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FULL RESEARCH PAPER

Mechanism and molecular markers associated with rust resistance in a chickpea interspecific cross (*Cicer arietinum* × *Cicer reticulatum*)

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Abstract A gene that controls resistance to chickpea rust (*Uromyces ciceris-arietini*) has been identified in a recombinant inbred line (RIL) population derived from an interspecific cross between *Cicer arietinum* (ILC72) × *Cicer reticulatum* (Cr5-10), susceptible and resistant to rust, respectively. Both parental lines and all RILs displayed a compatible interaction but differed in the level of infection measured as Disease Severity (DS) and Area Under the Disease Progress Curve (AUDPC). Histological studies of the seedlings of resistant parental Cr5-10 line revealed a reduction in spore germination, appressorium formation, number of haustoria per colony and colony size, with little host cell necrosis, fitting the definition of partial resistance. A Quantitative Trait Locus (QTL) explaining 31% of the total phenotypic variation for DS in seedlings and 81% of the AUDPC in adult plants in the field was located on linkage group 7 of the chickpea genetic map. The AUDPC displayed a bimodal distribution with high frequency of susceptible lines and both the AUDPC and markers showed the same distorted segregation. Consequently, it was hypothesised that a single dominant gene (proposed as *Uca1/uca1*) controlled resistance to rust in adult plants. This allowed us to locate the gene on the genetic linkage map. Two Sequence Tagged Microsatellite Sites (STMS) markers, TA18 and TA180 (3.9 cM apart) were identified that flank the resistance gene. These findings could be the starting point for a Marker-Assisted Selection (MAS) programme for rust resistance in chickpea.

Keywords *Cicer* sp. · Partial resistance · QTL · STMS · *Uromyces ciceri-arietini*

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Introduction

Chickpea (*Cicer arietinum*) crops are extremely important in semi-arid areas of central, south and southeast Asia, southern Europe, northern and eastern Africa, the Americas and Australia. The crop is the second most important pulse in terms of area under cultivation throughout the world after dry beans, but ranks third in terms of production, following dry beans and peas (FAOSTAT 2005).

The yield potential of present-day chickpea varieties exceeds 4 t ha^{-1}; however, actual yield is on average less than 0.8 t ha^{-1}. The gap between potential and actual average yield is mainly due to diseases and poor management practices. The most important chickpea diseases on a global scale are fusarium wilt and ascochyta blight (Nene and Reddy 1987). Chickpea rust, caused by *Uromyces ciceris-arietini*, is widespread in the Mediterranean, southeast Europe, south Asia, east Africa and Mexico, but is usually considered to be of only local importance. It has been reported as a significant problem affecting chickpea production in central Mexico and Italy (Ragazzi 1982; Díaz-Franco and Pérez-García 1995). No sources of resistance to rust have been identified in chickpea and so far only measures relating to sowing dates and chemical control have been proposed (Díaz-Franco and Pérez-García 1995). Only a certain degree of slow-rusting has been reported recently in chickpea, and tends to be more frequent in wild *Cicer* relatives (Rubiales et al. 2001).

Cicer arietinum is the only cultivated species of the genus *Cicer*, but the resistance present in some wild relatives is accessible for chickpea breeding by conventional methods such as crosses successfully obtained using *C. reticulatum* and *C. echinospermum* (Ladizinsky and Adler 1976). These species form a crossability group with cultivated *C. arietinum*. A second crossability group proposed by the same authors is formed by *C. pinnatifidus, C. judaicum* and *C. bijugum*. However, no successful crosses have been reported between the species of the second group with cultivated chickpea (Ahmad and Slinkard 2004). The use of Marker-Assisted Selection (MAS) could accelerate plant breeding in relation to certain agronomic characteristics, including disease resistance, enabling combinations of resistance genes to be assembled.

A Recombinant Inbred Line (RIL) population derived from the interspecific cross ILC-72 (*C. arietinum*) × Cr5-10 (*C. reticulatum*) was available in our group. A genetic map was developed using this population and RAPD (Random Amplified Polymorphic DNA), ISSR (Inter-Simple-Sequence-Repeats) and STMS (Sequence Tagged Microsatellite Sites) markers. A Quantitative Trait Locus (QTL) for ascochyta blight resistance was found using this population (Cobos et al. 2006). Parental lines used to obtain this population also differed in rust resistance (Rubiales et al. 2001). Therefore, the aim of this study was to characterise the nature of resistance derived from the wild accession Cr5-10 by studying phases of the infection process microscopically. In addition, we studied the genetic inheritance of rust resistance and attempted to map this character using the RIL population cited above.

