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Short Communication

Resistance to race 1 of *Fusarium* wilt of chickpea (*Cicer arietinum* L.): Genetics and mapping of resistance genes in an intraspecific cross

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Abstract

***Fusarium* wilt causes 10% annual loss in India. Studies on F_1, F_2, F_3 and F_{10} recombinant inbred line populations (RILs) of chickpea cultivars ICCV2 (resistant) and JG 62 (susceptible) against *Fusarium* race 1 suggested that resistance in this cross is governed by two recessive genes h_1 and h_2. The linkage map constructed using 126 F_{10} RILs comprised of 49 molecular and 7 phenotypic markers with nine linkage groups. The h_1 gene governing *Fusarium* resistance was linked to RMMFP1 and TA59 markers. TA59 marker assigned this locus to cluster of *Fusarium* resistance genes on linkage group 2 of the interspecific genetic map.**

Key words: Chickpea, *Fusarium* wilt resistance, Genetic linkage map, Molecular markers

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world. High yield potential in chickpea is not realized (Patil et al. 2014) because of constraints such as drought and biotic stresses. About 10% annual losses in India occur due to vascular disease *Fusarium* wilt caused by the ascomycete *Fusarium oxysporum* f.sp. *ciceri.* The present paper attempts to (1) determine the genetics of resistance to race 1 of *Fusarium* in chickpea in the cross of ICCV2 x JG 62 and (2) to generate elite DNA markers to tag the *Fusarium* resistance gene in intraspecific cross to aid in marker assisted selection.

F_1, F_2, F3 and 126 F_{10} RILs of the *Fusarium* wilt resistant cultivar ICCV2 and susceptible cultivar JG62 were studied along with the checks C104, K850 (Late wilting checks) and WR315 (resistant check) during rabi 1999 in *Fusarium* wilt sick plots at ICRISAT with a spacing of 60 x 20 cm in conserved soil moisture. After JG62 started wilting, disease scoring was done after every 3 days for one month and then after every 10 days. Plants with characteristic vascular clotting were recorded as dead due to disease. 126 F_{10} RILs were tested again during rabi 2000 in wilt sick plots.

Standard procedures were used to isolate genomic DNA, PCR, electrophoresis and sequencing (Sambrook et al. 1992). Chi-square (χ^2) values for goodness of fit were calculated and compared with table values for appropriate degrees of freedom (n-1). MAPMAKER V2.0 (Lander et al. 1987) was employed for linkage analysis. Loci were first divided into linkage groups at a LOD-score of 3 by two-point analysis using the 'group' command. Marker order in linkage groups was determined with the 'try' command of the program, and confirmed by multipoint analysis applying the 'ripple' function. Final map distances were calculated applying the 'Kosambi' function provided by the program.

All the JG 62 plants wilted by 27 DAS while only 11% of ICCV 2 plants wilted at 80 DAS and all the F_1

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plants wilted between 27-34 DAS and thus resistance to *Fusarium* wilt is recessive. 206 F2 plants studied gave a good fit to 15 S : 1 R and the susceptible plants (189 plants) when grouped based on the time of wilting gave a good fit to 9 (Early wilters, EW; 111 plants) : 6 (34 to 48 DAS; Late wilters, LW; 78 plants) : 1 (R) (χ^2 = 1.53, P = 0.3-0.5). These results suggest the presence of two recessive genes governing resistance in this cross. The 100 F_3 families (each comprising 20 individuals) segregated in a 7 S (47 families) : 8 S/R (50 families) : 1 R (3 families) (χ^2 = 1.9, P= 0.3-0.5) ratio wherein the 50 families segregated in to two equal groups segregating in 15 : 1 and 3: 1 ratios of susceptible and resistant families. Thus the 100 F_3 progenies gave a good fit to a ratio of 7:4:4:1 (χ^2 = 1.9, p = 0.5-0.7) expected from the segregation of two independently segregating recessive resistance genes supporting the results from F_2. 126 F_{10} generation RILs also gave a good fit to 1EW:1LW1:1LW2:1R) (χ^2 = 0.008; P = 0.99) as expected for two independently segregating genes. LW1 are those that wilted along with K850 (45DAS) and LW2 are those that wilted with C104 (25DAS).

The presence of any one of the three genes h_1 or h_2 or H_3 confer the late wilting phenotype, whereas a combination of two of the three loci is necessary for complete resistance (van Rheenen et al. 1992). The genotype of JG62 is $H_1H_1H_2H_2h_3h_3$ (Singh et al. 1987). F_1 generation being recessive excludes the presence of H3 in ICCV2 indicating that the genotype of ICCV2 is $h_1h_1h_2h_2h_3h_3$.

Parents were screened with 200 RAPD, 23 MP-PCR, 50 ISSR and 85 STMS markers and 765 combinations of RMMFPs (Random Mixing of Microsatellite Flanking Primers). A linkage map was constructed with 61 polymorphic markers (6 RAPD, 5 MP-PCR primers, 1 ISSR, 37 STMS markers, and 12 RMMFPs) and morphological characters (double podding, seed size, stem colour, seed coat colour, flower colour, and seed type already recorded for the same material (Cho et al. 2002) and *Fusarium* wilt resistant genes using 126 F_{10} RILs. Heterozygous loci were scored as missing data and not considered for mapping.

