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Research Article

# Marker assisted characterization of wilt resistance in productive Chickpea genotypes

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**Abstract**

Fusarium wilt caused by *Fusarium oxysporum* f. sp. c*iceris* is one of the most wide spread disease of chickpea (*Cicer arietinum)*. Studies indicated two major independent loci $H_1$ and $H_2$ determine the resistance to race-1 in chickpea. The dominant alleles at both $H_1$ and $H_2$ loci result in susceptible early wilting and recessive at any one ($h_1h_1H_2$_ or $H_1$_$h_2h_2$) produce susceptible late wilting and recessive at both the loci ($h_1h_1h_2h_2$) result in resistance. The primer pair CS-27F/ CS-27R, termed allele specific associated primer (ASAP) linked to the allele for susceptibility at $H_1$ locus and RAPD marker A07C linked to $H_2$ locus of susceptibility for fusarium wilt were reported. However, single marker is not sufficient to identify resistant and susceptible genotypes as there are two independent loci governing resistance. In this study fifteen diversified genotypes including different genetic backgrounds were subjected to screening with two markers. The CS $27_{700}$ marker linked to $H_1$ locus was absent in all other genotypes except JG 62 and Karikadle. Other marker A07C $_{417}$ linked to $H_2$ locus was present in all genotypes except WR 315 and GBS 964. This clearly indicated that both markers CS 27 and A 07C were present in susceptible (early wilting $H_1H_1H_2H_2$) genotypes i.e. JG 62 and Karikadle. On the contrary both the markers were absent in resistant ($h_1h_1h_2h_2$) genotypes i.e. WR 315 and GBS 964. Presence of any one marker in other genotypes (K850, Bheema, Vijay, A1, BG256, KAK-2, JG-11, BGD103, ICCV-2, ICC4958 and ICCV506) indicates susceptible but late wilter. Thus, the DNA markers enable to identify susceptible and resistant lines at very early stage of growth. These markers can be used in Marker Assisted Selection to speed up conventional breeding.

**Key words: DNA markers, Allele Specific Associated Primers, gene pyramiding, wilt resistance**

## Introduction

Chickpea (*Cicer arietinum*.L. 2n=2x=16) is a major pulse crop in the Indian subcontinent and the Mediterranean region. It ranks third in the world among pulses after pea and common bean with an area of 11.67m ha, production of 9.30 mt and productivity of about 800kg/ha (FAOSTAT Database http://faostat.fao.org/site/567/default.aspx 2007). One of the major constraints in realization of the full yield potential (4 tons/ha) of chickpea is the vascular wilt caused by the deuteromycetes fungal pathogen *Fusarium oxysporum* f.sp. *ciceri*. Annual yield losses due to wilt have been estimated at 10–90% (Singh and Reddy, 1991). Persistence of the pathogen in soil and its capacity to survive there for years even in the absence of host (Haware *et. al* 1996) renders its control difficult. Instead, the use of wilt resistant chickpea cultivars, when they are available, is the most effective and eco friendly method of managing the disease (Sharma *et.al*. 2005).

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However characterization of a large number of germplasm lines, varieties and breeding lines for resistance to specific races of the pathogen is tedious, laborious, expensive, time consuming and is affected by inoculum load and environmental conditions. Marker-assisted selection (MAS) based on the use of DNA markers linked closely to wilt resistance genes can be used to characterize large numbers of chickpea genotypes. Two major independent loci $H_1$ and $H_2$ determine the resistance to race1 in chickpea. In present study the primers, CS 27F/CS 27R $_{700\ bp}$ (ASAP) and A 07C $_{417\ bp}$ linked to the allele for susceptibility at $H_1$ and $H_2$ locus of fusarium wilt respectively were used to characterize the wilt resistance in different chickpea genotypes.

## Material and Methods

### Plant material

Fifteen diversified (Resistance, susceptible & late wilter of fusarium wilt; national and local checks), productive chickpea genotypes viz; JG62, Kari kadle, K850, Bheema, Vijay, $A_1$ (National check), BG256,

KAK-2, JG-11, BGD103, ICCV-2, ICC4958, ICCV506, WR315 and GBS964 were used to characterize against fusarium wilt.

**Genomic DNA Extraction:**

The total genomic DNA was extracted from vegetative buds and young leaves of all 15 individual genotypes by following CTAB extraction protocol with little modification (Doyle and Doyle, 1987). Quality and quantity of DNA was assessed by 1% Agarose gel electrophoresis. RNAse treated DNA samples were diluted to a working concentration of 20-25 ng/□l and stored for further PCR amplification.