Materials and methods

Plant material

The experiments were conducted with a RIL population consisting of 102 $F_{6:7}$ lines. The RILs were obtained from the interspecific cross between ILC72 (*C. arietinum*) × Cr5-10 (*C. reticulatum*) (Cobos et al. 2006). ILC72 is a kabuli chickpea from ICARDA that is susceptible to rust and Cr5-10 is a rust-resistant wild species accession.

Growth chamber experiment

Resistance to rust was studied in the RIL population and the parental lines at the seedling stage in a growth chamber. Three pots per RIL with five plants were randomly distributed in trays. Each tray contained pots of susceptible and resistant parents as controls. Inoculation consisted of spraying the plants with *Uromyces ciceris-arietini* urediospores from a bulk population collected at Córdoba, Spain in 1999 (CO-99). The urediospores (2 mg per plant) were diluted in pure talcum power (1:10) and uniformly sprayed over the seedlings at the fourth leaf stage. Plants were incubated in complete darkness for 24 h at 20°C and 100% relative humidity (RH), and then transferred to the growth chamber at 20°C under a 11/13 day/night photoperiod. Disease severity (DS) and infection type (IT) were rated 10 days after inoculation. DS was estimated as the percentage of leaf tissue covered by pustules. IT was assessed using the IT scale proposed by Stakman et al. (1962) where 0 = no symptoms, ; = necrotic flecks, 1 = minute pustules barely sporulating, 2 = necrotic halo surrounding small pustules, 3 = chlorotic halo, 4 = well-formed pustules with no associated chlorosis or necrosis.

Analysis of variance (ANOVA) was applied for DS using the data from the parental lines in each tray in

order to determine the homogeneity of the infection, according to following model:

$$X_{ij} = \mu + L_i + T_j + e_{ij}$$

where X_{ij} is the individual data, μ the general mean, L_i the effect of ith parental line, T_j the effect of the jth tray and e_{ij} is the experimental error.

Field experiment

The RILs were sown in an insect-proof cage in single rows 2 m long, 0.5 m between rows and 10 plants m^{-1}. RILs were distributed in eight blocks with parental lines included in each one. The experiment was repeated twice. Each block was surrounded by the susceptible parental line in order to ensure disease pressure and uniformity. The plants were inoculated by spraying with an aqueous spore suspension (200 mg spores l^{-1}). Plants were inoculated after sunset to benefit from the darkness and higher RH at night. DS was rated as a percentage of the host tissue covered by pustules per row at weekly intervals, starting from the time at which the susceptible check showed the first symptoms.

Scores were used to calculate the Area Under the Disease Progress Curve (AUDPC) of each line (Shaner and Finney 1977). Analysis of variance (ANOVA) was applied for the AUDPC using data from the parental lines in order to determine the homogeneity of the infection in the experimental plot, according to the following model:

$$X_{ij} = \mu + L_i + B_j + e_{ij}$$

where X_{ij} is the individual data, μ the general mean, L_i the effect of ith parental line, B_j the effect of the jth block and e_{ij} is the experimental error.

Histological observations

Components of resistance were studied microscopically in the parental lines. Three plants per genotype were grown under controlled conditions (20°C, 11/13 day/night photoperiod. Seedlings were inoculated when the fourth leaf was completely expanded by dusting with 1 mg of urediospores per plant, diluted in pure talcum powder (1:10). Plants were incubated for 24 h at 20°C in complete darkness and 100% RH, and then transferred to a growth chamber at 20°C. Three leaves per plant were collected 2 days after inoculation (d.a.i.) and processed to study the phases of fungal growth prior to stomatal penetration following the method described by Sillero and Rubiales (2002). Urediospores (~100 per leaf sample) were counted under X200 magnification with a Leica DM LS microscope using two different categories: number of germinated urediospores (a spore was considered as germinated when a germ tube was at least as long as the diameter of the spore) and germ tubes forming appressoria over stomata expressed as percentage of germinated spores.

In order to study the phases of the infection process after stomatal penetration, three plants per genotype and three leaf samples per plant were cut 2 d.a.i and stained with Trypan blue, following the procedure defined by Sillero and Rubiales (2002), with slight modifications. The leaves were fixed in acetic acid/ethanol (1:3, v/v) for 30 min, stained by boiling in 0.05% Trypan blue in lactophenol/ethanol (1:2, v/v) for 10 min and cleared in a nearly-saturated aqueous solution of chloral hydrate (5:2, wt/vol) to remove the Trypan blue from the chloroplast membranes. The early stages of the infection process were studied microscopically, using a phase contrast Leica DM LS microscope at X400 magnification. Between 20 and 40 random colonies were studied per leaf. The number of hyphal tips and haustoria were recorded for each colony. The colony size (CS) was measured using a Leica DM epifluorescence microscope at X200 magnification. The size of each colony was calculated using the formula of ellipse area: $CS = \pi LW$, where L and W are the length and width, respectively, of 20 randomly-chosen established colonies per leaf. L and W were measured with an eyepiece micrometer.

