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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 genes for *fusarium* wilt races 4 and 5

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Abstract An integrated molecular marker map of the chickpea genome was established using 130 recombinant inbred lines from a wide cross between a cultivar resistant to fusarium wilt caused by *Fusarium oxysporum* Schlecht. emend. Snyd. &. Hans f. sp. *ciceri* (Padwick) Snyd & Hans, and an accession of *Cicer reticulatum* (PI 489777), the wild progenitor of chickpea. A total of 354 markers were mapped on the RILs including 118 STMSs, 96 DAFs, 70 AFLPs, 37 ISSRs, 17 RAPDs, eight isozymes, three cDNAs, two SCARs and three loci that confer resistance against different races of fusarium wilt. At a LOD-score of 4.0, 303 markers cover 2077.9 cM in eight large and eight small linkage groups at an average distance of 6.8 cM between markers. Fifty one markers (14.4%) were unlinked. A clustering of markers in central regions of linkage groups was observed. Markers of the same class, except for ISSR and RAPD markers, tended to generate subclusters. Also, genes for resistance to races 4 and 5 of fusarium wilt map to the same linkage group that includes an STMS and a SCAR marker previously shown to be linked to fusarium wilt race 1, indicating a clustering of several fusarium-wilt resistance genes around this locus. Significant deviation from the expected 1 : 1 segregation ratio was observed for 136 markers (38.4%, $P<0.05$). Segregation was biased towards the wild progenitor in 68% of the cases. Segregation distortion was similar for all marker types except for ISSRs that showed only 28.5% aberrant segregation. The map is the most extended genetic map of chickpea currently available. It may serve as a basis for marker-assisted selection and map-based cloning of fusarium wilt resistance genes and other agronomically important genes in future.

Key words Chickpea · Genetic map · Molecular markers · Fusarium wilt · Disease resistance genes

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Introduction

Chickpea (*Cicer arietinum L.*) is the third most important food legume crop worldwide with major production areas on the Indian sub-continent, West Asia and North Africa (WANA). Despite considerable international investment in conventional breeding, productivity of the crop has not yet been significantly improved. The main constraint for increasing yield in WANA countries is the susceptibility of the plant to a foliar disease, ascochyta blight, caused by the ascomycete *Ascochyta rabiei* (Saxena and Singh 1987; Singh et al. 1992; Kaiser 1997), and on the Indian subcontinent to the vascular disease fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* (Srivastava et al. 1984; Jiminéz-Diaz et al. 1993). Consequently, chickpea breeding aims at high yielding cultivars that combine long-lasting resistance against fusarium wilt and ascochyta blight with tolerance to abiotic stresses such as drought and cold.

In recent years, the use of molecular markers has accelerated plant breeding in a number of areas including disease resistance, insect resistance and quality factors (see e.g. Melchinger 1990). The availability of DNA marker maps has facilitated marker-assisted selection,

positional cloning of resistance genes, and mapping of quantitative trait loci (QTLs) of agronomic interest in many crops (Patterson et al. 1988; review in Winter and Kahl 1995). For chickpea, there is a need to develop a DNA marker map of sufficient density for use in marker-assisted selection and the cloning of important genes.

Chickpea is a self-pollinated diploid (2n=2*x*=16) annual with a moderately sized genome of around 750 Mbp (Arumuganathan and Earle 1991) that evolved from its wild progenitor *Cicer reticulatum* by selection (review in van der Maesen 1987). Genetic variation within cultivated chickpea is minimal (Ahmad et al. 1992; Udupa et al. 1993; Labdi et al. 1996) and has prompted several researchers to use inter- rather than intra-specific crosses for linkage analysis of morphological and isozyme loci (Gaur and Slinkard 1990; Kazan et al. 1993) and RFLPs (Simon and Muehlbauer 1997). Also in other crops with more variable genomes, interspecific crosses are prefered to maximise polymorphism in linkage analysis. In chickpea, an intraspecific cross was used to map a gene for resistance to fusarium wilt race 1. Two sequence-characterized amplified region (SCAR) markers (Mayer et al. 1997) and an ISSR marker (Ratnaparkhe et al. 1998) were shown to be located close to this resistance locus. The ISSR marker was also linked to the gene for resistance to race 4 of fusarium wilt (Ratnaparkhe et al. 1998a) indicating close linkage of the two resistance genes. One of the SCAR markers (CS27) was also mapped in the population used here. A skeleton map based on 120 sequence-tagged microsatellite site (STMS, Beckman and Soller 1990) markers was recently presented (Winter et al. 1999).