The map consisted of 9 linkage groups with 7 phenotypic markers and 49 molecular markers. The mapped loci covered 262.8 cM with an average distance of 4.7 cM between two markers (Fig. 1). All linkage groups of intraspecific map contain at least one STMS marker (underlined in Fig. 1) also present

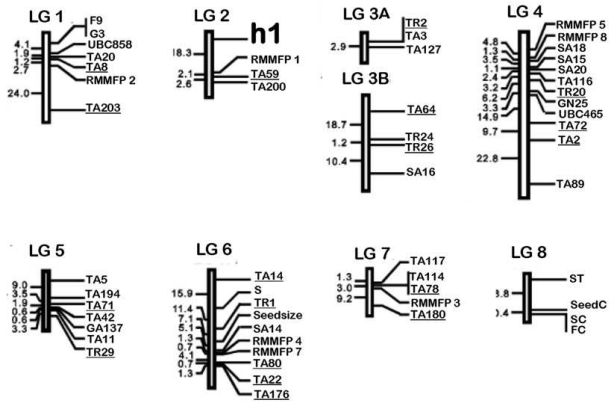

Fig. 1. Linkage map of intraspecific cross ICCV2 X JG62 of Chickpea. LG stands for Linkage groups. The underlined STMS markers are those which are present in interspecific map of Winter et al. (2000) and are used to assign the linkage group numbers. Agronomical traits mapped were: double podding (S) and seedsize on LG6, and seed type (ST), stem colour (SC), seed coat colour (SeedC) and flower colour (FC) on LG8. The *h1 Fusarium* resistance locus mapped to LG2 is presented in bold.

in the interspecific map of Winter et al. (2000). The order of markers in the two maps is similar, though map distances in some cases vary owing to the variations in recombination frequencies in different genomic regions of different populations.

The gene for double podding 'S' is flanked by STMS markers TR 1 and TA 14 in Linkage group 6 as reported by Cho et al. (2002) and seed size locus is flanked by MP-PCR marker Sa-14 and STMS TR1. The h_1 locus, was linked to markers RMMFP1 and TA59 that are located on the same side of the resistance gene. The h_2 locus remained unlinked. Loci for resistance to *Fusarium* wilt races 4 (Foc4) and 5 (Foc5) (Winter et al. 2000), QTLs for resistance to *Fusarium* wilt race 1 (Patil et al. 2014) are all mapped to linkage group 2 close to the STMS marker TA59 and is considered as hotspot of *Fusarium* wilt resistance genes.

STMS primers were mixed at random instead of using particular right and left primer to generate RMMFPs markers. Around 75% of RMMFPs were found to be co-dominant markers. Sequencing of three co-dominant RMMFP markers revealed that difference in the number of "TTA" repeat underlies the polymorphism. The region flanking the "TTA" repeat of RMMFP1 and RMMFP7 (that were amplified with common forward primer and different reverse primer)

RMMFP1

TTTCAACTTAAGACATGAAATTTGTTTTTAACGGTTCCTT(TTA)28AAAATTAAAATAACACATTACAATAGTAACCGACAA
TTTTTTTTGAATAATTATCTCCCTTCCTAAGGTAAAATATTAAAATAAAATAAAATGTCTGGATGTTACAGATTAGATCTTAAA
ATCTCAACACAGTAATTTGAGTTAATTTTTGAACTTAAAGTACCACATGCATTAAATAAT**ATAATTTAAGGACTAACATATTA
AACTTAG**

RMMFP2

TTTCAACTTAAGACATGAAATTTGTTTTTAACCGTTCCTT(TTA)27AAAATTAAAATATCACATTACAATAATAACCGACATT
AGAATAATTATCTCAATTCCTAATAAGGTAAAATATTAAAATAAAATAAAATGTCTGGATGITACAAAAGACACATTCAACCTA
GGAAAAAAAAACTCGGTTTCTATGCAATAATATTGATATATGATATTGAACAATAATTGAC**ATTATACAAATGAACTTTTGGA
ACGATC**

Fig. 2. Analysis of the sequences of RMMFP1 and RMMFP2. Black bold represents primer sequence and light grey color shows the regions that are similar between the markers on either side of the repeat

are 97.5% and 22.8% identical on either of the sides (Fig. 2) respectively, though they have been mapped to different linkage groups (Fig. 1). This data corroborates to that shown by Chouman et al. (2000) where chickpea microsatellites have shown to have conserved flanking sequences. With moderately dense intraspecific chickpea map (Thudi et al. 2011; Jaganathan et al. 2015) now available and by developing new techniques to generate more markers (like SNPs, RMMFPs), marker assisted selection and development of disease resistant cultivars can be achieved in near future.

Authors' contribution

Conceptualization of research (KA, JK, PW); Designing of the experiments (KA, JK, PW); Contribution of experimental materials (JK, PW); Execution of field/ lab experiments and data collection (KA); Analysis of data and interpretation (KA, JK, PW); Preparation of manuscript (KA, JK, PW).

Declaration

The authors declare no conflict of interest.

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