The means were compared (LSD test, $P<0.01$) for all microscopic components of resistance between genotypes. When the components of resistance were expressed as percentages, the data were angular-transformed (arcsin square root) prior to analysis of variance.

Markers and QTL analysis

For DNA extraction, about 0.1 g of young leaf tissue was excised, immediately frozen in liquid nitrogen and stored at −80°C. DNA was isolated using the DNAzol® method (INVITROGEN). Eight STMS and a gene-specific marker were analysed for incorpora-

tion into the genetic map previously developed in the RIL population by Cobos et al. (2006). STMS amplification was achieved according to the protocol defined by Winter et al. (1999) in 20 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, 2 µM of primer and 1 unit of Taq DNA polymerase (Biotools). 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 followed by a final extension at 60°C for 5 min. Amplification products were analysed in 3% MetaPhor agarose (Biowhitaker Molecular Application) in 1× TBE buffer. Amplification of a fragment of the gene encoding a replication factor C/activator 1 subunit from *C. arietinum* was performed following the protocol and sequences developed by Pfaff and Kahl (2003). In order to obtain polymorphism in the RIL population the RSA I ($BioLabs_{inc}$) restriction enzyme was used. Aliquots (10 µl) of PCR product was incubated with the enzyme following the manufacturer's instructions. The products of this incubation were electrophoresed in gels composed of a mixture of 1% agarose D-1 LE and 1% agarose LM-SIEVE (Pronadisa) in 1× TBE buffer and visualized by ethidium bromide staining.

Segregation of marker loci was statistically analysed for goodness of fit to the expected 1:1 ratio for a gene using the chi-square test. Linkage analysis was performed using MAPMAKER Vs 3.0 (Lander et al. 1987); a LOD score threshold of 3 and a maximum recombination fraction of 0.25 were applied as general linkage criteria to establish linkage groups. Kosambi's function was applied to estimate map unit distance.

Genotypes from linkage map and quantitative data were used as input data for QTL Cartographer software Vs 3.0 (Wang et al. 2004). First, single marker analysis using LRmapqtl (simple linear regression) was performed. Then, Simple Interval Mapping (SIM; Model 3 in Zmapqtl) and Composite Interval Mapping (CIM; Model 6 in Zmapqtl) were performed. Markers to be used as cofactors for CIM were selected by Forward–backward stepwise regression (FB method in SRmapqtl), using a window size of 3 cM. Threshold for QTL significance at α=0.01 was estimated by permutation analysis (Churchill and Doerge 1994) using 1,000 permutations. The determination coefficient (R^2) value of the best marker in a linkage group associated with a resistant reaction was used to estimate the proportion of the total phenotypic variation explained by the QTL.

Results

Mechanism of resistance

In growth chamber and field experiments, parental genotypes and all RILs showed a compatible reaction type (IT=4), with no macroscopically-visible necrosis. Differences in the development of the infection process in seedlings of resistant and susceptible parents were evident 2 d.a.i. Significant differences were observed both for spore germination and percentage of germ tubes forming appressoria over stomata (Table 1). Significant differences were also observed in events after stomatal penetration, such as number of infection attempts without haustorial formation, considered as early aborted colonies (Niks 1986; Sillero and Rubiales 2002); number of haustoria per colony; number of hyphal tips per colony and colony size (Table 1).

Genetic analysis of resistance

In the field, only two scores could be made in the second replication, because there was a strong presence of *Cuscuta* spp. at the final stages of the assay. The correlations between the scores of the two replications were high and significant (r=0.75 and 0.86 for scores 1 and 2, respectively) suggesting no differences between them. For that reason, only the three scores from the first replication were used to estimate the AUDPC. ANOVA for DS in the growth chamber experiment and AUDPC in the field using susceptible and resistant parents showed that there were no significant differences between trays or between blocks, respectively. These results suggest that the infection may be considered uniform. However, the variation coefficient of ANOVA for DS was very high (CV=45.1%) indicating that experimental error was important. For the AUDPC, this parameter was lower (CV=19.5%). Therefore, DS values were converted into relative values (rDS) expressed as a percentage with regard to the susceptible parent (= 100%) growing in each tray in the