Recombinant inbred lines (RILs, Burr et al. 1988; Lister and Dean 1993) were generated by single-seed descent from the F_2 to the F_6 and beyond until their genomes could be considered virtually homozygous. The RILs were increased to provide sufficient seed for the evaluation of many traits in different environments. Another advantage of RILs is that, contrary to the often used F_2 populations, dominant and co-dominant markers have a similar information content which allows the integration of rapidly applied and economic dominant markers such as random amplified polymorphic DNA (RAPD, Williams et al. 1990), DNA amplification fingerprints (DAFs, Caetano-Anollés et al. 1991), amplification fragment length polymorphism (AFLP, Vos et al. 1995) and inter-simple sequence repeats (ISSRs, Gupta et al. 1994) into a framework of codominant markers like STMSs. We have recently developed more than 200 STMS markers (Hüttel et al. 1999; Winter et al. 1999), 118 of which now provide a skeleton of highly informative; codominant markers relative to which other, mostly dominant, markers can be located. The genetic map presented here contains STMS, DAF, AFLP, ISSR, RAPD, SCAR and isozyme markers as well as loci for resistance to fusarium wilt races 4 and 5, and is the most comprehensive map of the chickpea genome available to-date. It will serve as the basis for the development of a high-density map that can be used for map-based cloning of resistance genes, marker-assisted selection, and mapping of QTLs of agronomic interest.

Materials and methods

Plant material and DNA extraction

The 130 F_6-derived F_7 RILs of the interspecific cross of the cultivated chickpea line ICC-4958 (fusarium wilt resistant)×*C. reticulatum* PI 498777 (susceptible) were used previously to generate a core STMS marker map (Winter et al. 1999). Also the extraction of DNA from young leaflets of individual plants of each line was described there.

Detection of DNA polymorphism: AFLP

AFLP procedures were performed as outlined by Vos et al. (1995) using commercially available kits (Gibco-BRL, Bethesda, USA). Initial screening for variability between parental lines, with AFLP kits recommended for either large or small genomes, detected satisfying numbers of polymorphism with the small-genome kit. In addition to the primers provided by the kit, *EcoRI* primers with two selective bases (CA, CC, CG, CT and GA) were purchased from BioSpring (Frankfurt, Germany) and used for PCR-amplification. For radioactive detection of amplification products the *EcoRI* primer was labeled with ^{32}P (Amersham Pharmacia Biotech Europe, Freiburg, Germany) and PCR-generated fragments separated on 5% denaturing polyacrylamide gels for 90 min. The gels were vacuum-dried and exposed to X-ray films (Kodak XAR, Amersham Pharmacia Biotech Europe, Freiburg, Germany) for 3 to 12 h.

DAF analysis

DAF was performed as described by Caetano-Anollés et al. (1991), with minor modifications as follows: PCR was carried out on a Perkin Elmer Geneamp 9700 thermal cycler using random 10-mer primers procured from Eurogentec Deutschland (Cologne, Germany), Operon Technologies (Alameda, USA) or Roth (Karlsruhe, Germany), respectively. Each 15-µl PCR reaction contained 1.5-µl 10×PCR buffer (Eurogentec Deutschland, Cologne, Germany), 2.5 mM of $MgCl_2$, 10 mM of dNTPs, 0.4 U of "Silverstar" DNA polymerase (Eurogentec Deutschland, Cologne, Germany), 40 pmol of oligonucleotide primer, and 1 ng/µl of template DNA. The DNA was first denatured for 2 min at 95°C, followed by 40 cycles of 15-s denaturation at 95°C, 1-min annealing at 35°C and 2-min elongation at 72°C, with a final elongation of 2 min at the same temperature. The reaction products were separated in 1.8% agarose gels, stained with ethidium bromide and viewed under ultraviolet light.

RAPD analysis

For RAPD analysis PCR was performed essentially as described for DAF. The major difference was that for RAPD around 6-times less primer and a more-complicated time regime was used. A 20-µl mix consisted of 10 ng of template DNA, 6.5 pmol of random primer, (Operon Technologies, USA), 0.4 mM of spermidine, 200 mM of each dNTP, 1×*Taq* polymerase buffer, and 0.8 units of *Taq* DNA polymerase (Boehringer Mannheim). The amplification reactions were carried out on a Perkin Elmer thermocycler or a PTC200 thermocyler (MJ Research). An initial denaturation step at 94°C for 4 min was followed by five cycles at 92°C for 30 s, 36°C for 2 min and 72°C for 90 s, then by 35 cycles at 92°C for 5 s, 40°C for 20 s, and 72°C for 90 s. The final elongation was at 72°C for 5 min. PCR products were separated on 2% agarose gels, stained with ethidium bromide and scored for the presence or absence of polymorphic bands.

Detection of RFLPs with cDNAs

RFLPs detected by anonymous lentil cDNAs as detected by Havey and Muehlbauer (1989) were used to determine polymorphism between ICC-4958 and *C. reticulatum* according to the methods described in Simon and Muehlbauer (1997).

SCAR analysis

A sequence characterized amplified region (SCAR), referred to as allele specific associated primers (ASAPs), as reported by Mayer et al. (1997), was used in this study. The SCAR (CS-27) we used was reported by Mayer et al. (1997) to be linked to the genes for resistance to fusarium wilt.

