Journal of Food Legumes 24(2): 91-95, 2011

Synteny relationships among the linkage groups of chickpea (*Cicer arietinum* L.)

R. BHARDWAJ[1], J.S. SANDHU[2], R.K. VARSHNEY[3], P.M. GAUR[3], LIVINDER KAUR[1] and YOGESH VIKAL[4]

[1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana – 141 004, India; [2]Indian Council of Agricultural Research, Krishi Bhavan, New Delhi – 110 014; [3]International Crops Research Institute for the Semi-Arid Tropics, Patancheru - 502 324 (AP), India; [4]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana - 141 004, India; E-mail: ruchipau@gmail.com
(Received: December 11, 2010; Accepted: March 29, 2011)

ABSTRACT

Ascochyta blight (AB) caused by *Ascochyta rabiei* L. is a devastating disease of chickpea world-wide. Resistant germplasm has been identified and an F_2 mapping population of chickpea cultivars with contrasting disease reactions to *Ascochyta rabiei* L. was used to identify the markers linked to the disease using STMS and ICCM markers. STMS markers showed 40% polymorphism between the parents. Bulked segregant analysis (BSA) was performed to identify markers linked to the AB resistance gene. Of the 252 markers, only 10 were found to be possibly linked. MAPMAKER version 3.0 gave the linkage map of LG5 region of the chickpea genome spanning a length of 275.2 cM. The AB resistance gene was present on LG5 flanked by the markers TA 42 and TR 35, with a distance of 45.0 cM and 38.6 cM, respectively. It was concluded that there was synteny in the linkage groups 1, 3, 5 and 7.

Key words: *Ascochyta rabiei*, BSA, Chickpea, ICCM markers, STMS markers

Chickpea (*Cicer arietinum* L.) is a self-pollinated diploid annual, with 2*n*=16 and genome size of approximately 750 Mbp (Arumuganathan and Earle 1991). It is the third most important grain legume crop in the world. It is grown in the Indian sub-continent, West Asia, North Africa (WANA), the Mediterranean basin, the Americas and Australia (Croser *et al.* 2003). However, the vulnerability of this crop to biotic stresses (Ascochyta blight, Fusarium wilt, nematodes and pests) and abiotic stresses (drought and cold) severely reduces the yield. Ascochyta blight, caused by *Ascochyta rabiei* L. [Pass] Labr., is a wide-spread foliar disease that causes extensive crop losses in most regions of the world where the crop is commonly grown. This is due to the fact that environmental conditions favourable to chickpea crop (>350 mm annual rainfall, 23-25° C) also favour the disease. Therefore, controlling this disease is essential to ensure stable chickpea production. Conventional breeding methods are underway but efforts to breed for resistance has not proved much fruitful. Molecular marker technology has made it possible to identify markers linked to resistance gene/s for ascochyta blight. However, Kusmenoglu *et al.* (1992) and Udupa and Baum (2003) found that low polymorphism in the cultivated chickpea limit the tagging of resistance genes for either ascochyta blight or fusarium wilt. Huttel *et al.* (1999) and Udupa *et al.* (1999) reported that the microsatellite based marker systems as sequence tagged microsatellite sites (STMS) markers which are polymorphic due to variable number of repeat motifs have proved that microsatellites were abundant in the chickpea genome and could efficiently be used for detecting the genetic variation within the cultivated chickpea. In the present study, efforts were made to map the ascochyta blight resistance gene using STMS markers developed by Udupa *et al.* (1999) and Winter *et al.* (1999) and a set of ICCM chickpea markers developed at ICRISAT, in an intraspecific cross of chickpea and also to study the colinearity between the linkage groups.

MATERIALS AND METHODS

***Mapping Population*:** The study employed 250 F_2 plants of chickpea derived from an intraspecific cross 'GL 769' × 'GL 90168'. These two parents 'GL 769' and 'GL 90168' are imparting susceptible and resistant reaction, respectively to ascochyta blight. The F_2 mapping population along with two parents was sown in ascochyta blight screening nursery in the year 2006-07 at Punjab Agricultural University Research Farm, Ludhiana.