Table 1 Percentage of events in the early developmental stages of infection units and the early stages of *Uromyces ciceris-arietini* infection in seedlings in the parental lines of the RIL population ILC72 × Cr5-10

Line	Spore germination (%)	Appressoria over stomata (%)	Infection units without haustoria (%)	No. haustoria/colony	No. hyphal tips/colony	Colony size (mm^2)
ILC72	83.5 a	22.8 a	1.2 a	2.3 a	9.8 a	0.2 a
Cr5-10	53.4 b	9.7 b	68.7 b	1.0 b	5.0 b	0.01 b

Data with different letter, per column, are significantly different (LSD, $P<0.01$).

controlled conditions assay. The frequency distribution of genotypes according to values of AUDPC in the RIL population followed a bimodal distribution (Fig. 1a) with a higher frequency of susceptible lines, suggesting the presence of major genes controlling this character. For rDS, the RIL population showed a distribution skewed towards low levels of infection (Fig. 1b).

Initial single marker analysis, using the map developed by Cobos et al. (2006) and applying simple linear regression, revealed a high association between some markers on LG7+8 and rust resistance reaction expressed as both rDS and AUDPC ($P<0.001$). A further analysis using SIM detected a putative QTL in the same region that belonged to LG7 on chickpea maps (Winter et al. 2000; Cho et al. 2004). The STMS TA18 was the only indicative marker present in LG7 of Cobos et al (2006), therefore eight STMS (TA28, TAA59, TS62, TA78, TA114, TA117, TA140, TA180) from LG7 of the maps reported by other authors (Winter et al. 2000; Tekeoglu et al. 2002; Cho et al. 2004) were selected in order to saturate this genomic region. Only three of them (TA180, TA28 and TA78) were polymorphic in our population and were integrated in our LG7 as expected. A gene-specific primer, corresponding to a replication factor C/activator 1 subunit of *C. arietinum*, located in LG7 (Pfaff and Kahl 2003) was also polymorphic and located in the same linkage group. Hence, LG7 of the map used in this study now includes one ISSR, one replication factor gene, four STMS and seven RAPDs covering a total distance of 98.7 cM (Fig. 2). Most markers exhibited distorted segregation ($P>0.05$) with respect to the expected Mendelian inheritance ratio of 1:1 and a tendency towards the cultivated susceptible parent phenotype was observed. Some differences were observed in the order of markers when compared to the LG7 map defined by Winter et al. (2000) and Tekeoglu et al. (2002) (Fig. 2).

After integration of the new markers in LG7, the current chickpea genetic map was used to detect QTL associated with resistance to rust. Employing SIM, a QTL peak for rDS with a LOD score of 7.4 was detected close to the RAPD marker $OPX13_{750}$ on LG7, explaining 30.5% of the total phenotypic variation. For the AUDPC, STMS marker TA18 had the highest LOD score (27.28) and explained 73.7% of the total variation. CIM was applied for rDS and AUDPC using forward–backward stepwise regression to select cofactors. An unique strong QTL for both rDS and AUDPC, with maximum LOD score values of 7.4 and 31.9 respectively, was mapped in the expected genomic region close to STMS TA18 (Fig. 3). Alleles associated with resistance were present in the resistant parent. The significance level detected by permutation tests was 2.5 for both traits.

There was evidence to suggest that the rust resistance found in this population for AUDPC in field, even when it results in a quantitative phenotypic expression (partial resistance), can be regarded as a qualitative character. Firstly, almost all resistance is explain by a single, fairly large QTL. Furthermore, as mentioned previously, the frequency of genotypes with AUDPC values showed a bimodal distribution with high frequency of susceptible lines, and markers on LG7 also showed distorted segregation with a tendency towards the susceptible parent alleles. AUDPC values were divided into two categories taking in account the point where the distribution reaches a minimum between both modal: (1) resistant lines (AUDPC<300) and (2) susceptible lines (AUDPC>300). The distorted segregation of these two categories ($0.05>P>0.001$) was similar to the 1:1 deviation exhibited by markers located in the QTL region. Consequently, it was hypothesised that a major gene (proposed as *Uca1*/*uca1*) controls rust resistance. A clear co-segregation between *Uca1*/*uca1* and markers was detected and this locus was mapped between TA18 and TA180 in the same genomic region as the QTL (Fig. 4).

