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A linkage map of chickpea (*Cicer arietinum* L.) based on populations from Kabuli × Desi crosses: location of genes for resistance to fusarium wilt race 0

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Abstract Two recombinant inbred line (RIL) populations derived from intraspecific crosses with a common parental line (JG62) were employed to develop a chickpea genetic map. Molecular markers, flower colour, double podding, seed coat thickness and resistance to fusarium wilt race 0 (FOC-0) were included in the study. Joint segregation analysis involved a total of 160 markers and 159 RILs. Ten linkage groups (LGs) were obtained that included morphological markers and 134 molecular markers (3 ISSRs, 13 STMSs and 118 RAPDs). Flower colour (*B*/*b*) and seed coat thickness (*Tt*/*tt*) appeared to be linked to STMS (GAA47). The single-/double-podding locus was located on LG9 jointly with two RAPD markers and STMS TA80. LG3 included a gene for resistance to FOC-0 ($Foc0_1$/$foc0_1$) flanked by RAPD marker $OPJ20_{600}$ and STMS marker TR59. The association of this LG with FOC-0 resistance was confirmed by QTL analysis in the CA2139 × JG62 RIL population where two genes were involved in the resistance reaction. The STMS markers enabled comparison of LGs with preceding maps.

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Introduction

Chickpea (*Cicer arietinum* L.) is an annual, autogamous legume, the only cultivated species of the genus *Cicer*. The crop is grown in more than 30 countries in arid and semiarid areas of Central, South and Southeast Asia; southern Europe; northern and eastern Africa; in the Americas; and Australia. Its considerable nutritive value makes it a valuable source for both food and feed (Williams and Singh 1987; Gil et al. 1996). The crop is the second pulse in area under cultivation worldwide after dry beans, but ranks third in terms of production, following dry beans and peas (FAOSTAT 2004). For breeding purposes, chickpea is frequently divided into two main types: Desi, with small, sharp and dark-coloured seeds and Kabuli, having large, smooth, cream-coloured seeds.

Two fungal diseases, ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Lab. and fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceris* (Nene and Reddy 1987), are major constraints for chickpea production. In the Mediterranean Basin, many of the traditional Kabuli-type cultivars are susceptible to fusarium wilt, and sources of resistance (mainly Desi varieties) have been included in plant breeding programs (Singh 1987; Kaiser et al. 1994). To date, eight races of the pathogen have been reported (0, 1A, 1B/C, 2, 3, 4, 5 and 6), which are discriminated by the reactions they elicit in a differential set of cultivars (Haware and Nene 1982; Jiménez-Díaz et al. 1993). Some of them were also characterised by race-specific PCR products (Jiménez-Gasco and Jiménez-Díaz 2003). In Spain, race 0 is the most widespread, followed by races 5 and 6 (Jiménez-Díaz et al. 1993). Halila and Strange (1996), after analysing the reaction of some Tunisian isolates on differential chickpea lines, found that all of them belong to race 0. Race 1B/C was also identified in one isolate from Tunisia (Jiménez-Gasco et al. 2001). Races 0 and 1B/C, causing yellowing syndrome (Haware and Nene 1982; Jiménez-Díaz et al. 1993; Kelly et al. 1994), which is less virulent than the

wilting syndrome prompted by races 1A, 2, 3, 4, 5 and 6 (Jiménez-Díaz et al. 1993).

Resistance to fusarium wilt race 0 (FOC-0) is governed by one or two independent genes (Tekeoglu et al. 2000; Rubio et al. 2003). In addition, resistance to races 1, 2 and 4 rely on no more than three loci (Upadhyaya et al. 1983; Muehlbauer and Singh 1987; Gumber et al. 1995; Kumar 1998; Tullu et al. 1999). A combination of two recessive homozygous genes and a third dominant gene is necessary to confer complete resistance fusarium wilt race 1 (Upadhyaya et al. 1983; Smithson et al. 1983). For race 5, only one resistance gene has been described and mapped so far (Tekeoglu et al. 2000; Winter et al. 2000).