**PCR amplification and Electrophoresis:**

Two primers viz; Allele Specific Associated Primer (ASAP) CS 27F/CS 27R and RAPD primer A 07C (both from Sigma Aldrich Ltd.) were used to amplify 15 diversified genotypes (Table. 1). PCR amplification was carried out using Master Thermal Cycler 5331-Eppendorf version 2.30, 31-09, Germany. PCR cycle conditions for ASAP were as follows: Initial denaturing step at 95$^{o}$C for 5 min followed by 40 cycles of 94$^{o}$C for 20 sec, 62 $^{o}$C for 1 min 72 $^{o}$C for 1 min and in the last cycle, primer extension at 72 $^{o}$C for 8 min was provided. Where as for A 07C: Initial denaturation was at 95 $^{o}$C for 5 min followed by 40 cycles of 94 $^{o}$C for 1 min, 36 $^{o}$C for 1 min, 72 $^{o}$C for 2 min and final 8 min extension at 72 $^{o}$C. The PCR products amplified with both the markers were resolved on 2% agarose gel containing ethidium bromide. The amplified products were visualized and photographed under UV light (Alpha Innotech Corporation, USA). The amplicons were analysed by AlphaImager version 4.1.02 software.

**Results and Discussion**

Legume breeders have taken interest in chickpea and have contributed many varieties performing well in the fields during last several decades. Progress has been done in chickpea research using several biotechnological tools in the last 10 years. However, research in cereals like wheat and rice has been accelerated due to the integration of conventional and modern biotechnology. Similar progress needs to be achieved in a legume like chickpea, where several diverse genotypes among the germplasm are unexploited. Those genotypes can be screened using molecular markers for their use in gene pyramiding and molecular breeding.

Earlier studies indicated genetics of wilt resistance against race 1 indicated three independent loci designated as $H_1$, $H_2$ and $H_3$ govern resistance to wilt (Singh *et. al.* 1987). However some studies indicated two major independent loci, $H_1$ and $H_2$ determine resistance to race 1 in chickpea (Brinda and Ravikumar, 2005; Upadhyaya *et. al*. 1983 a & b). The dominant alleles at both $H_1$ and $H_2$ loci result in susceptible early wilting and recessive at any one ($h_1h_1H_2$_ or $H_1$_$h_2h_2$) produce susceptible late wilting and recessive at both the loci ($h_1h_1h_2h_2$) result in resistance.

Development of molecular markers in chickpea has been relatively slow due to minimal polymorphism in its genome (Kazan and Muehlbauer, 1991; Mayer *et.al*.1997). However the markers linked to different wilt resistance genes were identified and mapped (Sharma and Muehlbauer, 2007).The first wilt resistance gene to be tagged in chickpea was $H_1$ with CS 27F/CS 27R (ASAP) (Mayer *et. al*. 1997), which amplifies a fragment of 700 bp linked to the allele for susceptibility. However, this marker alone is not sufficient to identify resistant and susceptible genotypes as there are two independent loci governing resistance to Fusarium wilt. So, RAPD Marker A $07C_{417}$ linked to $H_2$ locus of susceptibility for fusarium wilt was identified (Soregaon *et.al*. 2007). Using both the markers one can go for effective characterization of chickpea genotypes for fusarium wilt.

In the present experiment 15 diversified genotypes which include bold seeded varieties and breeding lines were subjected to screening with two markers.

The ASAP (CS 27F/CS 27R) marker linked to $H_1$ locus was absent in all other genotypes except JG62 and Karikadle (Fig.1A), where as other marker A 07C linked to $H_2$ locus was present in all genotypes except WR315 and GBS964 (Fig.1B). Presence of both the markers CS 27F/CS 27R and A 07C clearly indicated susceptible early wilting ($H_1H_1H_2H_2$) genotypes i.e. JG62 and Karikadle. On the contrary, absence of both the markers indicated resistant ($h_1h_1h_2h_2$) genotypes i.e. WR315 and GBS964. As remaining genotypes viz. K850, Bheema, Vijay, $A_1$, BG256, KAK-2, JG-11, BGD103, ICCV-2, ICC4958 and ICCV506 showed presence of any one marker, grouped as susceptible but late wilter (Table. 2).

Both the markers are linked to susceptibility alleles and susceptibility is dominant over resistance. Thus markers enable to identify susceptible and resistant lines at very early seedling stage of growth. Such of the lines showing presence of both or any one of the markers can be eliminated in the early generation. Conversely, lines without these DNA markers are the ones that carry resistant genes at the two major loci. These markers can be used in MAS to speed up conventional breeding. Ravikumar *et. al* (2007) also used these two markers to study the inheritance pattern of Fusarium wilt resistance alleles in different populations.

Except KAK-2 and ICCV-2 all other genotypes are '*Desi*' types, which are small and brown with 90% area under cultivation in India. KAK-2 and ICCV-2

are '*Kabuli*' types with bold and cream colored seeds grown in 10% area. Resistant genotypes identified using markers can be used in breeding with '*Kabuli*' type to develop "Bold seeded Fusarium wilt Resistant genotype"

Screening of the chickpea genotypes causing wilt resistance genes can be facilitated with marker assisted selection (MAS). Chickpea breeders are aiming to exploit MAS for resistance breeding. Efficiency of MAS, however, depends upon closeness of the marker to the gene.