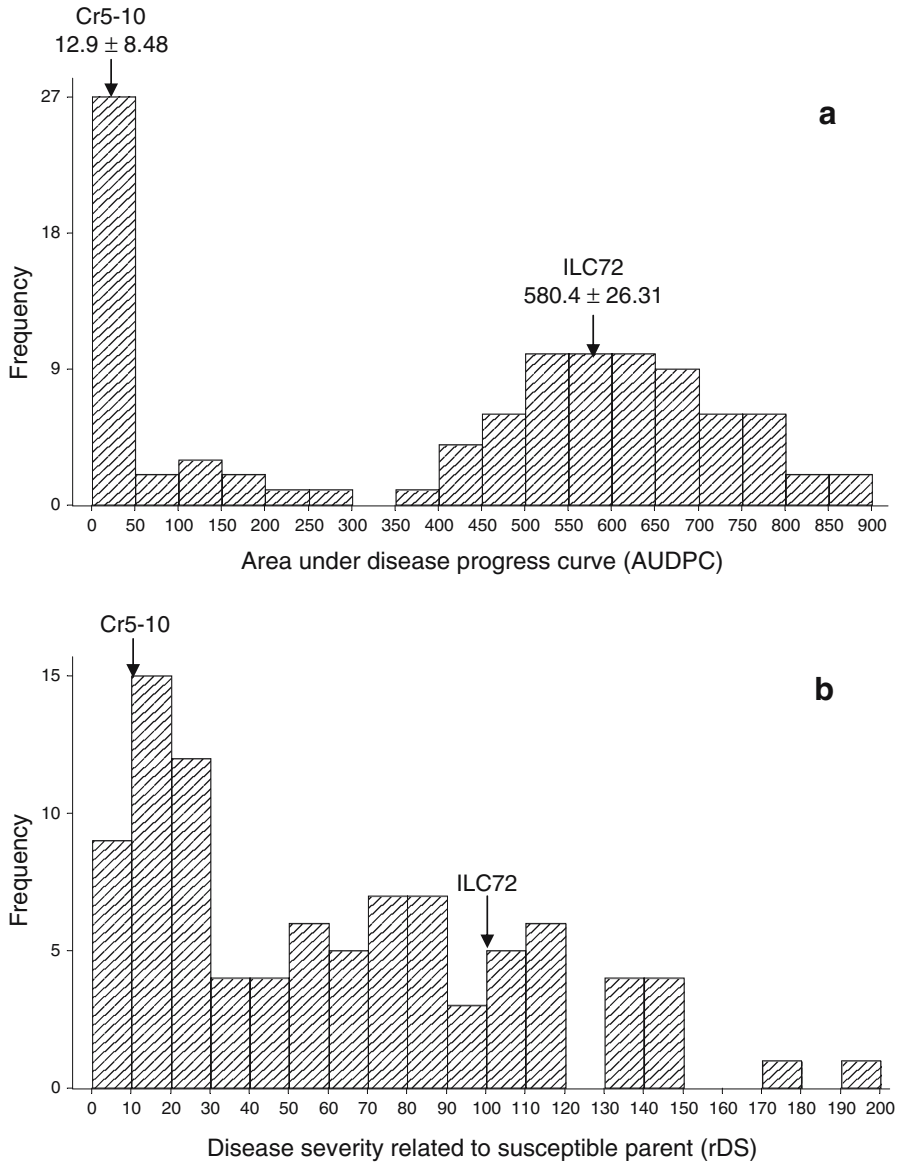

Fig. 1 Frequency distribution for rust resistance estimated as the AUDPC in adult plants (**a**) and rDS in seedlings (**b**) for the chickpea RIL population ILC72 × Cr5-10. *Arrows* indicate the mean values for parental lines

Discussion

The resistant reaction to chickpea rust, present in the wild chickpea parental genotype, is mainly an incomplete non-hypersensitive response. These results agree with the findings of Rubiales et al. (2001) in chickpea. In other legume-rust pathosystems, quantitative non-hypersensitive types of resistance are predominant, e.g. in pea – *U. viciae-fabae* (Pal et al. 1980) and in groundnut – *Puccinia arachidis* (Subrahmanyam et al. 1993).

