have been identified and mapped by Udupa and Baum (2003) in *C. arietinum*. Also, Cho et al. (2004) employing a different RIL population found QTLs for resistance to pathotypes I and II of *A. rabiei*. One of those QTLs could be the same as QTL-1 reported by Santra et al. (2000) in an interspecific cross because of the presence of the common STMS GAA47 (Tekeoglu et al. 2002). Flandez–Galvez et al. (2003) using F_2 progeny derived from a chickpea cross between desi (resistant)×kabuli (susceptible) genotypes detected three QTLs in a genomic region coincident with LG4 in the interspecific crosses (common markers STMS TA130 and TA146) (Winter et al. 2000; Tekeoglu et al. 2002). Therefore, comparison among maps obtained from different crosses is only possible by using locus specific markers such as STMS or SCARs (Paran and Michelmore 1993).

Using an intraspecific cross, we identified a QTL for resistance to blight in line ILC3279 in a genomic region that was highly saturated with RAPD markers (Millán et al. 2003). In the present study, the objective was to convert RAPD markers that are linked to this QTL into locus specific SCAR markers in our material, and to validate other QTLs reported by other authors using STMS markers.

Material and methods

Plant material

A population of 106 $F_{6:7}$ RILs derived from an intraspecific cross between lines of *C. arietinum*, ILC3279×WR315, was used. ILC3279 is a resistant kabuli line from the former Soviet Union (maintained by the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria) and WR315 is a susceptible desi landrace from Central India (maintained by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT); Patancheru, India). The RILs were advanced from F_2 to F_6 by the single seed descent (SSD) procedure.

Evaluation for Ascochyta blight

The RILs were screened in the field during 2002 and 2003 at Córdoba (southern Spain). They were sown on 30 January 2002 and 4 February 2003 in single rows 2 m long and spaced 0.3 m apart and with a within row spacing of 10 cm. The lines were distributed in two replications with blocks including the parental lines. Single rows of a susceptible spreader (cv. 'Blanco Lechoso') were sown every four rows and, additionally, rows of the spreader were sown around the experimental field in order to increase disease pressure and its uniformity. Inoculum was provided by infested chickpea debris collected from previous years and was distributed within the plot area on 3 April 2002 and on 8 April 2003. After inoculation, the field was sprinkle irrigated frequently to maintain a moist environment favourable for disease development. Disease reaction of each RIL, parents and spreader was evaluated using a 1 (highly resistant) to 9 (highly susceptible) rating scale based on the severity of the infection on leaves, stems and pods as proposed by Singh et al. (1981). Plants were scored weekly for five consecutive weeks starting from the time at which the susceptible check showed disease symptoms. The five scores were used to calculate the area under the disease progress curves (AUDPC) of each line (Campbell and Madden 1990).

Analysis of variance was applied to the AUDPC data over the 2 years according to the following model:

$$x_{ijk} = \mu + Y_j + R/Y_{k(j)} + L_i + YL_{ji} + e_{ijk}$$

where x_{ijk} is the individual datum, μ the general mean, Y_j the effect of jth year, L_i the effect of ith RIL, $R/Y_{k(j)}$ the effect of the kth repetition within the jth year, YL_{ji} the effect of the jith year×RIL interaction and e_{ijk} is the residual error.

Genotypic analysis

The RIL population was phenotyped for flower colour (*B*/*b*, pink/white) and for 17 RAPD decamer primers and one ISSR that produced markers associated with ascochyta blight resistance (Millán et al. 2003). The population was also genotyped for three STMS markers (GAA47, TA72 and TA146) associated with two QTLs for ascochyta blight resistance previously identified in a chickpea interspecific cross (Tekeoglu et al. 2002), two of which were also reported associated with QTLs in intraspecific crosses (Udupa and Baum 2003; Flandez–Galvez et al. 2003; Cho et al. 2004).

For DNA extraction, about 100 mg of young leaf tissue was excised, frozen immediately in liquid nitrogen and stored at −80°C. DNA was isolated using the CTAB method of Lassner et al. (1989) with the modifications described by Torres et al. (1993).