Microsatellites

The conditions for the detection of STMS and ISSR polymorphisms as well as corresponding primer sequences have been described elsewhere (Ratnaparkhe et al. 1998a; Hüttel et al.1999; Winter et al. 1999). Sequences of PCR primers used for DAF, RAPD and ISSR analysis are available upon request.

Isozyme analysis

Isozyme analysis was carried out as described by Tullu (1996). Abbreviations used here are: aconitase (ACO), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), 6-phosphogluconate dehydrogenase (6PGD), glucose-6-phosphate dehydrogenase (G6PD).

Screening for fusarium wilt resistance

Rating of the disease reaction was performed according to Tullu et al. (1998), and Ratnaparkhe et al. (1998a, b). Fungal inoculum was prepared from a single-spored isolate grown on filter paper on potato-dextrose agar (PDA). From completely overgrown filters, colonies with a wild-type appearance were removed, placed on fresh filter paper in Petri dishes and dried for 5 days in a laminar flow hood. The paper was then cut into pieces and used to prepare the primary inoculum. The conidial concentration was adjusted to 1×10^6 spores per ml with a hemacytometer. Twelve to twenty seeds of each RIL were grown in the greenhouse (21° to 26°C) in plastic trays filled with sterile Perlite. When the seedlings reached the three- to four-nodal stage, they were removed, pruned while submerged in the spore suspension for 5 min and re-planted into Perlite. Plants were scored as susceptible or resistant over the next 2 months.

Linkage analysis

For linkage analysis Mapmaker V2.0 (Lander et al. 1987), kindly provided by Prof. Lander, was used. Loci were first divided into linkage groups at a LOD-score of 4 by two-point analysis using the ”group” command. Marker order in the linkage groups was determined using the ”try” command of the program and the order of STMS markers described by Winter et al. (1999) as a starting order. Marker orders obtained in that way were scrutinised by multipoint analysis applying the ”ripple” function. Markers that could not be assigned a unique placement for reasons other than lack of recombination were eliminated from the data set. Final map distances were calculated by applying the ”Kosambi” function (Kosambi 1944) provided by the program.

Results

General features of the map

The integrated molecular marker map of the chickpea genome is shown in Fig. 1. Of the 354 markers used for mapping, 303 were linked in 16 linkage groups (LGs) that span a total of 2077.9 cM. At a LOD-score of 4, 53 (14.9%) of the markers were unlinked. The smallest LG (16) is made up of two AFLP markers without recombination, whereas the largest (LG 1) comprises 39 markers spanning 373.9 cM. There are seven other LGs that are larger than 150 cM (LGs 2 to 8), while the others are smaller than LG9 (40.5 cM). The average marker density in LGs is 6.8 cM with 1-cM representing around 0.36 Mbp of the 750 Mbp chickpea genome.

Distribution of markers

The distribution of markers is not random. There is a tendency for clustering at three levels that allow some insight into the distribution of the respective sequences in the chickpea genome. Firstly, all marker types (except RAPD) tend to cluster at specific genomic regions. Secondly, a tendency for clustering of markers of the same type is observed. Finally, marker subtypes, such as STMS markers containing a particular microsatellite motif, or AFLPs derived from amplification of *Eco*RI-*Mse*I restriction fragments with a primer that contains a particular combination of specific bases, tend to cluster together. Examples for clustering at the first level can be found in all larger LGs. Generally, these LGs include one core region where different marker types, mostly STMSs and AFLPs, but also some of the few mapped isozyme loci and ISSR markers are tightly linked. These core regions are often subdivided into regions where one type of marker is more abundant than the other. For example, the central region of LG3 is made up of a cluster of five AFLP markers that cover about 10 cM and are linked at a distance of 4.9 cM to a group of ten $(TAA)_n$-containing STMSs that span only 6.6 cM. Another good example for level-2 and -3 clustering of different marker subtypes is found in LG 2 where, within 5.6 cM, four AFLP markers cluster that all have the same selective bases at the *Eco*RI- and *Mse*I-linker site. Examples of clustering of DAF markers are found at one end of LGs 3 and 6. However, intermingling of different marker types can also be observed. Adjacent to regions of high marker density, neighboring chromosomal areas are often poorly covered. The clustering of markers in certain, mainly central, regions of LGs cannot be attributed to segregation distortion, because these clusters are to a similar extent visible also in normaly segregating parts of the genome.