***Phenotypic screening for ascochyta blight resistance*:** The phenotypic screening of F_2 population for ascochyta blight resistance was done using cut twig screening technique as per Sharma *et al.* (1995). The resistant and susceptible individuals were identified to find out the genetics of ascochyta blight resistance. The individual 250 F_2 plants were tagged using Zeol tag. The tender shoots of parents and individual F_2 plants were cut with the help of scissor. Individual shoot was wrapped with moist cotton swab at the base and then transferred into test tube (15x100 mm) containing fresh water. The tubes were placed in test tube stands. Twigs were inoculated by spraying spore suspension ($1x10^5$/ml) of isolate 2 of *Ascochyta rabiei* and covered with moist dasuti cloth chambers. After 48 h, cloth chambers were removed and the plants were kept wet by spraying water daily up to 13 days from 10.00 – 16.00 h. After 13 days of inoculation, the severity of disease was recorded on 1-9 disease scale in order to perform phenotyping of the F_2 population. The plants scoring disease rating up to 5 were considered resistant, while >5-9 were rated susceptible. Chi-square analysis was applied to test the goodness of fit to find out the appropriate genetic ratio.

***Plant DNA extraction and genotyping using PCR*:** DNA was extracted following CTAB (Cetyl Tri-methyl Ammonium Bromide) protocol suggested by Saghai Maroof *et al.* (1984) modified in the DNA extraction laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. Polymerase chain reaction (PCR) was used for DNA amplification. Reaction mixture of 5 µl volume was prepared from the following components for one reaction: Milliq H_2O – 1.55 µl, Buffer 10X – 0.5 µl, $Mgcl_2$ 10 mM- 1µl, Primer 5pmoles/µl –0.5µl, DNTPs 2mM –0.25µl, Taq polymerase (Bioline Inc.) 0.5U/µl – 0.2µl, DNA 5ng/µl –1 µl. The marker amplifications were performed in ABI thermal cycler using a touchdown amplification profile. For Winter *et al* series primers, the amplification cycles were: initial denaturation of 3.00 min at 95°C followed by 5 cycles of denaturation for 20s (sec) at 94°C, touchdown from 65°C to 60°C (59°C) with 0.5°C decrease in each cycle followed by extension at 72°C for 30s. The next 30 cycles were, denaturation at 94°C for 20s, annealing at 59°C for 50s and extension at 72°C for 30s followed by final extension of 20 min at 72°C and then hold at 4°C. Majority of the Winter *et al* series primers were amplified at 65-60°C touch down profile. Very few were amplified at 55-45°C and 60-55°C. The ICCM primers were amplified at TD PAGE Bioline (61-54°C) with PCR profile: initial denaturation of 3.00 min at 94°C followed by 10 cycles of denaturation for 15s at 94°C, touchdown from 61°C to 54°C (59°C) with 0.5°C decrease in each cycle followed by extension at 72°C for 30s. The next 35 cycles were, denaturation at 94°C for 10 s, annealing at 54°C for 20 s and extension at 72°C for 30 s followed by final extension of 20 min at 72°C and then hold at 4°C. 6% polyacrylamide gels were used for separation and visualization of PCR amplified microsatellite products. The components of PAGE gel were: Dist.H_2O – 52.5 ml, 10x TBE – 7.5ml, Acryl-bisacrylamide -15ml, 10 per cent APS (ammonium per sulphate) – 400-450 µl, TEMED (N, N, N'N'-tetramethylethylenediamine)—90-100 µl.

Parental polymorphism was carried out using STMS markers developed by Udupa *et al.* (1999) and Winter *et al.* (1999) and a set of ICCM markers (Spurthy *et al.*2010). Total 252 markers were screened on parents. Two types of markers *viz*., labeled with fluorescent dyes and unlabeled primers were used in the present study. The forward primers of the labeled markers had been labeled with different dyes (PET- Red colour, NED- Yellow colour, FAM-Blue colour, VIC- Green colour) at 5' end so as to screen them on capillary electrophoresis with the use of these fluorescent dyes. Genotyping of unlabelled primers was done on 6% PAGE gels. The labeled PCR product of 1ml was mixed with 7µl of HIDIFORMAMIDE (PE- Applied Biosystems, California), (maintains stability of DNA) and 0.25µl of LIZ 500 (PE- Applied Biosystems, California), (which is a size standard for determining allele size) and the total volume was made to 10µl with distilled water. DNA framgents were denatured at 94°C for 4 min and size fractioned using capillary electrophoresis on an ABI-3130*xl* automatic genetic analyzer (PE- Applied Biosystems, California). The electrophoretic data were exported to the GeneScan 3.7 software (PE- Applied Biosystems, California) to allot the size peak patterns, using the internal LIZ-500 size standard and Genotyper 3.7 (PE-Applied Biosystems, California) for allele sizing. The unlabeled primers were resolved on 6% PAGE gel for better separation and visualization of PCR amplified microsatellite products.