Marker-assisted selection (MAS) could become an efficient strategy to accelerate pyramiding of different resistance genes. However, MAS requires intraspecific linkage maps saturated with co-dominant markers. Due to the low genetic variability within *C. arietinum*, most of the available chickpea genetic maps (Gaur and Slinkard 1990a, 1990b; Kazan et al. 1993; Simon and Muehlbauer 1997; Winter et al. 1999; Tekeoglu et al. 2002; Collard et al. 2003), including the most extensive one available to date (Winter et al. 2000), have been developed using interspecific crosses. To overcome this problem, STMS markers have been developed for chickpea (Hüttel et al. 1999; Winter et al. 1999). Many of these single-locus, co-dominant markers detect polymorphism even between closely related chickpea accessions and thus enable the transfer of linkage information among different populations (Cho et al. 2002; Udupa and Baum 2003; Flandez-Galvez et al. 2003; Winter et al. 2003; Cho et al. 2004). Using transferable STMS markers linked to resistance genes for fusarium wilt races 1, 3, 4 and 5 (Mayer et al. 1997, Ratnaparkhe et al. 1998; Tullu et al. 1998; Winter et al. 2000; Benko-Iseppon et al. 2003; Sharma et al. 2004), clustering of all these loci on the same linkage group (LG) (i.e. on LG2 of Winter et al. 2000) was demonstrated (Tekeoglu et al. 2000; Sharma et al. 2004).

Until now, resistance genes against FOC-0 could not be assigned to any LG, and only a single RAPD marker ($OPJ20_{600}$) could be grouped with a FOC-0 resistance gene in two different recombinant inbred line (RIL) populations (Rubio et al. 2003). To further improve the intraspecific genetic map of chickpea and to locate agronomically interesting genes, we mapped two RIL populations segregating for resistance to FOC-0 with STMS markers.

Materials and methods

Plant material

Two $F_{6:7}$ RIL populations advanced by the single-seed-descent method from F_2 to F_6 (Johnson and Bernard 1962) were used in this study. They were derived from two crosses, CA2156 × JG62 and CA2139 × JG62, and comprised 79 and 80 lines, respectively. CA2156 is a white-flowered, single-podded Spanish Kabuli landrace susceptible to FOC-0; JG62 is an Indian Desi cultivar with purple flowers and double podding. CA2139 is a Spanish Kabuli landrace, with white flowers and single podding. Although the two latter cultivars are both resistant to FOC-0, different genes control the resistance reaction (Rubio et al. 2003). Parental Kabuli and Desi lines also differ in terms of seed coat thickness, a character that exhibits monogenic inheritance [thick/thin (*Tt*/*tt*), Gil and Cubero 1993]. CA2156 and CA2139 have thin seed coat, whereas JG62 has a thick seed coat. We followed Muehlbauer and Singh (1987) nomenclature for the symbols of flower colour genes; the *P*/*p* locus produces pink/blue petals when *B* is present and white petals when *b* is present. The single- or double-podding character is controlled by one locus (*Sfl*/*sfl*), and double podding is recessive (Muehlbauer and Singh 1987).

Both RIL populations were sown in the field at Córdoba, Spain, and were phenotyped for morphological characters. Seed coat thickness was measured in two desiccated circular samples (3 mm ϕ) obtained from five seeds per line as reported by Gil and Cubero (1993). Scoring for resistance to race 0 was performed at Beja, Tunisia, for 2 years (1999 and 2001) in a wilt-infected field (Rubio et al. 2003).

PCR analysis

For DNA extraction, about 100 mg young leaf tissue was excised, immediately frozen in liquid nitrogen and stored at −80°C. DNA was isolated using the CTAB method (Lassner et al. 1989), with the modifications described by Torres et al. (1993). The 102 RAPD decamer primers (prefix 'OP-' from Operon Technologies, Almeda, Calif., USA; prefix 'Mer-' Cornell University, Ithaca, N.Y., USA) tested in previous studies for polymorphisms (Hajj-Moussa et al. 1996) were selected. Optimal reaction conditions were established according to Williams et al. (1990). Amplification was performed in a PE Applied Biosystems GeneAmp 9700 thermal cycler in 25-µl volumes including 20–40 ng plant genomic DNA, buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100), 1.5 mM MgCl2, 125 µM each dNTP, 0.32 µM primer and 0.6 U *Taq* DNA polymerase (Bioline). Forty cycles of denaturation at 94°C for 20 s, annealing for 1 min at 36°C, and 1 min elongation at 72°C, with a final extension at 72°C for 8 min were performed.