To date, resistances commonly used in breeding against rusts were mainly those acting after haustorial formation (hypersensitive response). The great disadvantage of this type of resistance is that it is often ephemeral (Niks and Rubiales 2002). Other resistance mechanisms that might prove more durable are those that stop the pathogen at the cell penetration stage or even earlier, prior to stomatal penetration. However, the limited differences among genotypes in germination and germ tube directional growth commonly found within host species suggest that reduction of

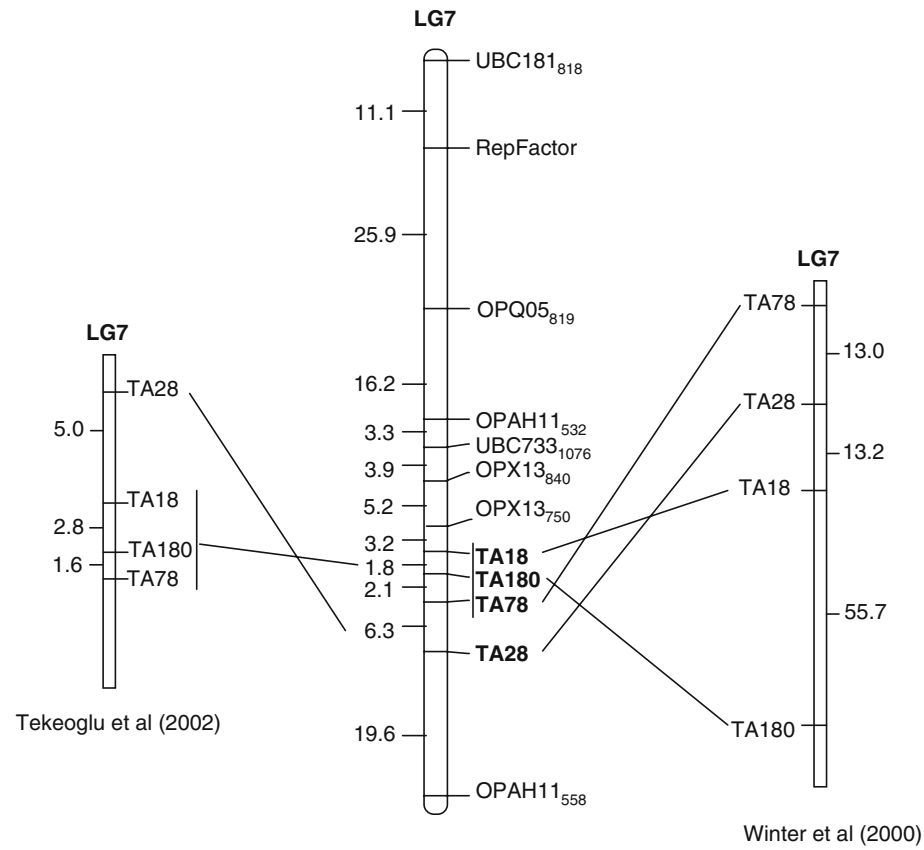

Fig. 2 Comparison of LG7 in the genetic map derived from the RIL population ILC72 × *C. reticulatum* (Cr5-10) (*middle*) with genetic maps of FLIP 84-92C × *C. reticulatum* (PI599072) (Tekeoglu et al. 2002) (*left*) and ICC-4958 × *C. reticulatum* (PI 489777) (Winter et al. 2000) (*right*). Corresponding markers in the different crosses are connected by *solid lines*

urediospore germination and/or fungal development on the leaf surface are of marginal importance, at best, in reducing infection levels, and offer few perspectives for exploitation in resistance breeding (Niks and Rubiales 2002). Reduced spore germination identified in the chickpea resistant parent employed in this study is more relevant for breeding purposes and deserves further investigation to determine its mechanistic basis. Differences in the germination of *Puccinia helianti* spores have been recently reported in sunflower and associated with the excretion of coumarins on the leaf surface (Prats et al. 2007). Similarly, *Rhynchosporium secalis* spore germination is inhibited in certain barley cultivars, although the responsible factor(s) are unknown (Lehnackers and Knogge 1990). Differences in stomatal recognition for appressorial formation could be due to morphological features of the host stomata over which they are developed, as described in bean – *U. appendiculatus* (Wynn 1976), or *Hordeum chilense*–*Puccina hordei* pathosystems (Rubiales and Niks 1996).

Mechanisms of resistance acting after stomatal penetration are also operative in the wild chickpea resistant parent reported in this paper, with a high proportion of colonies failing to form any haustoria (68.7%), and when the colonies were established, their haustorial number was reduced, and this may result in a smaller colony size. Early studies comparing host and non-host resistance to rust fungi showed that non-host resistance is typically expressed before the formation of the first haustorium (Heath 1981). However, pre-haustorial resistance can also be identified in host interactions, playing a major role in so-called partial resistance, which may be more durable than resistance controlled by hypersensitive resistance genes (Niks 1986; Niks and Dekens 1991; Rubiales and Niks 1995; Niks and Rubiales 2002). This pre-haustorial resistance is usually under polygenic control (Niks 1986) but has also been described in monogenic resistances such as *Lr34* (Rubiales and Niks 1995) and *Lr46* (Singh et al. 1998).

