Segregation of *Fusarium* wilt susceptibility linked DNA marker in susceptible–resistance cross of chickpea

*Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *Ciceri* is a major obstacle to increase yield in chickpea, particularly in dry areas. Therefore, chickpea breeding aims at combining resistance against biotic stresses like *Fusarium* wilt in agronomically acceptable cultivars. Evaluation of a large number of host cultivars, segregating populations and breeding lines for resistance to specific races of the pathogen under field conditions is laborious, time consuming, costly and is affected by inoculum concentration and environmental conditions [Jimenez-Gasco et al 2001]. Maintaining uniform wilt-sick plot for evaluation is a major challenge. There is a lot of variation in intensity of pathogen within small experimental plots. Eight races of the pathogen have been reported [Kelly et al 1994], of which race 1 is more prevalent in all the chickpea growing regions of India and considered economically more important.

Marker assisted selection (MAS) based on the use of DNA markers linked closely to wilt resistance genes can be used to screen a large number of chickpea breeding lines for the presence of these genes and to pyramid them in agronomically superior genotypes. Genetic stocks for wilt resistance were identified in cultivated chickpea accessions and the genetic analysis suggest 2-3 major genes influencing resistance [Brinda and Ravikumar 2004; Upadhyaya et al 1983a; 1983b]. Mayer et al [1997] developed an allele specific associated primer (ASAP) CS-27700 linked to susceptibility to race 1 at H1 locus, which can be used in breeding. The selection on markers only without phenotypic evaluation was found to be efficient even for small population size in several crops [Stuber, 1999]. However, inconsistencies were observed in the effect of marker or QTL depending on the parental genotypes employed for pyramiding resistance. Minor genes were suggested to be involved in race specificity and resistance [Concibido, 2004]. Therefore, the markers-resistance/susceptibility linkage requires further validation across crosses, which are regularly employed in resistance breeding programme in India. In the present study an attempt has been made to study the segregation of the ASAP marker linked to H1 locus in F2 generation.

The highly susceptible genotype JG 62 was crossed to resistant genotype WR 315. The susceptible JG 62 has dominant alleles at both H1 and H2 loci; conversely WR 315 has recessive alleles. The F1 was grown to produce F2 population. The ASAP marker (Mayer et al 1997) is linked to H1 locus and susceptibility. The parents, F1 and 119 F2 plants were analyzed for the presence of ASAP marker.

Total genomic DNA was extracted from vegetative buds and leaf tissues of parents, F1 and F2 following the Rapid DNA extraction protocol of Edwards et al [Edwards, 1991] with required modifications. The quantity and quality of DNA was assessed and stored at 40°C for PCR amplification. The DNA was used for Polymerase Chain Reaction following the protocol of Mayer et al [1997]. The primers used for PCR amplification are CS-27F (AGCTGGTCGCGGTCAGAGGAAGA) and CS-27R (AGTGGTGCGGATGGGCCATGGTG). The amplicons were separated on 2% agarose gel at 5V/cm of gel for 2 hours, using 1x TAE buffer at pH 8.0. The gels were scored for the presence or absence of specific marker (CS-27700) linked to H1 locus and tested for single locus goodness of fit for 3:1 segregation.

The specific fragment linked to H1 was present in the susceptible parent JG 62 and the F1, while it was absent in the resistant genotype WR 315 (Fig 1). The ASAP marker is linked to susceptibility locus H1 and the susceptibility (H1) is dominant over resistance (h1). Among the 119 F2 segregants studied, presence of fragment CS-27700 ASAP marker linked to H1 was observed in 90 plants and it was absent in the remaining 29 plants (Fig 2; Table 1). The goodness of fit for 3:1 segregation ratio showed single locus segregation of the linked marker (Table 1). Thus supporting the contention that the parents differ at the locus linked to the dominant marker. Brinda and Ravikumar [2004] also revealed the similar results at F5 generation of a cross. The results clearly indicate that the ASAP marker linked to H1 shows normal segregation in the cross JG 62 x WR 315. Therefore the above cross is suitable to study the linkage between marker and wilt susceptibility for further utilization of the marker in breeding.

All the F2 plants have been advanced to succeeding generations using Single Seed Descent (SSD) method to develop RIL’s. The RIL’s will be used to study the linkage between marker and wilt reaction. Further, the parents segregate for both H1 and H2 loci. The molecular marker linked to H1 locus is already tagged and validated in this cross. Therefore the RIL’s developed from this cross will be suitable for identifying molecular markers linked to H1 locus of wilt resistance. We are advancing the plants through single seed descent method and they are in advanced stage. Attempts will be made to develop markers linked to H2 locus. The study is in progress.

### Table 1. Single locus goodness of fit for 3:1 ratio of presence and absence of Allele Specific Associated Primer (ASAP) marker (CS-27700) in chickpea progenies.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number of genotypes with fragment (+)</th>
<th>Number of genotypes without fragment (-)</th>
<th>X²</th>
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<tr>
<td>CS-27700</td>
<td>90</td>
<td>29</td>
<td>0.007</td>
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References