Twenty-two ISSR sequences reported by Ratnaparkhe et al. (1998) were surveyed for polymorphisms, and analysis was performed following their protocol. The 25-µl reaction volumes contained 30 ng genomic DNA in 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.24 µM primer and 1 U *Taq* polymerase (Bioline). The thermal cycler was programmed for 35 cycles of the following temperature

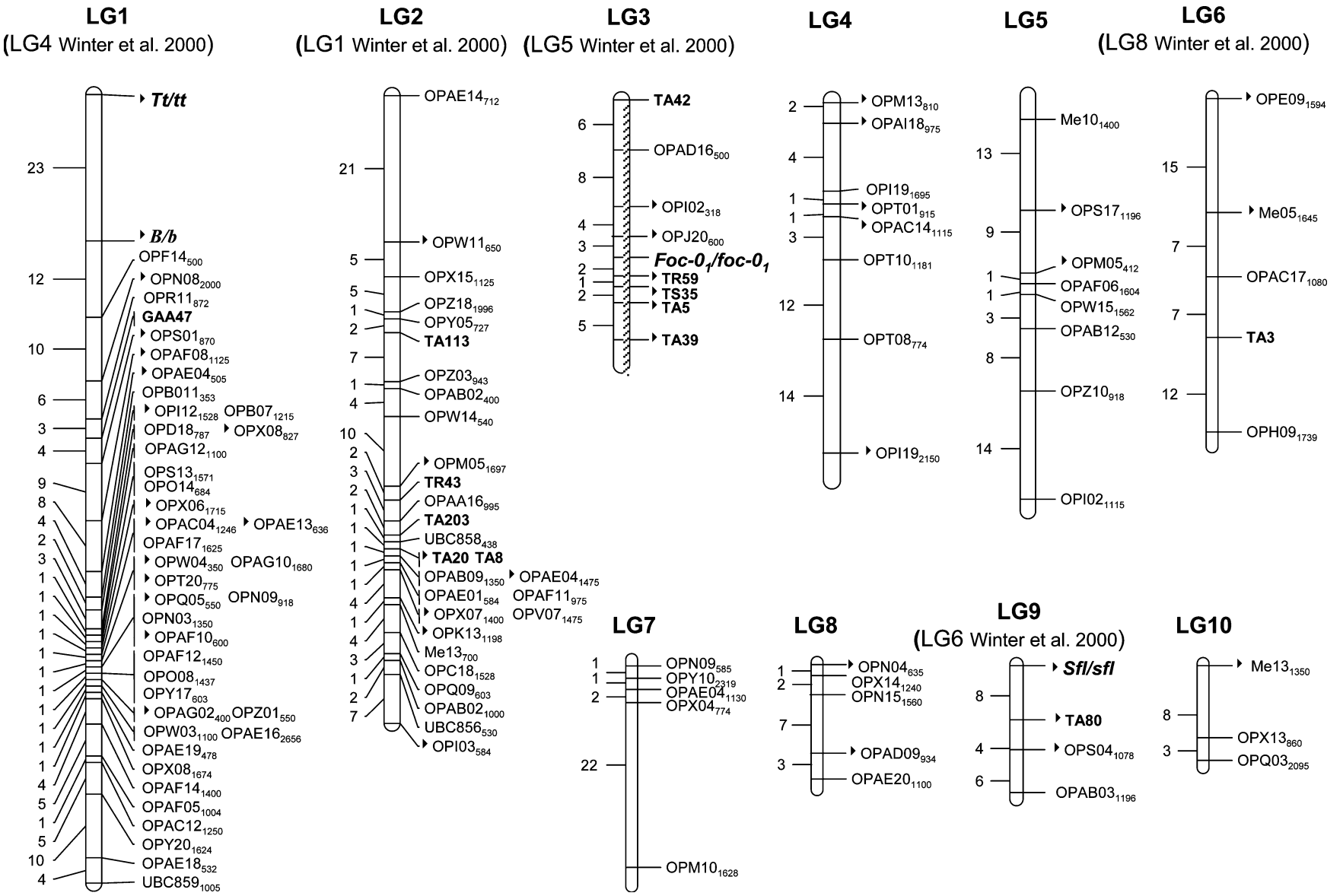

Fig. 1 Joint map of chickpea recombinant inbred line (RILs) from crosses CA2139 × JG62 and CA2156 × JG62. Shared markers are indicated by *arrows*. The STMS markers are in *boldface*. Map distances are indicated in centiMorgans. The linkage groups (*LG*s) have been numbered according to the number of clustered markers. Between *parentheses* are correspondences to LGs of Winter et al. (2000) reference map

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 in Winter et al. (1999). Selected primers showing polymorphism in one or both populations were chosen based on their location in previous linkage maps (Winter et al. 2000; Tekeoglu et al. 2002). Amplification conditions were as described by Winter et al. (1999), with slight modifications: 25-µl reactions containing 20–40 ng plant genomic DNA, buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.2 µM primer and 1 U *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, followed by a final extension at 60°C for 5 min.