Optimal reaction conditions for RAPD analysis were established according to Williams et al. (1990). Amplification was carried out in 25 μl reactions containing 20–40 ng of plant genomic DNA, buffer (50 mM KCl, 10 mM Tris–HCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 125 μM of each dNTP, 0.32 μM of decamer primers (Operon Technologies, CA, USA) and 0.6 units of *Taq* DNA polymerase (Bioline). Amplification was achieved in a PE Applied Biosystems GeneAmp 9700 thermal cycler, programmed for 40 cycles with the following temperature profile: 20 s at 94°C, 1 min at 36°C, 1 min at 72°C. Cycling was concluded with a final extension at 72°C for 8 min.

The ISSR analysis was performed following the primer sequences and protocol of Ratnaparkhe et al. (1998). The 25 μl reaction volumes contained 30 ng of genomic DNA in 10 mM Tris–HCl, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.24 μM of primer and 1 unit of *Taq* polymerase (Bioline). The Thermal Cycler was programmed for 35 cycles of the following temperature profile: 94°C for 30 s, 50°C for 30 s and 72°C for 2 min followed by a final extension at 72°C for 10 min.

The STMS primer sequences were described by Winter et al. (1999). Amplification conditions were established according to Winter et al. (1999) with modifications in 25 μl reactions containing 20–40 ng of plant genomic DNA, buffer (50 mM KCl, 10 mM Tris–HCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of primer and 1 unit of *Taq* DNA polymerase (Bioline). After denaturing the DNA for 2 min at 96°C the reaction mixture was subjected to 35 cycles of the following temperature profile: 96°C for 20 s, 55°C for 50 s and 60°C for 50 s and a final extension at 60°C for 5 min.

SCAR development

The RAPD fragments were excised from agarose gels and the DNA was purified using the QIAquick Gel Extraction Kit (Qiagen). The purified DNA was cloned into the pGEM-T Vector System I (Promega Corporation, USA). For each RAPD marker, three inserts were sequenced in both forward and reverse orientations using the Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and with an ABI3700 DNA Analyzer (Applied Biosystems).

The consensus sequence from each RAPD marker was used to design SCAR primers (software OLIGO version 6.45) which were synthesized by Sigma-Genosys (UK). They were 19–25 nucleotides long, including the ten bases of the RAPD, with similar T_m values for each primer pair and were not susceptible to the formation of dimers and hairpins. They were synthesized by Sigma-Genosys. SCARs and their likely translated products were compared with DNA and protein sequences available in GenBank (http//www.ncbi.nlm.nih.gov/) for similarities using the BLAST algorithm (Altschul et al. 1997).

Genomic DNA from parental, one resistant RIL, and one susceptible line was used as template to optimize annealing temperature and amplification conditions for SCAR primers. PCR amplifications were carried out with a TGradient thermocycler (Biometra, Goettingen, Germany) in 25 μl reaction volumes. Each PCR reaction contained 50–75 and 10–30 ng of plant genomic DNA for $SCK13_{603}$ and for the rest of the markers, respectively, buffer (50 mM KCl, 10 mM Tris–HCl, 0.1% Triton X-100), 2 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of primer and 1 unit of *Taq* DNA polymerase (Bioline). The thermal profile for PCR was an initial denaturation at 95°C for 5 min followed by either 40 cycles for OPM02/935 or 30 cycles for the rest of the SCAR markers of 95°C for 1 min, 50– 70°C for 30 s, and 72°C from 20 s to 1 min 20 s with a final extension at 72 °C for 8 min.

Amplification products from the RAPD, ISSR and SCAR amplifications were electrophoresed in gels composed of a mixture of 1% agarose Seaken and 1% Nu-Sieve agarose (Hispanlab SA) in 1x TBE buffer. STMS were analysed in 2.5% Metaphor agarose (Biowhitaker Molecular Application) also in 1x TBE buffer.

Marker data were tested for the expected 1:1 segregation ratio using χ^2 goodness of fit . Linkage analysis was performed using JOINMAP 3.0 (Van Ooijen and Voorrips 2001), a LOD score threshold of 3 and a maximum recombination fraction of 0.25 were employed as general linkage criteria to establish linkage

groups. Kosambi's function was applied to estimate map unit distance (Kosambi 1944). QTL analysis was performed using MAPQTL 5 software (Van Ooijen 2004). Interval mapping (IM) with a mapping step size of 1 cM was used to identify putative disease resistant QTLs in every linkage group considering the evaluations made in the two cropping seasons. Significance of QTLs was empirically determined with the permutation test consisting of 1000 replications (Churchill and Doerge 1994).