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**Table.1. Sequences of ASAP and RAPD Markers employed in the study.**

| Sl. No | Primer Name | Sequence (5'- 3') |
|---|---|---|
| 1 | CS 27F | AGCTGGTCGCGGGTCAGAGGAAGA |
| 2 | CS 27R | AGTGGTCGCGATGGGGCCATGGTG |
| 3 | A 07C | GAAACGGGTGC |

**Table.2. Reaction of different diversified genotypes with two Markers**

| Sl .No | Genotypes | CS-27 | A 07C | Wilt Reaction |
|---|---|---|---|---|
| 1 | JG 62 | + | + | Susceptible (Early wilter) |
| 2 | Kari Kadle | + | + | |
| 3 | K850 | _ | + | Susceptible (Late wilter) |
| 4 | Bheema | _ | + | |
| 5 | Vijay | _ | + | |
| 6 | A1 | _ | + | |
| 7 | BG 256 | _ | + | |
| 8 | KAK-2 | _ | + | |
| 9 | JG-11 | _ | + | |
| 10 | BGD 103 | _ | + | |
| 11 | ICCV-2 | _ | + | |
| 12 | ICC 4958 | _ | + | |
| 13 | ICCV 506 | _ | + | |
| 14 | GBS 964 | _ | _ | Resistant |
| 15 | WR 315 | _ | _ | |

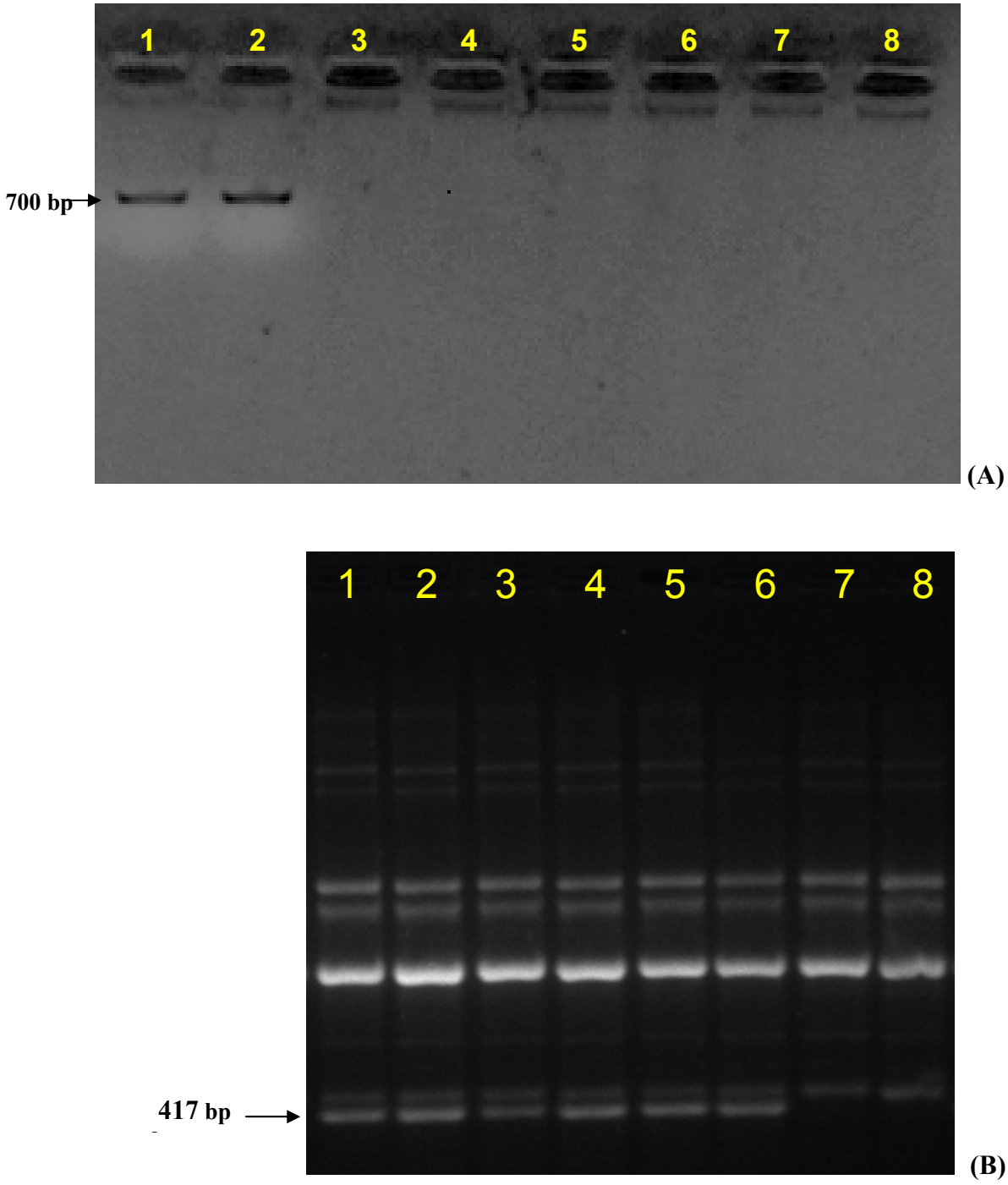


Fig. 1. Screening of DNA Markers (A) CS-27 and (B) A 07C across different diversified genotypes. 1-JG 62; 2-KariKadle; 3-K850; 4-Bheema; 5-JG11; 6-BGD103; 7-GBS964; 8-WR 315.