***Bulked segregant analysis (BSA) and selective genotyping*:** BSA was performed essentially as defined by Michelmore *et al.* (1991). Equal amount of DNA from 10 resistant and 10 susceptible plants were bulked. The individuals having the disease score of 1 in the phenotypic screening of the disease by the cut twig method were considered as resistant and 10 plants of this score were pooled to constitute the resistant bulk. Similarly, the individuals with the disease score of 9 were considered as susceptible and 10 plants of this score were pooled to constitute the susceptible bulk and the DNA concentration was normalized to 5ng/l.

Primers giving rise to polymorphic bands between the bulks were further tested for possible linkage to resistance locus by selective genotyping of 10 resistant and 10 susceptible F_2 plants. Here, the marker analysis was performed on 5 ng DNA of each individual F_2 plant and two parents. Bands present in all individuals of one group but absent in all individuals of other group were assumed to be linked to one or other resistance locus, and the respective primers were tested on the whole population.

***Linkage analysis*:** MAPMAKER version 3.0 was used to generate the linkage map of the region and identifying markers linked to *Ascochyta* blight resistance. Maximum LOD score 3.0 and recombination fraction 50 were used for identifying linkage groups. MAPCHART version 2.1 developed by Voorripps (2002) was used for the graphical presentation of the linkage groups.

RESULTS AND DISCUSSION

***Screening for disease resistance*:** Phenotypic screening revealed the parent 'GL 769' had 9 score whereas the resistant parent 'GL 90168' had disease score of 2 and was found to be truly resistant to ascochyta blight under artificially created epiphytotic conditions. The F_1 plants of this cross were also resistant. The F_2 population evinced the digenic control of resistance as evident from 13R: 3S (χ^2 = 0.240) genetic ratio. Thus, resistance was found to be dominant with one dominant and one recessive gene control. The digenic control of resistance to ascochyta blight was also reported by Kusmenoglu (1990), Mahendra Pal *et al.* (1999) and Santra *et al.* (2000).

***Parental polymorphism survey*:** Of the total 252 chickpea markers screened, 100 were found polymorphic among the parents. The size of the amplified products ranged from 0.1-0.7 Kb with most of the markers falling in the range of 0.2-0.4

Kb. The polymorphic amplification products were designated according to their size (base pairs). Approximately 40% of the polymorphism was found in the present study. 40% intraspecific polymorphism using microsatellites was also reported by Winter *et al.* (1999) and Lichtenzveig *et al.* (2005). However, high levels of intraspecific polymorphism (66%) using STMS markers was reported Sethy *et al.* (2006) which attributed to the preferential isolation of the GA/CT repeat motifs, that have been reported to be highly polymorphic in other plant systems like rice, bean, tomato and pea-nut by Cho *et al.* (2000), Gaitan Solis *et al.* (2002) and He *et al.* (2003). But the same STMS markers generated 77% interspecific polymorphism in a cross between chickpea and *C. reticulatum* as reported by Sethy *et al.* (2006).