Results

Field evaluation for ascochyta blight

The AUDPC of the RIL population followed a normal distribution in both years (Fig. 1). Mean values for both resistant and susceptible parents were significantly different in both years. The analysis of variance showed highly significant variation among RILs for AUDPC and the RIL×year interaction was also significant (Table 1).

Linkage analysis

Two linkage groups (LG4a and LG4b) with 4 and 21 markers, respectively, were obtained (Fig. 2). Both linkage groups were numbered according to the extensive map of Winter et al. (2000) and taking in account the position of indicative STMS markers. Considering the common STMS markers (GAA47, TA72, TA146) both linkage groups may be also coincident, respectively, with LG VIII and IV of Tekeoglu et al. (2002) and LG4A and LG4B of Cho et al. (2004). All marker segregation ratios fit to Mendelian inheritance of 1:1. Three of the selected primers employed in a previous study (Millan et al. 2003) generated new polymorphic bands also associated with blight resistance ($OPR11_{870}$, $OPY10_{370}$ and $UBC881_{465}$). Eleven of the markers located in LG4b (10 RAPD and 1 ISSR) were the same as those in the linkage group associated with resistance to ascochyta blight in the cross ILC3279×CA2156 (Millán et al. 2003). Eight new RAPD and two STMS (TA72 and TA146) markers were added in the present study and mapped in the linkage group reported by Millan et al. (2003). These 21 markers on LG4b covered a genetic distance of 38.1 cM with a tight cluster of markers in the middle. The second linkage group included four different markers: one RAPD ($OPR11_{870}$), one ISSR ($UBC881_{465}$), the flower colour locus (*B*/*b*) and the STMS marker GAA47.

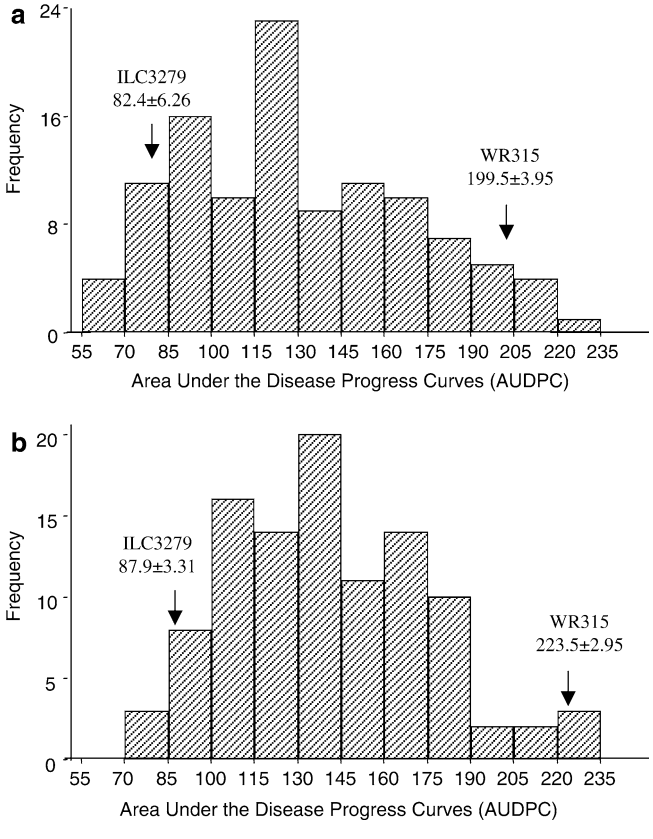

Fig. 1 Frequency distribution for AUDPC of the chickpea RIL population from the cross ILC3279×WR315 scored for Ascochyta blight resistance during 2 years, **a** 2002 and **b** 2003. Average values for parental lines are *arrowed*

Table 1 Analysis of variance for resistant reaction to blight as AUDPC of a RIL population of chickpea growing during 2 years

Variation source	*df*	Mean square
Year	1	12619.77
Repetition (year)	2	12831.76
RIL	103	4806.44***
Year×RIL	101	643.22*
Error	201	477.78

QTL analysis

Due to the significant year–RIL interaction (Table 1) we have analysed data for AUDPC in each year separately. In year 2002, simple linear regression analysis revealed a significant association between LG4a and ascochyta blight severity. Interval mapping allowed us to localize a QTL in this linkage group (QTL_{AR1}) explaining around 15% of the total phenotypic variation with a maximum LOD value of 2.1 (Fig. 3) (significance level detected by permutation test was 1.5).