Amplification products from the RAPD and ISSR protocols were separated in gels composed of a mixture of 1% agarose and 1% Nu-Sieve agarose (Hispanlab, Spain) in one-time Tris-borate-EDTA (TBE) buffer. STMSs were analysed in 2.5% Metaphor agarose (Biowhittaker Molecular Applications, Rockland, Me., USA) and in one-time TBE buffer.

Statistical analysis

Segregation data from two $F_{6:7}$RIL populations with a common parental line, JG62, was used to obtain a joined map. Goodness-of-fit to the expected 1:1 segregation ratio of markers was calculated by χ^2 tests. Linkage analysis was performed using JoinMap, version 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 LGs. Kosambi's function was applied to estimate map unit distances (Kosambi 1944). Prior to the joint map, two separate maps were produced for each population. In order to create a composite map, we applied the principles described by Román et al. (2004): (1) the genes encoding similar morphological phenotypes were homologous in both populations, and (2) polymorphic RAPD and ISSR amplification products with the similar molecular weight obtained by amplification with the same primer represented the same allele and the same locus in the respective LG. Missing data were assigned to all the individuals of a family when the marker did not segregate.

Results of reaction to FOC-0 in RIL population CA2139 × JG62 fit the expected 3:1 (resistant to susceptible) segregation ratio for two independent genes (Rubio et al. 2003). In this case, resistance was treated as quantitative trait (percentage of dead plants), because the allelic state for both resistance genes of the resistant lines could not be reliably determined. QTL analysis was performed using MapQTL, version 5, software (van Ooijen 2004). Interval mapping (Lander and Botstein 1989; van Ooijen 1992) with a mapping step size of 1 cM, was applied to identify putative resistance QTL in each LG for each data set from the two years of evaluations separately. The significance of QTL was determined empirically, employing the permutation test of Churchill and Doerge (1994) with 1000 replications. The coefficient of determination (R^2) for the marker most tightly linked to a QTL was used to estimate the proportion of the total phenotypic variation explained by the QTL.

Results

Linkage maps for the two RIL populations

After survey of parental lines, 58 RAPD decamer primers, 6 ISSRs, 14 STMSs and four morphological loci (*B*/*b*, *P*/*p*, *Sfl*/*sfl* and *Tt*/*tt*) generated a total of 145 polymorphisms in the RIL population from the cross CA2139 × JG62. Two RAPD markers deviating significantly from the expected segregation ratio were excluded from the study. In addition, the single-/double-podding character displayed a slightly skewed segregation (expected 1:1, $0.05 > P > 0.01$). Linkage analysis revealed 11 LGs including 125 molecular markers that covered 330.03 cM. A total of 14 markers including 11 RAPDs, two STMSs (TA14 and TA103), and the *P*/*p* locus were unlinked; the latter segregated for pink:blue flowers in 48 coloured-flower lines.

Starting from the above primers, polymorphisms in a second RIL population (CA2156 × JG62) were studied. Fifty-six RAPD, seven STMS, three morphological markers (*B*/*b*, *Sfl*/*sfl*, *Tt*/*tt*) and the $Foc0_1$/$foc0_1$ locus (data from Rubio et al. 2003) were subjected to linkage analysis. Seven LGs including 52 molecular markers and the map position of *Sfl*/*sfl* and $Foc0_1$/$foc0_1$ loci were obtained. By decreasing the maximum LOD score to 2.4, the flower colour locus *B*/*b* could be grouped with 23 RAPDs and GAA47. Twelve RAPD markers and the locus *Tt*/*tt* remained unlinked. This map covered a total of 176.4 cM.