***Bulked Segregant Analysis (BSA)*:** By definition, if a resistant allele (R) was tightly linked to a given STMS band, then that band would be present in parents (RR × rr). When gel was run in which resistant and susceptible (S) phenotypes, segregating in a cross between resistant and susceptible homozygous were grouped separately, then any band that was present in all resistant progeny, but absent in all susceptible progeny, must therefore be linked in coupling phase with resistance. Using each of the 100 polymorphic markers, BSA was carried out on F_2 individuals of two pooled DNA samples representing two extremes of distribution. Out of the 100 polymorphic markers, 82 primers showed band in both the resistant and susceptible bulks, indicating that these were not linked to the gene of interest. 17 markers showed exact banding pattern among resistant and susceptible bulks. These seventeen markers *viz.*, TAASH, TA103II, TA200, TA71, TA106, TA125, TA64, TA42, TA78, TA80, TR8, CaSTMS9, TA180, TR26, TR35, TS17x and TA110 showed the exact genetic ratio for probable linkage on the bulks. The 17 markers giving rise to polymorphic bands between the bulks were further tested for possible linkage to the resistance locus by selective genotyping of 10 resistant and 10 susceptible F_2 individuals, to find out the markers segregating in the same genetic ratio as the phenotypic ratio. From these 17 markers, only 10 markers *viz.*, TR35, TA71, TA110, TA125, TA42, TA78, TR8, TA200, TR26 and TA106 showed the probability of linkage. Then these 10 markers were applied to the population to find out the markers showing the linkage pattern. BSA was also applied by Rakshit *et al.* (2003) to identify DAF markers linked to the locus contributing resistance to *Ascochyta rabiei* in chickpea and found that three out of five markers were polymorphic between the bulks, parents and also in selective genotyping were linked to the resistance locus.

***Marker segregation*:** Ten STMS markers were tested on the F_2 population to find out the linked marker to AB resistance gene. The markers TA110, TA125, TA78, TA200, TR26 and TA106 segregated in the expected 3R: 1S F_2 Mendelian ratio and the markers TA71, TA42 and TR8 segregated in 13R: 3S ratio, whereas the marker TR35 showed segregation distortion. Some markers in chickpea intraspecific cross that showed segregation distortion were also reported by Flandez-Galvez *et al.* (2003). Distorted marker segregation is the systematic deviation from an equal representation of alleles among the functional gametes involving all the chromosomes and could be caused by selection processes at the gamete or zygote stage or/ and post zygotic selection as reported by Gadish and Zamir (1987), Zamir and Tadmor (1986) and Lyttle (1991) or due to uneven recombination of homeologous chromosomes which results in the biased estimation of linkage marker distance. The marker that showed skewed segregation in the present study was in favour of the resistant parent; it therefore appeared that the site of crossing over for that marker was higher on the male side. Thus, marker segregation distortion is a general phenomenon and is observed in the segregating generations of the inter or intraspecific crosses.

***Linkage Analysis*:** Linkage analysis was carried out using MAPMAKER version 3.0 for determining the linkage between the gene and the marker and for establishing tentative location of the ascochyta blight resistance gene. The markers were screened on F_2 plants and their blight resistance gene was confirmed by working out the recombination distance in centimorgan (cM). The ascochyta blight resistance data was converted to parents 1(A, susceptible), 2 (B, resistant) and heterozygous (H) alleles and computed with the marker data for determining the distance between the ascochyta resistance gene and the marker. The seven markers (TA200, TA110, TA125, TA71, TA42, TR35 and TA78) grouped in one linkage group with the gene of interest and three markers (TR8, TR26 and TA106) were found unlinked (Fig 1) using maximum LOD score 3.0 and recombination fraction of 0.5. MAPCHART version 2.1 developed by Voorripps (2002) was used to construct the linkage map of LG 5, spanning a total length of 275.2 cM. The AB resistance gene was found to be associated with two flanking markers TA42 and TR35, with a distance of 45.0 cM and 38.6 cM, respectively. The region of AB resistance gene has been identified on LG 5. It is also evident from the results that only one gene was flanked by the markers and we were not able to define the second gene. The possibility could be none of the polymorphisms detected in the present study were actually linked to the other gene and that might be the minor gene for resistance. Same findings were also encountered in a study by Rakshit *et al.* (2003). Some of the markers belonging to the different linkage groups in earlier studies by Winter *et al.* (1999), Winter *et al.* (2000), Udupa and Baum (2003) and Flandez-Galvez *et al.* (2003), were clubbed into single group in the present study. The marker STMS marker TA200 has been mapped on LG I in a study by Flandez-Galvez *et al.* (2003), TA125 has been mapped LG 3 Flandez-Galvez *et al.* (2003) and on LG I by Winter *et al.* (1999). Similarly, marker TA110 has been mapped on LG 2 by Udupa and Baum (2003) and Winter *et al.* (2000) but the same was also mapped on LG 7 by Winter *et al.* (1999). The markers TA71 and TA42 have come from the same linkage group i.e LG 5