The relation of linked to unlinked markers varies for different marker types

The percentage of unlinked markers of a particular type varies for the different marker types. As summarized in

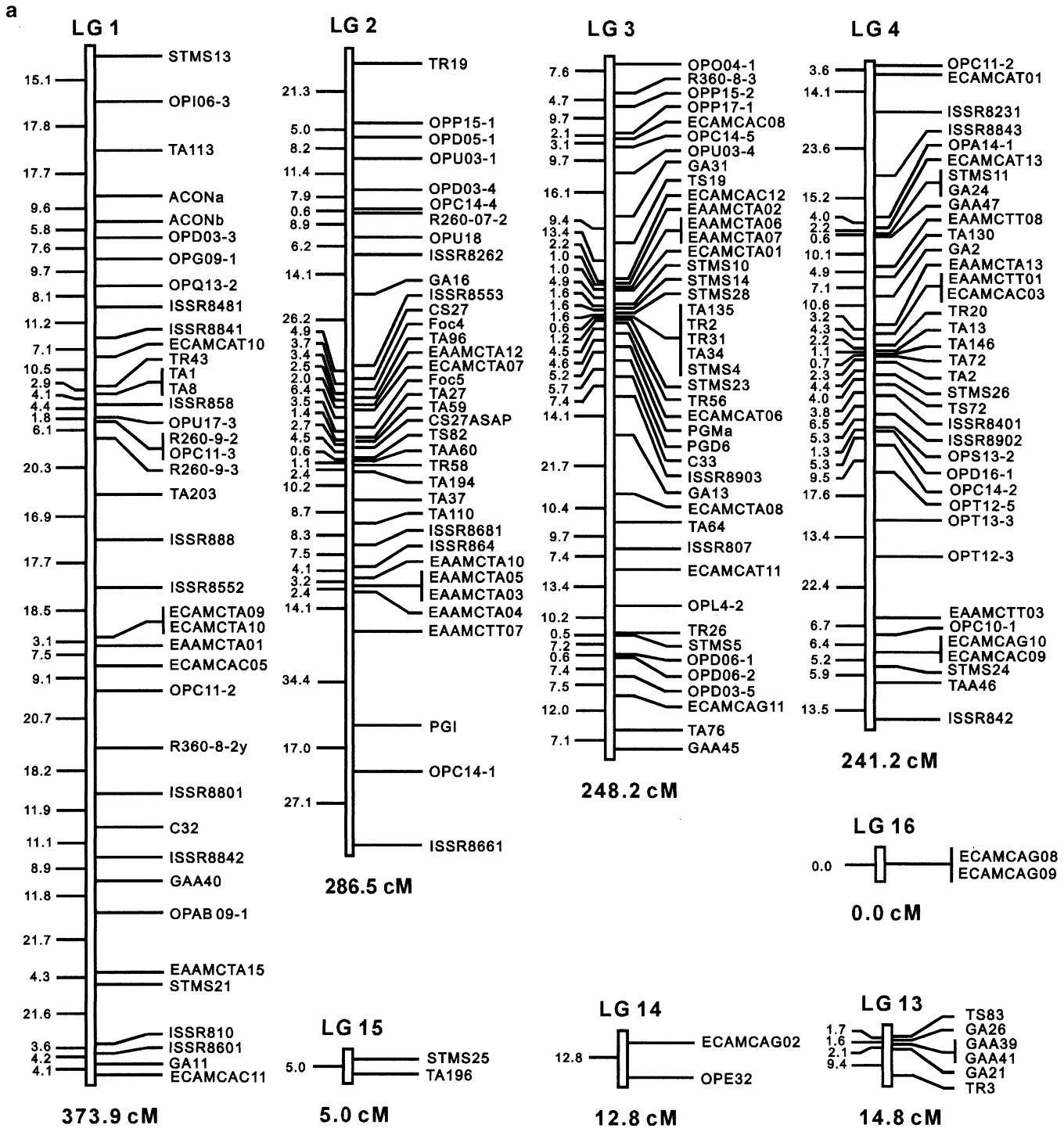

Fig. 1 Integrated map of the chickpea genome. Marker identification: STMS markers are described as *STMS* (Hüttel et al. 1999), or *TA*, *TR*, or *TS* (Winter et al. 1999) followed by a number. AFLP markers are characterized by the abbreviation for the rare cutting enzyme *Eco*RI (*E*) and selective bases (as e.g. C and A), followed by the abbreviation for the frequently cutting enzyme *Mse*I (*M*) and selective bases (as e.g. T and C). The *numbers* indicate the polymorphic bands. ISSR markers are identified by *ISSR and a number*, DAF and RAPD markers either by *OP* (Operon) *followed by a number*, or by *R* (Roth) *followed by a number*. *C32, C33 and S1E1* represent anonymous cDNAs. Random primer sequences for DAF, RAPD and ISSR markers are available on request. Isozyme abbreviations are as described in Materials and methods. Loci for resistance to fusarium wilt races 4 and 5 are abbreviated by *Foc4* and *Foc5*, respectively. The filled bars in LG 8 indicate that these markers are linked at LOD-scores of 3.99 and 3.96, respectively