The presence in both RIL populations of 48 common molecular markers (41 RAPDs and six STMSs) and three morphological traits (*B*/*b*, *Sfl*/*sfl*, *Tt*/*tt*) (Fig. 1) enabled joining of maps obtained independently (Fig. 1). Groupings were coincident for 41 of common markers (85.4%). Missing coincidence was due to the lack of polymorphism of markers joining expected clusters in one or the other population (e.g. locus *B*/*b* was unlinked to RAPD markers in CA2139 × JG62, because GAA47 was only polymorphic in the CA2156 × JG62 RIL population).

Integrated map

Joint segregation analysis including a total of 160 markers and 159 individuals resulted in 10 LGs comprising a total of 138 markers ($Foc0_1$/$foc0_1$, *B*/*b*, *Sfl*/*sfl*, *Tt*/*tt*, three ISSR, 13 STMS and 118 RAPDs, Fig. 1).

The joint map covered a total genetic distance of 427.9 cM.

LG1 included 56 RAPD markers, one STMS (GAA47) and the two morphological characters flower colour (*B*/*b*) and seed coat thickness (*Tt*/*tt*). This group covered 123.9 cM. As demonstrated previously in F_2 populations, the maximum genetic distance between the two morphological characters is 21 cM (Gil and Cubero 1993). In the $F_{6:7}$ RIL populations studied here, distances were similar (23 cM). The presence of GAA47 may indicate that LG1 corresponds to LG4 of Winter et al. (2000), and group VIII of Tekeoglu et al. (2002). Because GAA47 is also linked to the anthocyanin pigmentation locus *P* (Santra et al. 2000), it may be the same as our flower colour locus *B*/*b*. Notably, a high marker density opposite to morphological loci can be observed.

LG2 was composed of 29 loci including 22 RAPD, two ISSR and five STMS (Fig. 1), and comprised 86.7 cM with a maximum distance of 20.5 cM between markers. Five of these markers were polymorphic in both populations. This group corresponds to LG1 of Winter et al. (2000), because it contains STMS loci TR43, TA8, TA113 and TA203.

LG3 carries the gene for resistance to FOC-0 ($Foc0_1$/$foc0_1$) flanked by RAPD marker $OPJ20_{600}$ 3 cM apart, and STMS TR59 separated by only 2 cM (Fig. 1). Three RAPDs ($OPAD16_{500}$, $OPI02_{318}$, and $OPJ20_{600}$) and four STMS markers (TA5, TA39, TA42, and TS35) complete this LG. Linkage between $Foc0_1$/$foc0_1$ and $OPJ20_{600}$ was previously reported (Rubio et al. 2003), and linkage to STMS TA39 in our CA2156 × JG62 RIL population permitted us to select new STMS markers according to their location in the same LG (LG5) of the map of Winter et al. (2000), and other maps (Tekeoglu et al. 2002; Udupa and Baum 2003). Map distances and marker orders of the STMS markers in our map differs from previous maps, possibly due to the intraspecific nature of our segregating populations.

The single-/double-podding locus (*Sfl*/*sfl*) was located on LG9 jointly with two RAPD makers and STMS TA80 (Fig. 1). The association between TA80 and *Sfl*/*sfl* was reported by Rajesh et al. (2002) using near isogenic lines was afterwards confirmed in an RIL population (Cho et al. 2002). Considering the presence of TA80, this group matches to LG6 of Winter et al. (2000).

The LG6 could be assigned to LG8 of the Winter et al. (2000) map, because both include the TA3 locus. Thus, our LG6 probably represents the smallest chickpea chromosome H (Vlácilová et al. 2002).

The rest of the LGs (LG4, LG5, LG7, LG8 and LG10) only included RAPD markers and could not be aligned to previously reported LGs.

QTL analysis in population CA2139 x JG62

Linear regression and interval mapping analysis identified a QTL for resistance to FOC-0 in RIL population CA2139 × JG62. A significant association between the QTL and all markers on LG3 were detected for disease evaluation data from both years. This QTL explained between 34.8% (year 1999 data) and 37.8% (year 2001 data) of the variation of the character with maximum LOD-scores between 7.3 and 8.16, respectively (Fig. 2). Thus, QTL mapping confirms that LG3 carries at least one of the FOC-0 resistance genes, from the resistant parent JG62.