A genomic region on LG7 of the chickpea map has been found associated with resistance to rust in this

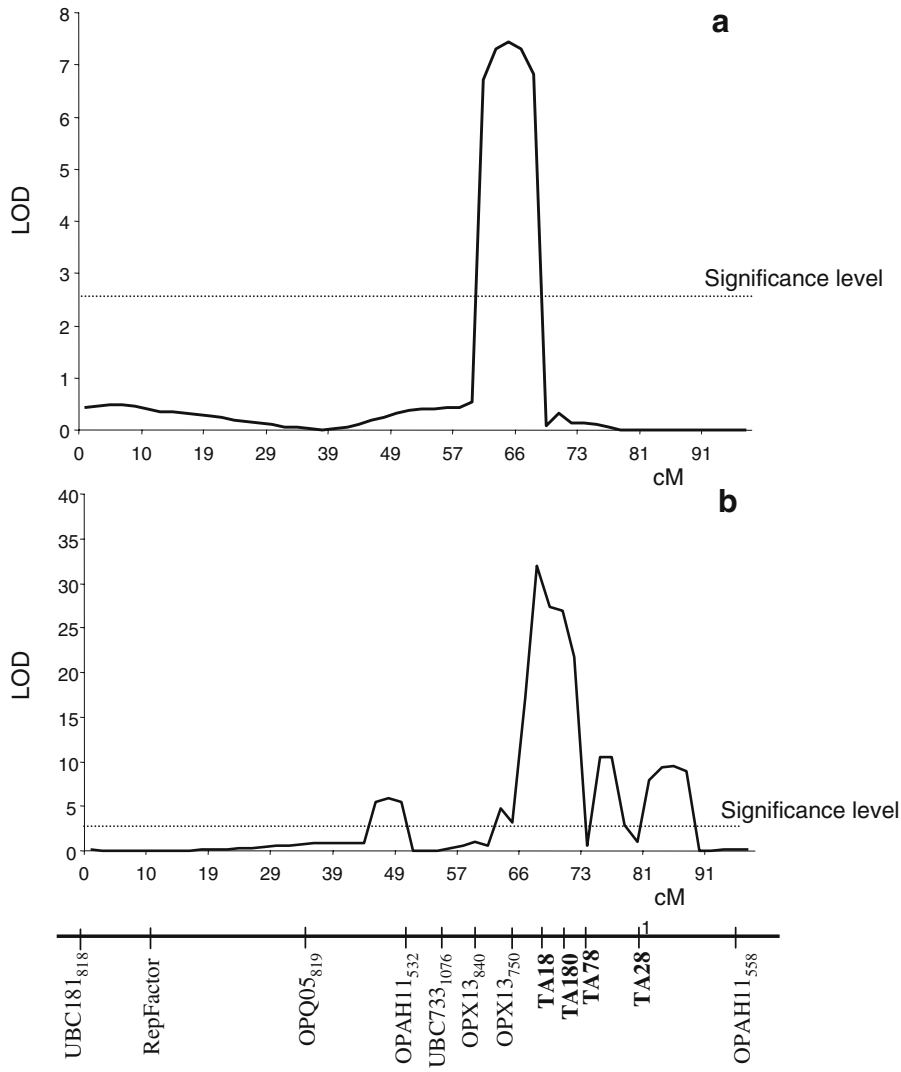

Fig. 3 LOD profile of QTL analysis of rust resistance obtained in the chickpea RIL population ILC72 × Cr5-10 using CIM. (**a**) QTL in the seedling experiment; (**b**) QTL in the adult plant assay

study. STMS markers from LG7 common across mapping populations in previous studies were integrated in the RIL population map reported in this paper. Differences between our results and the map defined by Tekeoglu et al. (2002) were only minor, where the STMS TA28 was close to TA18, whereas in this study, it was near to TA78. In both cases the four STMS TA18, TA180, TA78 and TA28 clustered in a narrow window of around 10 cM. More significant differences were observed with the map of Winter et al. (2000) where the distances between markers were greater and TA180 was further away from the rest of the markers. The reason for this discrepancy might be due to the greater number of markers in the Winter et al. (2000) map in this genomic region, which could affect both the estimated distance and the order of the markers. In fact, in a previous map using the same RIL population and a lower number of markers, Winter et al. (1999) also found differences in the distances and the order of the markers. In this study, with the introduction of four new loci in the LG7+8 of Cobos et al. (2006), minor differences were observed in the order of markers in this genomic region. More recently, similar results have been reported for the position of these four STMS – with TA18, TA180 and TA78 tightly linked and TA28 a little further – using populations from interspecific (Abbo et al. 2005) and intraspecific (Cho et al. 2004) crosses.