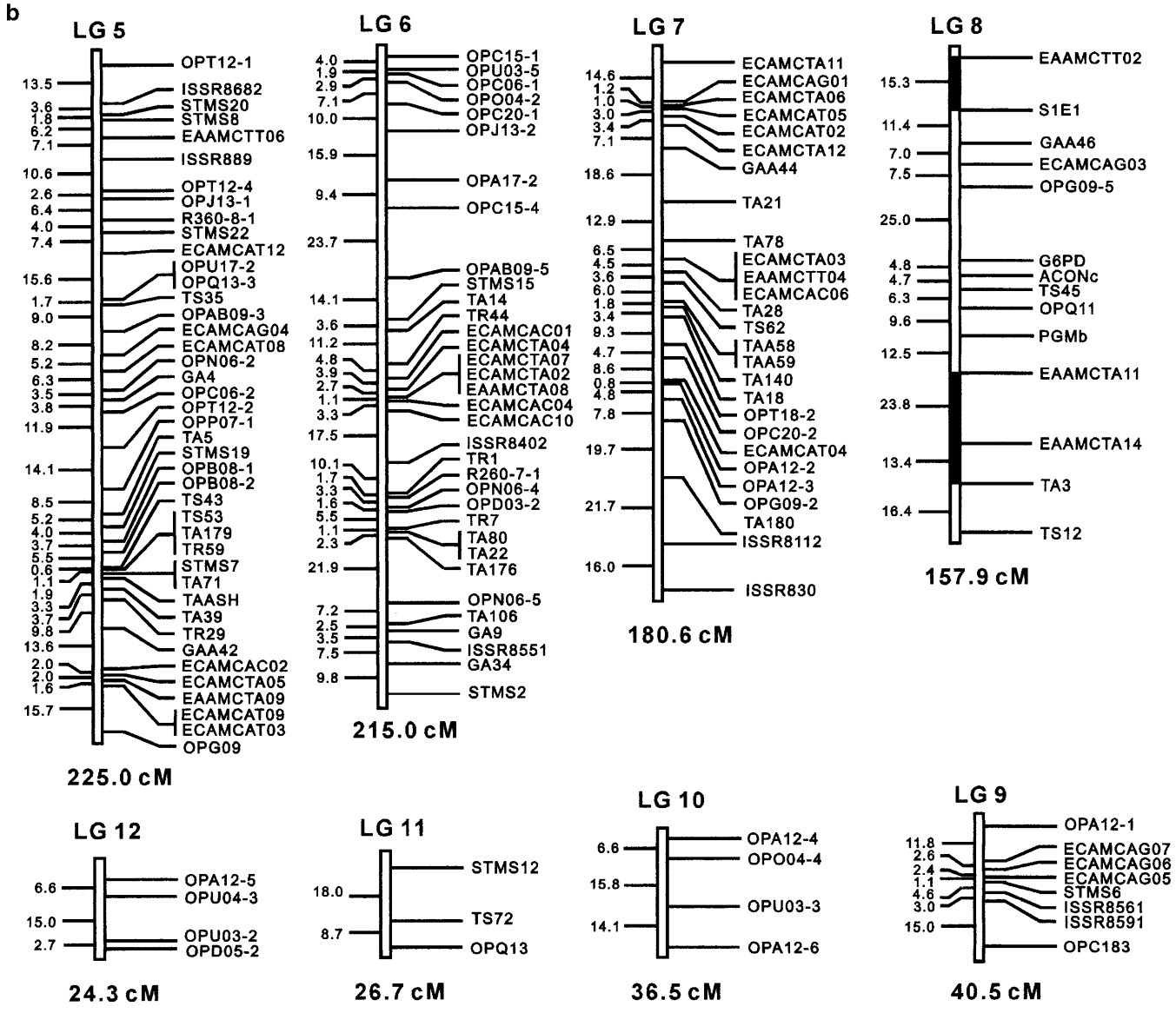

Fig. 1b

Table 1, at LOD-score 4, only 1.4% of AFLP markers but 76% of RAPD markers are unlinked. Of the isozyme and resistance gene markers, only the locus for resistance to fusarium wilt race 0 is unlinked. However, this locus tends to be linked at LOD-scores of 2.9 and 2.6, respectively, to markers STMS 24 and ECAMCAC09 on linkage group 4. When the LOD-score, as a criterion for linkage, is reduced from 4 to 3, the total number of unlinked markers is reduced from 14.4% to 10.4%.

Residual heterozygosity and segregation distortion

RILs in F_6 to F_7 are expected to contain 2 to 1% residual heterozygous loci. These can be detected by codominant markers such as STMSs. Indeed, in the present study heterozygotes were detected at a frequency of around 1 to 2% that is in accordance with expectations. We scored heterozygous loci as missing data and did not consider them for mapping.

A high number of skewed segregation of markers was observed in the RILs. A general tendency in favor of *C. reticulatum* alleles existed, because in 68% of the cases the offspring contained the wild parent's allele, whereas at 31% of the loci the *C. arietinum* allele was present. Only six markers showed the perfect Mendelian inheritance of 1:1. The general tendency towards the preferential inheritance of the alleles of a particular parent, as an extreme, resulted in distorted segregation at 136 of the 354 marker loci (38.4%) as judged from χ^2 tests ($P<0.05$).