Discussion

Although results of Tekeoglu et al. (2000) indicate that resistance to FOC-0 may be controlled by only one recessive allele at the $Foc0_1$/$foc0_1$ locus, a second independent locus ($Foc0_2$/$foc0_2$) could be implicated in some genotypes (Rubio et al. 2003). For the first time, one of these resistance genes, $Foc0_1$/$foc0_1$, present in JG62, has been mapped. This locus is tightly flanked by two molecular markers ($OPJ20_{600}$ and TR59). TR59 was the marker that explained a higher percentage of the total variation (37.8%). The presence of at least one resistance locus on LG3 was confirmed by QTL analysis in the CA2139 × JG62 RIL population where either of two independent genes confers resistance (Rubio et al. 2003).

The STMS markers permitted us to confirm that this locus is located in a different LG than the earlier mapped loci for resistance to fusarium wilt races 1, 3, 4 and 5; all of them clustered in LG2 of the map of (Winter et al. 2000) and on other LGs in other maps (Tekeoglu et al. 2002; Sharma et al. 2004). However, because Rubio

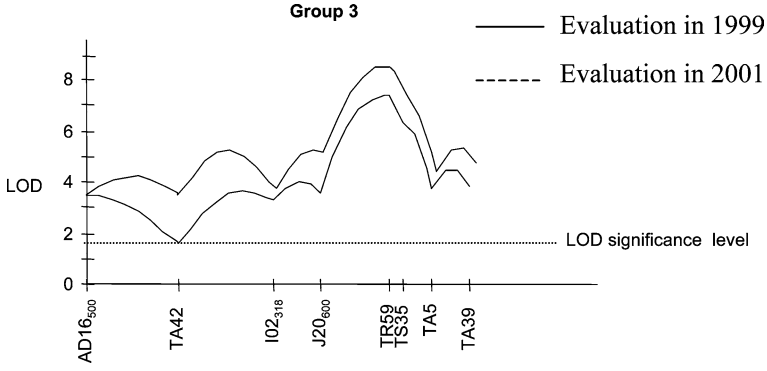

Fig. 2 Interval QTL mapping analysis of resistant to fusarium wilt race 0 in the chickpea RIL population CA2139 × JG62

et al. (2003) demonstrated the presence of at least two resistance genes for race 0, more work is needed to saturate the maps with markers to identify also the second locus.

In this study, the association between the flower colour locus and STMS marker GAA47 on LG1 (Fig. 1) as reported by Tekeoglu et al. (2002) was confirmed, and also linkage of seed coat thickness. Seed colour and thin seed coat are very important quality traits of chickpea cultivars for the Mediterranean area, because Kabuli-type chickpeas with white, fast-cooking seeds are preferred for human consumption (Gil et al. 1996). Thus, this particular part of LG1 encompassing GAA47 probably harbours genes that confer the most easily visible differences between Kabuli and Desi chickpeas. Because there is evidence that Kabuli chickpeas were selected from Desis based on seed quality characters (Moreno and Cubero 1978; Gil and Cubero 1993), it may well be that selection focussed on LG1. Moreover, a QTL for ascochyta blight resistance was detected between the flower colour locus and GAA47 (Santra et al. 2000; Tekeoglu et al. 2002); there is increasing demand to saturate this agronomically and economically interesting LG with preferably codominant STMS markers for potential use in MAS.

Another genomic region of particular interest defined by this study is LG9, because it harboured the double-podding gene. Because this character has a positive influence on yield stability (Rubio et al 1999), better coverage with markers would improve the precision of molecular breeding.

Our results once again demonstrate that STMS markers detect high levels of polymorphism, even between cultivated chickpea lines, and that linkages found in populations derived from inter- and intraspecific crosses are conserved in many cases (Winter et al. 2003, this study). Moreover, anchoring genomic areas of interest with STMS markers has been a very profitable strategy allowing saturation of the genomic region surrounding the $Foc0_1/foc0_1$ locus on LG3 with ease once an STMS marker linked to the gene had been identified. Today, we consider marker density around this locus in LG3 sufficient for targeted selection of resistant offspring, although our failure to detect markers linked to the second locus for resistance to FOC-0 segregating in our population clearly demonstrates the demand for more highly polymorphic, co-dominant markers such as STMSs for molecular breeding in chickpea. In this respect, it should be noted that even the often observed clustering of STMS markers (Winter et al. 1999) is not a disadvantage, because it allows choosing from several different markers for the same genomic region, one of which will almost certainly detect polymorphism in any given population.