Significant mapping association between both linkage group (LG4a and LG4b) and resistance to ascochyta blight was found in the year 2003 ($P < 0.001$ for most markers). The IM showed the presence of one QTL in each linkage group (QTL_{AR1} and QTL_{AR2}) for resistance to blight (Fig. 3). Maximum values of LOD score were 5.6 and 5.13 in LG4a and LG4b, respectively. The proportion of the total phenotypic variation explained for both QTLs (R^2) was 34 and 21%, respectively. A major QTL peak was mapped in the middle of LG4b in a genomic region where nine markers (three SCAR, two

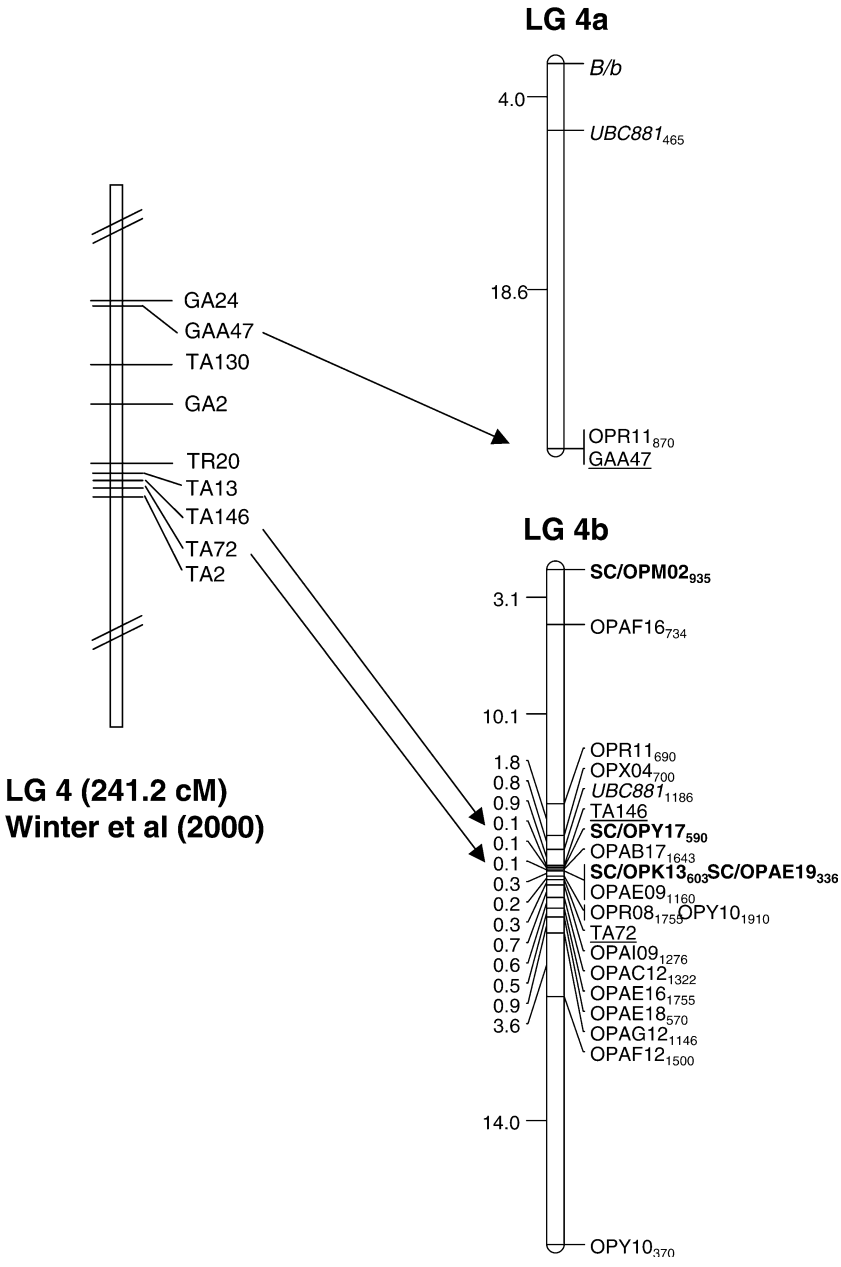

Fig. 2 Linkage groups obtained in the chickpea RIL population ILC3279×WR315 and their alignment through common STMS markers included in LG4 of Winter et al. (2000). SCAR markers are in *bold*. STMS markers are *underlined*. ISSR markers are in *italic*. RAPD are the predominant markers. Map distances are in cM

STMS and four RAPD) cover a genetic distance of 0.8 cM.