Different marker classes exhibited different amounts of segregation distortion. As can be seen in Table 1, segregation distortion was most pronounced for RAPDs (47%), but much less so for AFLPs (28.5%). Nevertheless, segregation distortion was less related to the class of affected markers than to the genomic region where they resided. An example indicating that whole genomic

Table 1 Number of analysed markers and segregation distortion of different marker types

Marker-type	Number of markers	Unlinked markers (%)	Distorted segregation (%)
STMS	118	12 (10.1%)	47 (39.8%)
DAF	96	16 (16.6%)	40 (42.1%)
AFLP	70	1 (1.4%)	20 (28.5%)
ISSR	37	8 (21.6%)	14 (37.8%)
RAPD	17	13 (76.4%)	8 (47.0%)
Isozymes	8	0 (0.0%)	3 (37.5%)
R-Gene	3	1 (33.3%)	1 (33.3%)
cDNA	3	0 (0.0%)	1 (33.3%)
SCAR	2	0 (0.0%)	2 (100%)
Total	354	51 (14.4%)	136 (38.3%)

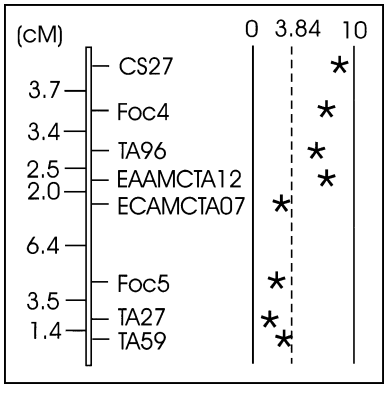

Fig. 2 Segregation distortion of different marker types around fusarium resistance loci Foc4 and Foc5 as a function of their genomic location. *Numbers* on the left indicate the map distances between markers in cM. CS27 is a SCAR marker (Mayer et al. 1997), EAAMCTA12 and ECAMCTA07 are AFLP markers, TA96, TA27 and TA59 are STMSs. The *asterisks* between the solid lines to the right indicate the χ^2 values for the different markers, the *dotted line* indicates the χ^2 treshold value of 3.84 (P=0.05), above which markers segregate distortedly

regions display similar levels of segregation distortion, irrespective of which markers are concerned, is illustrated in Fig. 2 for the region surrounding the fusarium resistance gene cluster on LG 2. Another example, where isozyme markers are also involved, can be found on LG3. Here, the whole upper region of the LG from AFLP marker ECAMCTA08 to DAF marker R360 –8-3 is affected. STMS marker GA13 next to the AFLP marker, and the following ISSR marker ISSR8903, have χ^2 values above ten. The segregation of all following markers, including the two isozyme markers PGD6 and PGMa, the adjacent cluster of STMS markers and the AFLP markers up to EAAMCTA06, is heavily distorted (χ^2 values above ten). Segregation distortion of the next AFLP-, STMS- and DAF-markers is less pronounced with χ^2 values less than ten. However, segregation distortion becomes more severe at DAF markers OPP15–1 and R360–8-3, which again display χ^2 values above ten. There are also more examples where markers of all types are affected by segregation distortion.

Discussion

General features of the chickpea genetic map

The current genomic map of chickpea is made up of 303 markers that comprise 2077.9 cM, rendering it the most extensive linkage map for chickpea available to-date. Previous chickpea maps covered 550 cM (Simon and Muehlbauer 1997) or 613 cM (Winter et al. 1999), respectively. The average distance between markers organised into linkage groups is around 7 cM, though the observed clustering and non-random distribution of markers resulted in large fluctuations in marker density. Also, the size of a LG does not necessarily reflect the number of linked markers since, for instance, LG1 with its 373 cM consists of 39 markers, whereas in LG 5, 42 markers cover only 225 cM. As the physical size of the chickpea genome was estimated to be 750 Mbp (Arumuganathan and Earle 1991), 1 cM would relate to 360 kbp on average, which is half the value of 750 kbp/cM calculated for the so-called high-density map of tomato (Tanksley et al. 1992). However, 51 markers are still unlinked. There are eight large and eight small LGs in the current map, and the eight large groups probably represent the eight chickpea chromosomes. However, in view of so many unlinked markers, we conclude that large regions of the genome are still not covered by markers.

Clustering of markers at specific regions as well as subclustering of different marker types is shown in Fig. 1. This is true for all types of markers except RAPDs. Essentially similar observations concerning overall clustering and marker type-specific subclustering were made by Becker et al. (1995) in an intra-species map of barley. In their map, AFLP markers rarely interrupted groups of RFLP markers, but were mostly clustered adjacent to them. In soybean, the distribution of AFLP markers depended on whether a methylation-insensitive restriction enzyme such as *Eco*RI or a sensitive enzyme such as *Pst*I had been used for their generation. *Eco*RI/*Mse*I AFLP clusters are expected to represent centromeric regions, whereas *Pst*I/*Mse*I AFLP prevail in euchromatin (Young et al. 1999). In chickpea, clusters of markers were mostly located in central regions of LGs, whereas marker density in distal regions was low. Non-random distribution of markers, often with centrally located clusters, was also reported for sugar beet (Halldén et al. 1996) or wheat (Langridge et al 1995). In soybean, the apparently random distribution of SSRs reported in a first study (Akkaya et al. 1995) vanished and clustering became visible when more markers were applied (Cregan et al. 1999). Tanksley et al. (1992) explained these observations, also made in more advanced tomato and potato maps, reasoning that "centromeres and centromeric heterochromatin, and in some instances telomeres, experience up to 10-fold less recombination than other areas of the genome." There are also exceptions to the clustering of markers, as e.g. the random distribution of $(GA)_n$-containing STMSs in rice (Chen et al. 1997).