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References

Benko-Iseppon AM, Winter P, Huettel B, Staginnus C, Muehlbauer FJ, Kahl G (2003) Molecular markers closely linked to fusarium resistance genes in chickpea show significant alignments to pathogenesis-related genes located on *Arabidopsis* chromosomes 1 and 5. Theor Appl Genet 107:379–386

Cho S, Kumar J, Shultz JL, Anupama K, Tefera F, Muehlbauer FJ (2002) Mapping genes for double podding and other morphological traits in chickpea. Euphytica 128:285–292

Cho S, Chen W, Muehlbauer FJ (2004) Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109:733–739

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Collard BCY, Pang ECK, Ades PK, Taylor PWJ (2003) Preliminary investigation of QTLs associated with seedling resistance to ascohyta blight from *Cicer echinospermum*, a wild relative of chickpea. Theor Appl Genet 107:719–729

FAOSTAT-Agriculture (2004) Database. http://www.fao.org/waicent/portal/statistic.es.asp.

Flandez-Galvez H, Ford R, Pang ECK, Taylor PWJ (2003) An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged microsatellite site and resistance gene analog markers. Theor Appl Genet 106:1447–1456

Gaur PM, Slinkard AE (1990a) Inheritance and linkage of isozyme coding genes in chickpea. J Hered 81:455–461

Gaur PM, Slinkard AE (1990b) Genetic control and linkage relations of additional isozyme markers in chickpea. Theor Appl Genet 80:648–656

Gil J, Cubero JI (1993) Inheritance of seed coat thickness in Chickpea (*Cicer arietinum* L.) and its evolutionary implications. Plant Breed 111:257–260

Gil J, Nadal S, Luna D, Moreno MT, de Haro A (1996) Variability of some physico-chemical characters in Desi and Kabuli chickpea types. J Sci Agric 71:179–184

Gumber RK, Kumar J, Haware MP (1995) Inheritance of resistance to fusarium wilt in chickpea. Plant Breed 114:277–279

Hajj Moussa E, Millan T, Gil J, Cubero JI (1996) Variability and genome length estimation in chickpea (*Cicer arietinum* L.) revealed by RAPD analysis. J Genet Breed 51:83–85

Halila MH, Strange RN (1996) Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f.sp. *ciceri* race 0. Phytopath Medit 35:67–74

Haware MP, Nene YL (1982) Races of *Fusarium oxysporum* f.sp. *ciceris*. Plant Dis 66:809–810

Hüttel B, Winter P, Weising K, Choumane W, Weigand F, Kahl G (1999) Sequence-tagged microsatellite-site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217

Jiménez-Díaz RM, Crino P, Halila MH, Mosconi C, Trapero-Casas A (1993) Screening for resistance to fusarium wilt and ascochyta blight in chickpea. In: Singh KB, Saxena MC (eds) Breeding for stress tolerance in cool-season food legumes. ICARDA, Wiley, Chichester, UK, pp 77–95

Jiménez-Gasco MM, Jiménez-Díaz RM (2003) Development of a Specific Polymerase Chain Reaction-Based Assay for the identification of *Fusarium oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 5 y 6. Phytopathology 93:200–209

Jiménez-Gasco MM, Pérez-Artés E, Jiménez-Diaz RM (2001) Identification of pathogenic races 0, 1B/C, 5 and 6 of *Fusarium oxysporum* f.sp. *ciceris* with random amplified polymorphic DNA (RAPD). Eur J Plant Pathol 107:237–248

Johnson HW, Bernard RL (1962) Soybean genetic and breeding. Adv Agrom 14:149–221

Kaiser WJ, Alcalá-Jiménez AR, Hervás-Vargas A, Trapero-Casas A, Jiménez-Díaz RM (1994) Screening wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*. Plant Dis 78:962–967

Kazan K, Muehlbauer FJ, Weeden NF, Ladizinsky G (1993) Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theor Appl Genet 86:417–426

Kelly AG, Alcalá-Jiménez AR, Bainbridge BW, Heale JB, Pérez-Artés E, Jiménez-Díaz RM (1994) Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of to *Fusarium oxysporum* f. sp . *ciceris* infecting chickpea. Phytopathology 84:1293–1298

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

Kumar S (1998) Inheritance of resistance to *Fusarium* wilt (race 2) in chickpea. Plant Breed 117:139–142