SCAR development

Seven RAPD markers ($OPAC04_{1200}$, $OPAE09_{1160}$, $OPAE19_{336}$, $OPAI09_{1276}$, $OPK13_{603}$, $OPM02_{935}$, $OPY17_{590}$) located in LG4b were used to develop SCAR markers. The resulting bands from all of them were cloned in the vector pGEM-T and sequenced. SCAR primers were designed for six RAPD markers as shown in Table 2. The forward and reverse sequences obtained from $OPAE09_{1160}$ RAPD marker did not match to form a contig, and therefore primers could not be designed for this marker.

One unique band, of the expected size as the progenitor RAPD marker, was amplified for each marker using at least one pair of the designed primers (Fig. 4). The amplification with $SCY17_{590}$, resulted in length polymorphism, therefore it could be scored as a co-dominant SCAR. In three cases, $SCAE19_{336}$, $SCK13_{603}$ and $SCM02_{935}$ the RAPD polymorphisms were retained as the presence or absence of bands. These SCARs could be scored as dominant markers as the presence of the bands was always associated with resistance (Fig. 4). Primers designed for markers, $OPAI09_{1276}$ and $OPAC04_{1200}$, amplified products of the expected sizes in the DNA of both resistant and susceptible lines. Further optimization of PCR or using Hot Start PCR did not allow detection of polymorphism for these two markers (Table 2).

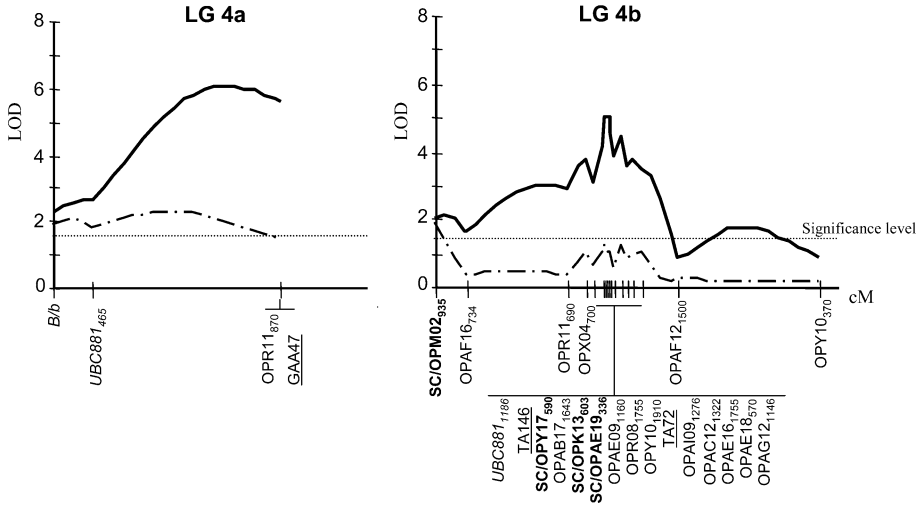

Fig. 3 QTL analysis in linkage groups obtained in the chickpea RIL population ILC3279×WR315 for ascochyta blight resistance evaluated during the years 2002 (*lines with dot*) and 2003 (*continuous line*). SCAR markers are in *bold*. STMS markers are *underlined*. ISSR markers are in *italic*. RAPD are the predominant markers

The whole population of the cross ILC3279×WR315 was analysed with markers $SCY17_{590}$, $SCAE19_{336}$, $SCK13_{603}$ and $SCM02_{935}$ (Fig. 4). They segregated in a 1:1 (presence:absence) ratio and cosegregated with their respective RAPD markers. Both RAPD and SCAR makers were located in the same positions in the linkage group (Fig. 2).

In a BLAST search, only two out of the six sequences, corresponding to SCARs $SCK13_{603}$ and $SCM02_{935}$, showed significant similarities to GenBank entries (Table 2).