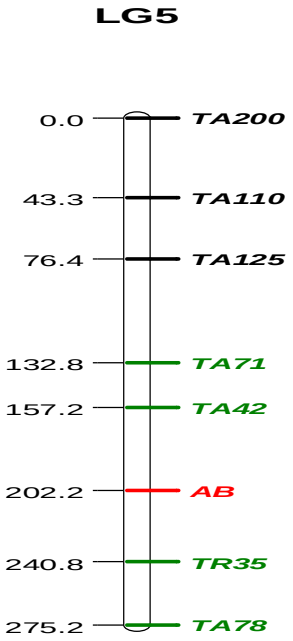

Fig 1. Genetic linkage map of the LG 5 of chickpea genome in the cross 'GL 769' × 'GL 90168'

by Udupa and Baum (2003). However, the marker TA71 was also reported to be present on LG 3 and LG 5, respectively by Winter *et al.* (1999) and Winter *et al.* (2000). The marker TR 35 has not been reported to be present on any linkage group, so, we have assigned this to LG 5. STMS marker TA 78 had been shown to be present on LG7 by Flandez-Galvez *et al.* (2003) and on LG 5 by Winter *et al.* (1999). This shows that there was reasonable synteny present between the linkage groups. The synteny between the LG 1 and LG 3; LG 3 and LG 4; LG 6 and LG 5 has been reported earlier by Flandez-Galvez *et al.* (2003). In the present study, the markers TA 200, TA 110 and TA 125 have been clubbed into markers of LG 5. The positions of the markers described in earlier studies showed that there is possibility of shuffling of the markers due to homology between the linkage groups.

Since TA 200 was found on LG I and TA125 was found on LG I and LG 3 (in two different studies) whereas TA 78 was reported on LG 5 and LG 3 (mentioned earlier). So, it is concluded that there was synteny in the linkage groups 1, 3, 5 and 7. Also in the present investigation, the length of LG 5 was found to be 275.2 cM whereas it was reported 68.1 cM by Winter *et al.* (1999) , 44.2 cM by Flandez-Galvez *et al.* (2003) and 39.5 cM by Udupa and Baum (2003). These inconsistencies e.g. a few tightly linked markers changed places relative to the other marker mayderive from differences in the recombination frequencies in distinct genomic regions between the two populations. These are visible as large differences in map distances between distantly related markers. Similar observations of variable distances between the markers in different segregating chickpea population were already reported by Kazan *et al.* (1993) and Simon and Muehlbauer (1999). These discrepancies can be explained in that recombination frequencies for specific region may change from one F_1 to another even in the population derived from crosses of the same parental lines (Rakshit *et al.* 2003).

Most of the genetic studies reported earlier were based on inter-specific crosses and have the disadvantage of identifying loci that may be polymorphic only between the more divergent genotypes but not between the more closely related genotypes. Such maps thus have little direct applications in breeding programmes that exploit intraspecific variation within the cultivated forms since they may not represent the true recombination distance map order of the cultivated genome. A genetic linkage map constructed from a cross within the cultivated gene pool especially in the frame work of targeting traits of breeding interest, would therefore be more desirable.

REFERENCES

Arumuganathan K and Earle ED. 1991. Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter **9:** 208-218.

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayres N and Cartinhour S. 2000. Diversity of microsatellite derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theoretical and Applied Genetics **100:** 713-722.

Croser JS, Ahmad F, Clarke HJ and Siddique KHM. 2003. Utilization of wild *Cicer* in chickpea improvement- progress, constraints and prospects. Australian Journal of Agricultural Research **54:** 429-444.

Ferguson ME, Burrow MD, Schulze SR, Bramel PJ, Paterson AH, Kresovich S and Mitchell S. 2004. Microsatellite identification and characterization in peanut. Theoretical and Applied Genetics **108:** 1064-1070.

Flandez-Galvez, Ford HR, Pang ECK and Taylor PWJ. 2003. An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged microsatellite site and resistant gene analog markers. Theoretical and Applied Genetics **106:** 1447-1456.

Gadish I and Zamir D. 1987. Differential zygotic abortion in an interspecific *Lycopersicum* cross. Genome **29:** 156-159.

Gaitan Solis E, Duque MC, Edward KJ and Tohme. 2002. Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization and cross species amplification in *Phaseolus spp.* Crop Science **42**: 2128-2136.

