Field Crops Research 111 (2009) 130–136

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Genetic analysis of agronomic traits in a wide cross of chickpea

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ARTICLE INFO

Article history:
Received 14 February 2008
Received in revised form 12 November 2008
Accepted 12 November 2008

Keywords:
Chickpea
Cicer reticulatum
Fusarium wilt resistance
Genetic map
Time to flowering
Quantitative trait loci

ABSTRACT

A genetic map composed of 10 linkage groups (LGs) covering 848.1 cM was constructed from a recombinant inbred line population from the cross *Cicer arietinum* (ICCL81001) × *Cicer reticulatum* (Cr5-9). The lines were genotyped with random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) and sequence tagged microsatellite site (STMS) markers, and phenotyped with respect to flowering time, flower colour, growth habit, seed size, seed coat reticulation, seed coat thickness and reaction to infection by two races of fusarium wilt. Segregation distortion was observed for 35% of markers, mainly clustered on LG4, LG7 and two unassigned linkage groups. *Rs*/*rs* (seed coat reticulation) together with a quantitative trait locus (QTL) accounted for >50% of the variation for seed coat thickness. Both *Hg*/*hg* (growth habit) and a flowering time QTL mapped to LG3A. The STMS marker TA142 was closely linked to this QTL. Two QTL for seed size were detected—one on LG4 linked to STMS markers GAA47 and STMS11, and the other on LG2 linked to STMS TA110. Two tightly linked QTL on LG2 closely linked to STMS marker TA59 controlled the resistance to both races of the pathogen causing fusarium wilt.

1. Introduction

Cicer arietinum L., the chickpea, is the only cultivated member of the 42 *Cicer* species. It is thought to have been domesticated in Turkey from *C. reticulatum* Lad. (Ladizinsky, 1975; Ladizinsky and Adler, 1976; Abbo et al., 2003; Kerem et al., 2007). The substantial differences in growth habit, pod dehiscence, seed size, shape, and the thickness and surface texture of the seed coat which now distinguish *C. reticulatum* from the cultivated chickpea, reflect the domestication process (Robertson et al., 1997; Kerem et al., 2007). The probable monophyletic origin of chickpea (Zohary, 1999; Abbo et al., 2003) is responsible for its narrow genetic base, which inhibits its improvement via conventional breeding (reviewed by Winter et al., 2003). There are, however, opportunities to expand the genetic diversity of the crop by accessing the secondary gene pool present in its wild relatives (Singh and Ocampo, 1997; Robertson et al., 1997; Singh et al., 2005). Some of these wild species have been identified as sources for improved tolerance/resistance to both biotic and abiotic stresses (Singh et al., 1998; Croser et al., 2003). However, the wide crosses necessary will inevitably also introduce undesirable traits, such as a reticulated and thick seed coat, small seed size, prostrate growth habit and pod dehiscence, although these can, in principle, be eliminated through backcrossing and selection.

Marker assisted selection (MAS) provides an efficient means of selecting specific alleles, and also for selecting against undesirable traits introduced as a result of a wide cross. The identification of appropriate markers requires the definition of linkage relationships, and some of the requisite mapping populations in chickpea have been developed from crosses between *C. arietinum* and *C. reticulatum* (Simon and Muehlbauer, 1997; Winter et al., 2000; Tekeoglu et al., 2002; Abbo et al., 2005). These maps have allowed for a range of genes and quantitative trait loci (QTL) to be linked to markers (e.g. Winter et al., 2000; Abbo et al., 2005; Cobos et al., 2006). A good example of the tagging of a major gene is the erect/prostrate growth habit, which is determined by *Hg*/*hg* (Muehlbauer and Singh, 1987), and found to be linked to the isozyme locus 6-PGD on LG (linkage group) 3 (Kazan et al., 1993; Winter et al., 2000). Similarly, a major seed size QTL has been located on LG4 (Cho et al., 2002; Abbo et al., 2005; Cobos et al., 2007), as well as some minor ones on LG1, LG6 (Abbo et al., 2005) and LG8 (Cobos et al., 2007).

Here we describe a genetic analysis of flower colour, growth habit, days to flowering, seed size, seed coat thickness, seed surface reticulation and resistance to *Fusarium* sp., based on segregation behaviour in a *C. arietinum* × *C. reticulatum* recombinant inbred

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doi:10.1016/j.fcr.2008.11.006

line (RIL) population. Our aim was to develop a genetic map as a means of tagging the genes and QTL underlying these traits, so that MAS could be deployed to accelerate the introgression of useful genes from the secondary gene pool of chickpea.

2. Materials and methods

2.1. Plant material

A set of 88 $F_{6:7}$ RILs, derived by single seed descent from the cross *C. arietinum* (ICCL81001) × *C. reticulatum* (Cr5-9) represented the mapping population. ICCL81001 is an early flowering (50% flowering achieved in 55 days) kabuli type, selected at the International Crops Research for the Semi-Arid Tropics (ICRISAT), resistant to *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) races 0 and 5 (Kumar and Haware, 1983). Cr5-9 is a local selection from *C. reticulatum* ILWC36, susceptible to both pathogen races and flowering late (73 days to reach 50% flowering).

2.2. Evaluation of agronomic traits

The RILs and the two parental lines were sown in the field at IFAPA Centro Alameda del Obispo in Córdoba (southern Spain, 37° 3′N latitude) in March 2001. Each plot consisted of a set of 2 m long rows, each sown with 20 plants of each RIL. The inter-row distance was 0.5 m. A row of one or other parent was placed every five RIL rows as a check. The following traits were monitored: flower colour (pink/white), growth habit (prostrate/non-prostrate), seed surface reticulation (strong/weak), number of days to 50% flowering, weight of 100 mature seeds, and seed coat thickness (Gil and Cubero, 1993). A greenhouse trial was carried out to assess the flowering time of the RILs under short day natural conditions. For this experiment the RIL population was sown in late November when the day length was ~10 h. Each RIL was represented by three plants grown in a 20-cm diameter pot.