Discussion

Most of the markers used in linkage analysis in the present study were also utilized to analyze a QTL for resistance to blight in an intraspecific RIL population allowing construction of a linkage group with 15 markers and a QTL for ascochyta blight resistance (Millán et al. 2003). In the present study, LG4b included 11 of the previously cited markers and seven markers that had previously been rejected by Millán et al. (2003) for linkage analysis in their study because they showed distorted segregation or a weak association with blight resistance.

Minor differences in the order of markers compared with the linkage group from Millan et al. (2003) were observed. These changes affected some markers placed in a highly saturated region (7.3 cM) and also two other markers (3.7 cM apart) on the distal end of LG4b. The population size used in the present study was larger than that of Millán et al. (2003) and it could explain discrepancies between the two linkage groups. Two STMS (TA72 and TA146) markers reported as tagging a QTL for blight resistance both in inter (Tekeoglu et al. 2002) and intraspecific crosses (Udupa and Baum 2003; Flandez–Galvez et al. 2003) were also integrated in LG4b .

The significant RIL×year interaction found in this study suggests variation between the years of either environmental conditions or pathotype of fungus or both. This variation may also explain the lack of significance of the LOD scores for LG4b and resistance to blight in the first year. Of the two QTLs (QTL_{AR1} and QTL_{AR2}) detected in our RIL population, QTL_{AR2} was related to ascochyta blight resistance only in the second year and was coincident with a QTL previously reported in an intraspecific RIL population (Millán et al. 2003). In both populations, the same source of resistance (ILC3279) was used, and the QTL explained a similar percentage of the total variation of the character (around 21%).

As a result of this work, we now have nine markers tightly linked (< 1 cM) to QTL_{AR2}, (three SCAR, two STMS and four RAPDs). The SCAR markers segregated in a 1:1 (presence: absence) ratio and were coincident with their respective RAPD markers. The SCAR markers are more useful than RAPD markers because they are locus specific and identifiable in different genetic backgrounds, thus reducing the chance of misclassifying individuals in segregating populations (Chowdhury et al. 2001). However, the development of polymorphic SCAR markers from all RAPDs was not possible. Three of the four SCARs ($SCY17_{590}$, $SCAE19_{336}$ and $SCK13_{603}$) were tightly linked to QTL_{AR2}. The use of co-dominant markers as STMS is desirable in MAS in order to detect heterozygous individuals in early generations. In our study only one SCAR marker ($SCY17_{590}$) was co-dominant, being sufficient to detect with a high probability heterozygous plants for QTL_{AR2} .

The QTL_{AR2} contained the STMS markers TA72 and TA146 and therefore corresponds with LG4 of the high density chickpea map developed by Winter et al. (2000) in an interspecific cross. Consequently, QTL_{AR2} may coincide with QTL-2 reported by Santra et al. (2000) detected in an interspecific RIL population with the resistant parental FLIP84-92C (kabuli type derived from a cross using the known resistant parent ILC72) and also located in LGIV by Tekeoglu et al. (2002) (common markers TA72 and TA146). This QTL also could correspond with one of the two QTLs conferring resistance

Table 2 Primer sequences for SCAR markers derived from RAPD markers associated with Ascochyta blight resistance in chickpea and the type of polymorphism observed. More significant similarity of DNA or protein sequences is indicated

SCAR markers	Primer sequence (5′ to 3′)[a]	Polym[b]	Accession number [c]	Similar GenBank entries[c]	
				Description	Accession number
$SCAC04_{1200}$	F1: ACGGGACCTGTCTGCCACCT R1: ACGGGACCTGCAATTTAAAGTTG	NP	AY860665	No significant homology	
	F2: ACGGGACCTGTCTGCCACCT R2: AAGTTGTTGGTTTTGTATGTTTGTC	NP			
$SCAE19_{336}$	F1: GACAGTCCCTCCATTATCTAAAC R1: GACAGTCCCTATGTGTGAGAAT	D	AY860667	No significant homology	
$SCAI09_{1276}$	F1: TCGCTGGTGTCTATAGTGGC R1: GGTGTGTGCCATAGGGAGAA	NP	AY860666	No significant homology	
$SCK13_{603}$	F1: GGTTGTACCCCATCCTCCCG R1: GGTTGTACCCTTGTGCCACTA	D	AY860669	*Poncirus trifoliate* citrus tristeza virus resistance gene locus	AF506028
$SCM02_{935}$	F1: ACGCCTCCTCCGCACAGTTA R1: ACAACGCCTCAATTTTAAAAAATAG	D	AY860668	*Lotus japonicus* genomic DNA, chromosome 2 *Arabidopsis thaliana* pentatricopeptide (PRP) repeat-containig protein *A. thaliana* selenium-binding protein-like	AP006647 NP_198751 BAB02421
$SCY17_{590}$	F1: GACGTGGTGACTATCTAGC R1: GACGTGGTGAAATAGATACC	C	AY860670	No significant homology	