He C, Poysa V. and Yu K. 2003. Development and characterization of simple sequence repeats (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. Theoretical and Applied Genetics **106:** 363-373.

Huttel B, Winter P, Weisind K, Chromosome W, Weigand F and Kahl G. 1999. Sequence tagged microsatellite markers for chickpea (*Cicer arietinum* L). Genome **42:** 210-217.

Kazan K, Muehlbauer FJ, Weeden NF and Ladizinsky G. 1993. Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theoretical and Applied Genetics **86:** 417-426.

Kusmenoglu I. 1990. Ascochyta blight of chickpea: Inheritance and relationship to seed size, morphological traits and isozyme variation. M.Sc. Thesis, Washington State University, Pullman, USA.

Kusmenoglu I, Muehlbauer FJ and Kazan K. 1991. Inheritance of isozyme variation in ascochyta blight resistant chickpea lines. Crop Science **32:** 121-127.

Lichtenzveig J, Scheuring C, Dodge J, Abbo S and Zhang HB. 2005. Construction of BAC and BIBAC libraries and their application for generation of chickpea (*Cicer arietinum* L.) map. Theoretical and Applied Genetics **110:** 492-510.

Lyttle TW. 1991. Segregation distorters. Annual Review of Genetics **25:** 511-557.

Mahendra Pal, Pandia R S, Kumar J and Singh B. 1999. Genetics of resistance to Ascochyta blight in chickpea. Indian Phytopathology **52:** 403-407.

Michelmore RW, Paran I and 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. Proceedings of Natural Academy of Sciences USA **88:** 9828-9832.

Rakshit S, Winter P, Tekeoglu M, JuarezMunoz J, Pfaff T, BenkoIseppon AM, Muehlbauer FJ and Kahl G. 2003. DAF marker tightly linked to a major locus for Ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Euphytica **132:** 23-30.

Saghai Maroof HA, Soliman KM, Jorgensen AR, and Allard RW. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of Natural Academy of Sciences USA **81:** 8014-8018.

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ and Muehlbauer FJ. 2000. Identification and mapping of QTLs conferring resistance to Ascochyta blight in chickpea. Crop Science **40:** 1606-1612.

Sethy NK, Shokeen B, Edwards KJ and Bhatia S**.** 2006. Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L). Theoretical and Applied Genetics **112:** 1416-1428.

Sharma YR, Singh G and Kaur L. 1995. A rapid technique for Ascochyta blight resistance in chickpea. International Chickpea and Pigeonpea Newsletter **2:** 34-35.

Simon CJ and Muehlbauer FJ. 1997. Construction of chickpea linkage map and its comparison with maps of pea and lentil. Journal of Heredity **88:** 115-119.

Spurthi NN, Hongyan Z, Varghese N, Datta S, Choi H, Horres R, Jungling R, Singh J, Kishor PBK, Sivaramkrishnan S, Hoisington DA, Kahl G, Winter P, Cook DR, Varshney RK. 2010. Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. Theoretical and Applied Genetics **120:** 1415–1441.

Udupa SM and Baum M. 2003. Genetic dissection of pathotype specific resistance to ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theoratical and Applied Genetics **106:** 1196-1202.

Udupa SM, Robertson LD, Weigand F, Baum M and Kahl G. 1999. Allelic variation at $(TAA)_n$ microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. Theoretical and Applied Genetics **97:** 299-307.

Voorripps R E. 2002. Map Chart: Software for the graphical presentation of linkage maps and QTLs. Journal of Heredity **93:** 77-78.

Winter P, Benkoiseppon A M, Huttel B, Ratnaparkhe M, Tullu A, Sonnante G, Pfaff T, Tekeoglu M, Santra D, Sant V J, Rajesh P N, Kahl G and Mubhlbauer F J. 2000. A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for fusarium wilt races 4 and 5. Theoretical and Applied Genetics **101:** 1155-1163.

Winter P, Ptaff T, Udupa SM, Huttel B, Sharma PC, Sahim S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ and Kahl G. 1999. Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Molecular Journal of General Genetics **262:** 90-101.

Zamir D and Tadmor Y. 1986. Unequal segregation of nuclear genes in plants. Botanical Gazzette **147:** 355-358.