2.3. Evaluation of resistance to infection by Foc races 0 and 5

The response to infection by *Foc* race 0 was measured under field conditions at Beja, Tunisia (36°43′N, 9°13′E) in a farm belonging to the National Institute for Agronomic Research (INRAT). The RILs were divided into four blocks each of 22 lines, with both parental lines present in each block. Plots were set out as described above (Section 2.2) and the disease response was measured by recording the percentage of dead plants present once the susceptible parent plants were all dead. The evaluation of response to infection by *Foc* race 5 was performed in a growth chamber at Cordoba. In this experiment, the RILs and parental lines were sown in trays (41 cm × 56 cm × 12 cm) filled with perlite at five lines per tray and 10 seeds per line. Inoculation followed the procedure described by Tullu et al. (1998), using *Foc* inoculum kindly provided by Dr. Fred J. Muehlbauer, Washington State University, Pullman, USA. Disease incidence was scored as the percentage of dead plants 4 weeks after inoculation.

2.4. Molecular markers and genotypic analysis

DNA was isolated from young leaves using DNAzol® (Invitrogen). Genotyping was carried out using 20 random amplified polymorphic DNA (RAPD) decamer primers (prefix "OP" from Operon Technologies, Alameda CA; prefix "Mer" from Cornell University, USA), one inter-simple sequence repeat (ISSR) sequence primer (prefix UBC, Ratnaparkhe et al., 1998) and 55 sequence tagged microsatellite site (STMS) primer pairs (Hüttel et al., 1999; Winter et al., 1999), selected on the basis of their map location. RAPD analysis was established according to Williams et al. (1990). RAPD amplifications were carried out in 20 μl reactions containing 20–40 ng template DNA, 1.5 mM $MgCl_2$, 125 μM dNTP, 0.32 μM primer and 0.6 U *Taq* DNA polymerase (Promega Biotech Iberica S.L.) in buffer (50 mM KCl, 10 mM Tris–HCl pH 8.8, 0.1% Triton X-100). The amplification programme consisted of 40 cycles of 94 °C/60 s, 35 °C/120 s and 72 °C/120 s, concluded with an incubation at 72 °C for 8 min. ISSR amplifications were carried out in 25 μl reactions containing 30 ng template, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.24 μM primer and 1 U *Taq* polymerase (Promega) in 10 mM Tris–HCl, 50 mM KCl, 0.1% Triton X-100. The PCR programme consisted of 35 cycles of 94 °C/30 s, 50 °C/30 s and 72 °C/120 s, followed by an incubation of 72 °C/10 min. STMS primer sequences and amplification followed Winter et al. (1999). After denaturing the template DNA for 2 min at 96 °C, the reactions were subjected to 35 cycles of 96 °C/20 s, 55 °C/50 s and 60 °C/50 s followed by an incubation of 60 °C/5 min. RAPD and ISSR amplicons were electrophoresed through 1% Seakem agarose and 1% Nu-Sieve agarose (Hispanlab, Spain) gels, and visualized by EtBr staining. Most of the STMS amplicons were electrophoresed through 2.5–4% Metaphor agarose gels (Biowhittaker Molecular Applications, Rockland, ME, USA), although some which required higher resolution were separated through 8% non-denaturing polyacrylamide gels and visualized by ethidium bromide.

2.5. Genetic mapping and QTL analysis

Goodness-of-fit to the expected 1:1 segregation ratio of markers in the RIL population was tested by χ^2. Linkage analysis was performed using JoinMap v4.0 with the maximum likelihood option (van Ooijen, 2006). Markers were grouped at a minimum LOD score threshold of 3.0 and a maximum recombination fraction of 0.25. Kosambi's function was applied to convert recombination percentages to centiMorgan (cM) map unit distances (Kosambi, 1944). QTL and markers were assigned to their LG as defined by Winter et al. (2000), based on their linkage to mapped STMS loci. QTL analysis was performed using MapQTL v5 (van Ooijen, 2004). The Kruskal–Wallis (van Ooijen et al., 1993), interval mapping (Lander and Botstein, 1989; van Ooijen, 1992) and the multiple-QTL models mapping method (MQM mapping) with cofactors (Jansen, 1994; Jansen and Stam, 1994) were applied to identify and validate putative QTL within each group. The significance of each QTL was determined empirically, employing the Churchill and Doerge (1994) permutation test, with 1000 replications and applying a 99% level of significance. 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 that QTL.

3. Results

3.1. The genetic map

The linkage analysis exploited 135 polymorphic markers (five ISSR, 72 RAPD, 58 STMS) and three trait loci (flower colour *B*/*b*, growth habit *Hg*/*hg* and reticulated seed *Rs*/*rs*). The resulting genetic map comprised 121 loci distributed across 10 LGs, with 14 markers remaining unlinked to any LG. The map covered 848.1 cM, with a mean inter-marker distance of 6.8 cM (Fig. 1). Only three small LGs could not be assigned to either one of the larger ones, or one of those defined by Winter et al. (2000), because no appropriate STMS markers were available. These were designated "unassigned linkage groups" (ULG1, ULG2 and ULG3). Indicative markers for LG3 fell into two LGs: LG3A with 16 STMS loci, and LG3B which contained TA76 and GAA45. The inheritance of most of the markers on LG1, LG2, LG3A, LG3B and LG5 fitted a 1:1 segregation ratio. About 35% of those which showed distorted