[a]Underlined nucleotides are derived from the progenitor RAPD primers
[b]*NP* no polymorphism; *D* dominant polymorphism; *C* co-dominant polymorphism
[c] From National Center for Biotechnologic Information (NCBI)

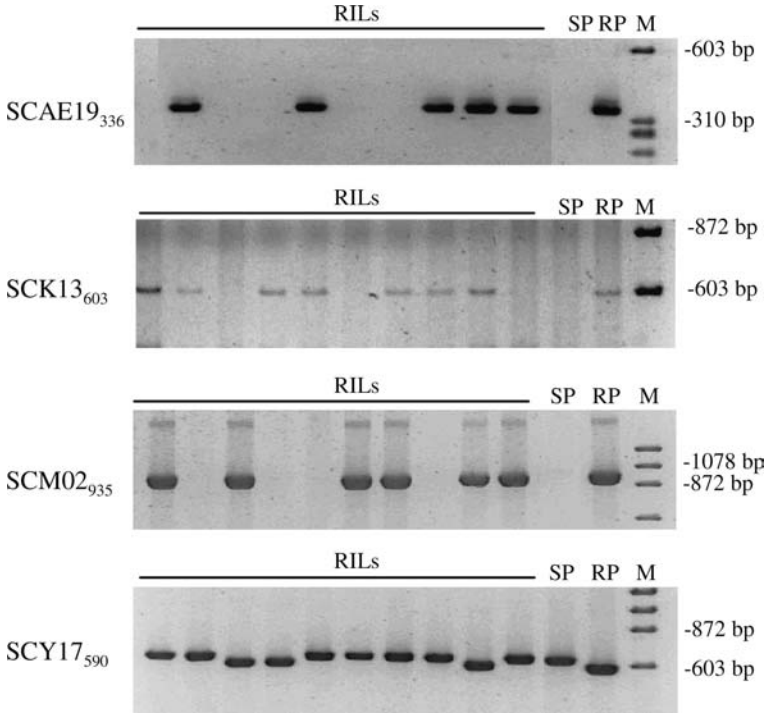

Fig. 4 Agarose gel showing the segregation of polymorphic SCARs markers $SCAE19_{336}$, $SCK13_{603}$, $SCM02_{935}$, $SCY17_{590}$ in the two parental lines and ten RILs of the population derived from the chickpea cross ILC3279×WR315. *RP* resistant parental *SP* susceptible parental. [a] Different RILs have been employed for each SCAR marker

to pathotype II of ascochyta blight reported by Udupa and Baum (2003) that used the line ILC3279 as source of resistance and was mapped in the same genomic region where the microsatellite TA72 on LG4 was present. Moreover, another putative QTL located in LG4 and linked to STMS TA146 was reported using an F_2 derived from an intraspecific cross, but in this case the resistant parent was ICC12004, a desi chickpea cultivar (Flandez–Galvez et al. 2003). Consequently, all these reports appear to refer to the same QTL which is present in different resistant lines. The high marker density of this interesting region obtained in our work might be helpful to determine whether the gene content of this important QTL contains a single gene or several linked genes.