Segregation distortion

Different marker classes exhibited different levels of segregation distortion. As can be seen from Table 1, segregation distortion was most pronounced for RAPDs (47%), but much less so for AFLPs (28%). Nevertheless, the extent of segregation distortion was not dependent on the marker type, but more on the overall segregation distortion of the region where they resided. Therefore, the difference in segregation behaviour of different marker types may be attributed to the fact that they are located in different genomic regions. The average segregation distortion was 38%, very close to that reported for RILs (39.4±2.5%) by Xu et al. (1997), who surveyed 53 different populations with a known number of distortedly segregating markers. For all other kinds of populations, segregation distortion was around 20% on average. To explain the relatively large extent of segregation distortion in RILs as compared to other populations, one has to take into account the special properties of RILs. Loci revealing only skewed segregation in the F_2, deviated significantly from Mendelian inheritance in RILs (Paran et al. 1995). A possible reason for this tendency is the higher number of meioses (12–14) experienced by RILs as compared to the two meioses leading from the F_1 to the F_2. Distorted parental alleles in an F_2 are more likely to produce gametes with distorted alleles, which may result in more distortion in the next generation.

Segregation distortion may cause severe drawbacks for the map-based cloning of resistance and other genes, as it may reflect recombination suppression at specific genomic regions and, hence, may lead to an underestimation of the physical distance between the gene of interest and the markers located next to it. For example, positional cloning of the Foc4 resistance gene on LG2 starting from the most closely linked markers could run into severe problems since the whole surrounding region is prone to segregation distortion (Fig. 2). Consequently, the physical distance between the gene and the closest markers could be much larger than expected from the average relation of 360 kbp / cM calculated for the whole genome. Therefore, it would be wise to first selectively increase the marker density in this region by bulked segregant analysis (Michelmore et al. 1991) or similar methods, and secondly to determine the physical distance between the markers by pulsed-field gel electrophoresis.

Tagging of fusarium wilt resistance genes

One important goal of genetic mapping in chickpea is the tagging of genes for resistance to important fungal pathogens such as *Ascochyta rabiei* and *Fusarium oxysporum* f.sp. *ciceri*. The RILs used here segregated for resistance to races 4 and 5 of the pathogen, and hence, two of these genes could be integrated into the current map. In a previous study, Mayer et al. (1997) developed two allele-specific associated primer (ASAP) pairs, namely UBC170 and CS27, from RAPD bands that were linked at 7% recombination to the locus for resistance to fusarium wilt race 1. Recombination between the two marker loci was 6% and both loci were located on the same side of the resistance locus. Analysis with additional markers placed the two loci near one end of LG VI on the linkage map of Simon and Muehlbauer (1997). These authors used RILs from an intra-species cross between C-104, a late-wilting chickpea accession and the resistant line WR-315. One of the ASAPs, CS27, that segregated also in the present cross, allowed us to infer the position of the locus for resistance to fusarium wilt race 1 on the map. CS27 is also linked to the genes for resistance to races 4 and 5 at distances of 3.7 and 18 cM, respectively, on LG2 in the present map. Therefore, we conclude that LG VI of Simon and Muehlbauer (1997) is the same as LG 2 in the current map. We further conclude that the genes for resistance to fusarium wilt races 1, 4 and 5 form a cluster in chickpea. Clustering of resistance genes for different races of a pathogen and also for different pathogens has been demonstrated in several plants including legumes (Kanazin et al. 1996; Yu et al. 1996) and may be the result of gene duplication, exon shuffling and recombination processes that are thought to have generated the different resistance genes from one or a few ancestral progenitor genes (reviews in Michelmore 1996; Hammond-Kosack and Jones 1997). It is interesting to note that the resistance loci and CS27 are flanked by several STMS markers on one side, and the microsatellite-based ISSR-marker 855 on the other side. Close linkage of microsatellite markers with resistance genes has also been documented for rice bacterial leaf blight (Blair and McCouch 1997). It is tempting to speculate that microsatellites, which are suspected to be involved in recombination processes (Vogt 1990), may also be involved in the evolution of resistance gene clusters in chickpea. However, it is also possible that close linkage of microsatellites is a consequence of the high overall density of microsatellites in the chickpea genome (Hüttel et al. 1999). For practical use in chickpea breeding, and especially marker-assisted selection, the close linkage of genes for resistance to fusarium wilt and several STMS markers is of great importance as it allows the use of at least one of the highly polymorphic markers for analysis of the segregation of wilt resistance genes in a wide range of germplasm.