SIM and CIM analysis basically identified the same activity regions in both seedling and adult

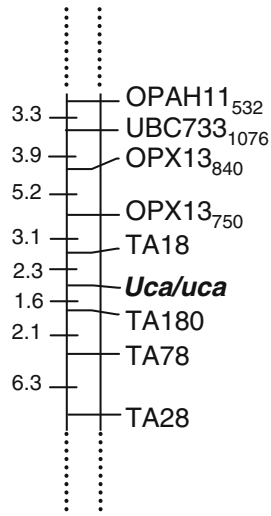

Fig. 4 Linkage map of ILC72 × Cr5-10 population showing the position of the *Uca1* gene for rust resistance in chickpea. Marker names are shown on the *right* and the estimated map distances are shown on the *left*. Recombinant fractions were converted to centi-Morgans using the mapping function defined by Kosambi (1944)

plants. Both QTL may be considered the same although QTL in the adult plant was stronger than in the seedling stage. It is a well-known fact in wheat that partial resistance is better expressed in adult plants than in seedlings (Rubiales and Niks 1995) and particularly in polycyclic (field) rather than monocyclic (growth chamber) inoculations (Parlevliet and van Ommeren 1975). In addition, it should be taken into account that environmental effects were higher in the seedling experiment.

The co-segregation between resistance/susceptibility score based on AUDPC data and markers reveal the qualitative nature of rust resistance in chickpea and describe molecular markers associated with partial resistance to rust in legumes. Partial resistance is usually assumed to be polygenically inherited. However, this is not always the case, as single genes causing partial resistance to rust have been described in peas (Vijayalakshmi et al. 2005) and wheat, such as *Lr34* (Dyck 1987; Rubiales and Niks 1996) and *Lr 46* (Singh et al. 1998) related to pre-haustorial resistance. In this study, a major gene controlling partial resistance to rust (*Uca1*/*uca1*) in chickpea is proposed in the adult stage of the plant. However, the variation observed for AUDPC within both resistant and susceptible groups may be due to environmental or/and minor gene effects. *Uca1*/*uca1* was located on LG7 of the chickpea map between two tightly-linked STMS markers (3.9 cM). Co-dominant STMS markers are desirable in MAS in order to detect heterozygous individuals in early generations. Moreover, STMS markers are easier to transfer between laboratories. They are ubiquitous and evenly distributed in all eukaryotic genomes (Tautz and Renz 1984). Therefore, the markers associated with rust resistance in chickpea could be present in other legumes. Comparative mapping revealed significant transferability of *Medicago truncatula* microsatellites in faba beans (40%), chickpea (37.3%) and peas (37.6%) (Gutiérrez et al. 2005). This transferability of microsatellites between species might allow us to identify the genomic region involved in rust resistance in other species. For instance, STMS TA78 and TA180 have been tested in *M. truncatula*, and were amplified (unpublished data). This could imply that the same region might be present in *M. truncatula*, a model species whose genome is being sequenced.

In conclusion, resistance to chickpea rust in the RIL population from the interspecific cross ILC72 (*C. arietinum*) × Cr5-10 (*C. reticulatum*) should be regarded as incomplete non-hypersensitive resistance, expressed by a reduction of rDS and AUDPC in the resistant lines. A QTL for partial resistance both in seedlings in the growth chamber and adult plants in the field was found in the same genomic region. A gene (*Uca1/uca1*) was located on LG7, between STMS markers TA18 and TA180 and is proposed for the control of resistance with the presence of modifier minor genes in the adult plant. These STMS markers and two other markers ($OPX13_{750}$ and TA78) are close enough to *Uca1*/*uca1* to allow reliable marker-assisted selection for rust resistance. Therefore, this gene for rust partial resistance can be more efficiently incorporated in the cultivars to be released using MAS, offering better prospects for durable resistance in chickpea breeding. Nevertheless, polymorphic markers in a given population might not be polymorphic in a different genetical background; therefore in order to solve this difficulty, more markers should be included in this region to be of practical value. In this paper, four markers tighly linked to the gene for resistance to rust are proposed for use in MAS. This major gene may be related to pre-haustorial resistance mechanisms.

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