The LG4a was strongly associated with resistance in both years (Fig. 3). This group may correspond to the linkage group containing QTL-1 which is linked to the anthocyanin pigmentation locus (P) and the STMS GAA47 on LGVIII, with both markers being 56.9 cM apart (Tekeoglu et al. 2002). Thus, in our LG4a, the flower colour locus (*B*/*b*) may be the same anthocyanin pigmentation locus (*P*), white colour being linked in coupling with the resistance QTL. In the map reported here, a shorter distance between *B*/*b* and GAA47 was obtained (22.6 cM). However, this discrepancy may be due to the intraspecific nature of the RIL population. Our QTL_{AR1} may also coincide with a QTL for resistance to pathotype II reported by Cho et al (2004) on their LG4A (indicative marker STMS GAA47). Because of this, we may also assert that the same QTL is present in both the resistant parents, [FLIP84-92C, used by Tekeoglu et al. (2002) and Cho et al (2004), and ILC3279, used in the present study]. Three DNA markers (2 RAPDs and 1 ISSR) mapped to QTL-1 by Santra et al. (2000) and located in LG VIII between the anthocyanin pigmentation locus (*P*) and GAA47 were monomorphic in our parent lines. In fact, many of the markers on the map of Santra et al. (2000) seem to be monomorphic in cultivated chickpea (Tekeoglu et al. 2002). Rakshit et al. (2003) employing the same RIL population of Santra et al. (2000) found a DAF marker tightly linked to QTL-1. In this study we report two new polymorphic DNA markers in this linkage group (one RAPD and one ISSR) in cultivated chickpea, but this low number of polymorphisms is insufficient for MAS. Further efforts must be made in order to saturate this interesting genomic region with markers so that they may be used in MAS. Taking into account the indicative marker GAA47 and the extensive map of Winter et al. (2000), QTL_{AR1} could be located in LG4. Therefore, QTL_{AR1} and QTL_{AR2} seem to be present in the same linkage group.

Two of the SCAR sequences were significantly similar to genes implicated in resistance of other cultivated plant species. $SCK13_{603}$, which is closely linked to QTL_{AR2}, is similar to a genomic region of *Poncirus trifoliata* that confers resistance to citrus tristeza virus ($E = 2e^{-14}$) (Yang et al. 2001). This locus was narrowed down to 300 kb and contains several putative disease-resistance genes similar to the rice *Xa21* gene, the tomato *Cf−2* gene and the *Arabidopsis thaliana RPS2* gene (Yang et al. 2001). The nucleotide sequence of the other SCAR ($SCM02_{935}$) revealed significant alignment with a sequence located on chromosome 2 of *Lotus japonicus* (*E* value $= 2e^{-28}$) (Kato et al. 2003) and the peptide corre-

sponding to this sequence showed significant similarities to selenium-binding proteins of *A. thaliana* (maximum *E* value = $3e^{-25}$) (Sato et al. 2000). Transgenic rice plants overexpressing a rice selenium-binding protein (OsSBP), homologous to mammalian selenium-binding proteins, showed enhanced resistance to the blast fungus, *Magnaporthe grisea*, and bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*. When such plants were infected with *M. grisea*, expression of defence-related genes and the accumulation of phytoalexin was accelerated (Sawada et al. 2004). It will therefore be interesting to use these markers in a chickpea BAC library in order to identify putative resistance genes. Currently, three chickpea BAC libraries have been developed (Rajesh et al. 2004; Lichtenzveig et al. 2005). In addition, it may be informative to ascertain the synteny of these SCARs with similar sequences in other plant species in which there is greater knowledge of the genes involved in resistance to pathogens.

For MAS it is usually imperative to saturate the appropriate regions of the genome with markers in order to obtain some that are sufficiently tightly linked to genes of interest to be of value. Polymorphic markers in a given population may not be polymorphic in a second one. For example, $OPAC04_{1200}$, one of the RAPD markers showing a strong association with ascochyta blight resistance in the ILC3279×CA2156 population (Millan et al. 2003), could not be used in the present study since it was not polymorphic in the parental lines. The character of white flower is another example since it is associated with resistance to ascochyta blight in both this study and in the one by Santra et al. (2000) but many kabuli lines with white flowers are susceptible to blight (Singh et al. 1983).

Conclusions

In this work, two QTLs are reported in an intraspecific RIL population of chickpea. These QTLs could be present in different resistant lines. The QTL named as QTL_{AR2} in this study has been mapped in a genomic region with a dense cluster of markers. Four SCAR markers have been developed and three of them are present in the cluster of markers along with two STMS markers. The precision of molecular breeding can be improved by selection using robust markers in breeding populations. QTL_{AR2} was located on a high saturated genomic region which will allow the identification of at least one tightly linked marker that could be used for cloning QTL_{AR2} with the aid of a chickpea BAC library. The sequence of the SCAR $SCK13_{603}$, closely linked to QTL_{AR2}, was significantly similar to genes related with resistance in other cultivated species. This result could be a starting point to find a candidate resistant gene for ascochyta blight in chickpea. Efforts should be made to saturate the genomic region of QTL_{AR1} with additional markers in order to provide markers for breeding purposes to fully study this important region of the chickpea genome.

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