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References

Ahmad F, Gaur PM, Slinkard AE (1992) Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L. Theor Appl Genet 83:620–627

Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB (1995) Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci 35:1439–1445

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9: 208–218

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995) Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249:65–73

Beckmann JS, Soller M (1990) Towards a unified approach to genetic mapping of eucaryotes based on sequence-tagged microsatellite sites. Bio/Technology 8:930–932

Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged-site markers diagnostic for the rice bacterial leaf blight resistance gene *Xa-5*. Theor Appl Genet 95:174–184

Burr B, Burr FA, Thompson KH, Albertsen MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Caetano-Anollés G, Bassam BJ, Gresshoff PM (1991) DNA amplification fingerprinting: a strategy for genome analysis. Plant Mol Biol Rep 9:292–305

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice. Theor Appl Genet 95:553–567

Cregan PB, Jarvik KT, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, Van Toai TT, Lohenes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1461–1490

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

Gupta M, Chyi Y-S, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionary diverse genomes using single primers of simple sequence repeats. Theor Appl Genet 89:998–1006

Halldén C, Hjerdin A, Rading IM, Säll T, Fridlundh B, Johannisdottir G, Tuvesson S, Åkesson C, Nilson N-O (1996) A high density RFLP linkage map of sugar beet. Genome 39:634–645

Hammond-Kosack KE, Jones JJ (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, isozyme, and morphological markers in lentil. Theor Appl Genet 77:395–401

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

Jiminéz-Diaz RM, Crino P, Halila MH, Masconi C, Trapero-Casas AT (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. John Wiley and sons, New York, pp 77–95

Kaiser WJ (1997) Inter- and intra-national spread of ascochyta pathogens of chickpea, faba bean, and lentil. Can J Plant Pathol 19:25–224

Kanazin V, Marek LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93:11746–11750

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

Kosambi D D (1944) The estimation of map distances from recombination values. Ann Eugen12:172–175

Labdi M, Robertson LD, Singh KB, Charrier A (1996) Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphisms. Euphytica 88:181–188

Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map in barley. Mol Breed 1: 389–395

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

Lister C, Dean C (1993) Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J 4:745–750

Maesen LJG van der (1983) Taxonomy, distribution and evolution of chickpea and its wild relatives. In: Witcombe JR, Erskine W (eds) Genetic resources and their exploitation – chickpea, faba beans and lentil. Martinus Nijhoff Publishers, The Hague, The Netherlands

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

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104:1–19

Michelmore RW (1996) Flood warning – resistance genes unleashed. Nature Genet 14:376–378

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

Patterson A, Lander E, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Paran I, Goldmann I, Tanksley SD, Zamir D (1995) Recombinant inbred lines for genetic mapping in tomato. Theor Appl Genet 90:542–548

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

Ratnaparkhe MB, Tekeoglu M, Muehlbauer FJ (1998b) Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor Appl Genet 97:515–519

Saxena MC, Singh KB (1987) The chickpea. CAB International, Wallingford, UK

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, Reddy MV, Haware MP (1992) Breeding for resistance to ascochyta blight in chickpea. In: Singh KB, Saxena MC (eds) Disease resistance in chickpea. ICARDA, Aleppo, Syria, pp 23–54

Srivastava SK, Singh SN, Khare MN (1984) Assessment of yield losses in some promising grain cultivars due to fusarium wilt. Indian J Plant Protec 12:125–126

Tanksley SD, Ganal MW, Price JP, de Vincente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni H, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Patterson AH, Pineda O, Röder M, Wing RA,Wu W, Young ND (1992) High density molecular linkage maps of tomato and potato genomes. Genetics 132:1141–1160

Tullu A (1996) Genetics of fusarium wilt resistance in chickpea. PhD thesis, Crop and Soil Sciences Department, Washington State University, Pullman, USA

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

Udupa SM, Sharma A, Sharma AP, Pai RA (1993) Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. J Plant Biochem Biotechnol 2:83–86

Vogt P (1990) Potential genetic functions of tandem repeat DNA sequence blocks in the genome are based on a highly conserved "chromatin folding code". Hum Genet 84:301–336

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

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

Winter P, Kahl G (1995) Molecular marker technologies for crop improvement. World J Microbiol Biotechnol 11:449–460

Winter P, Pfaff T, Udupa SM, Hüttel B, Sharma PC, Sahi 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

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2 backcross, double-haploid, and recombinant inbred populations in rice (*Oryza sativa* L). Mol Gen Genet 253:535–545

Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution. Theor Appl Genet 99:785–790

Yu YG, Buss GR, Saghai Maroof MA (1996) Isolation of a superfamily of candidate resistance genes in soybean based on a conserved nucleotide binding site. Proc Natl Acad Sci USA 93:11751–11756