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lassner MW, Peterson P, Yoder JI (1989) Simultaneous amplification of multiple DNA fragment by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Mol Biol Rep 7:116–128

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

Moreno MT, Cubero JI (1978) Variation in *Cicer arietinum* L. Euphytica 27:465–485

Muehlbauer FJ, Singh KB (1987) Genetics of chickpea. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, UK, pp 99–125

Nene YL, Reddy MV (1987) Chickpea diseases and their control. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, UK, pp 233–270

Ooijen JW van (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811

Ooijen JW van (2004) MapQTL 5, Software for the mapping of quantitative trait loci in experimental populations. Plant Research International Wageningen, The Netherlands

Ooijen JW van, Voorrips RE (2001) JoinMap 3.0, Software for the calculation of genetic maps. Plant Research International Wageningen, The Netherlands

Rajesh PN, Tullu A, Gil J, Gupta VS, Ranjekar PK, Muehlbauer FJ (2002) Identification of an STMS marker for double-podding gene in chickpea. Theor Appl Genet 105:604–607

Ratnaparkhe MB, 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

Román B, Satovic Z, Pozarkova D, Macas J, Dolezel,, Cubero JI, Torres AM (2004). Development of a composite map in *Vicia faba*, breeding applications and future prospects. Theor Appl Genet 108:1079–1088

Rubio J, Moreno MT, Cubero JI, Gil J (1999) Effect of the gene double pod in chickpea yield, yield components and stability of yield. Plant Breed 117:585–587

Rubio J, Moussa E, Kharrat M, Moreno MT, Millán T, Gil J (2003) Two genes and linked RAPD markers involved in resistance to *Fusarium oxysporum* f. sp . *ciceri*s race 0 in chickpea. Plant Breed 122:188–191

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ, Muehlbauer FJ (2000) Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. Crop Sci 40:1606–1612

Sharma KD, Winter P, Kahl G, Muehlbauer FJ (2004) Molecular mapping of *Fusarium oxysporum* f. sp. *ciceris* race 3 resistance gene in chickpea. Theor Appl Genet 108:1243–1248

Simon CJ, Muehlbauer FJ (1997) Construction of a chickpea linkage map and its comparison with maps of pea and lentil. J Hered 88:115–119

Singh KB (1987) Chickpea breeding. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, UK, pp 127–162

Smithson JB, Kumar J, Singh H (1983) Inheritance of resistance to Fusarium wilt in chickpea. Int Chickpea Newsl 9:21–22

Tekeoglu M, Tullu A, Kaiser WJ, Muehlbauer FJ (2000) Inheritance and linkage of two genes that confer resistance to fusarium wilt in chickpea. Crop Sci 40:1247–1251

Tekeoglu M, Rajesh PN, Muehlbauer FJ (2002) Integration of sequence tagged microsatellite sites to the chickpea genetic map. Theor Appl Genet 105:847–854

Torres AM, Weeden NF, Martin A (1993) Linkage among isoenzymes, RFLP and RAPD markers in *Vicia faba*. Theor Appl Genet 85:937–945

Tullu A, Muehlbauer FJ, Simon CJ, Mayer 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

Udupa SM, Baum M (2003) Genetic dissection of pathotype-specific resistance to ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106:1196–1202

Upadhyaya HD, Smithson JB, Haware MP, Kumar J (1983) Resistance to wilt in chickpea. II. Further evidence for two genes for resistance to race 1. Euphytica 32:749–755

Vlácilová K, Ohri D, Wrána J, Cihaliková J, Kubaláková M, Kahl G, Dolezel J (2002) Development of cytogenetics and physical genome mapping in chickpea (*Cicer arietinum* L.). Chromosome Res 10:695–706

Williams PC, Singh U (1987) Nutritional quality and the evaluation of quality in breeding programmes. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, UK, pp 329–356

Williams SJ, Kubelik AR, Livak KJ, Rafalski A, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Winter P, Pfaff T, Udupa SM, Hüttel B, Sharma PC, Sahim S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ, Kahl G (1999) Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol Gen Genet 262:90–101

Winter P, Benko-Iseppon AM, Hüttel 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* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance gene for fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163

Winter P, Staginnus C, Sharma PC, Kahl G (2003) Organisation and genetic mapping of the chickpea genome. In: Jaiwal PK, Singh RP (eds) Improvement strategies for leguminosae biotechnology. Kluwer, UK, pp 303–351