Characterization of *Fusarium* Wilt Resistance in Diverse Chickpea (*Cicer arietinum* L.) Genotypes using Established Molecular Markers

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Abstract

Chickpea cultivation is much affected due to wilt caused by *Fusarium oxysporum* f. sp. *ciceris* race 1. Two major loci H_1 and H_2 determine the disease reaction to race 1 of this pathogen in chickpea. Dominant alleles at both loci ($H_1\_H_2\_$) result in early wilting reaction, homozygous recessive at either of the loci ($H_1\_h_2h_2$ or $h_1h_1H_2\_$) results in late wilting reaction while homozygous recessive at both loci ($h_1h_1h_2h_2$) leads to resistance. An Allele Specific Associated Primer (ASAP), CS-27_{700} and an RAPD marker, A07C_{417} are linked to susceptibility at H_1 and H_2 loci, respectively. In the present study sixteen diverse genotypes from different genetic backgrounds were screened using the above two established molecular markers. The ASAP marker CS-27_{700} linked to H_1 locus was present in five genotypes viz., ICC-12236, Karikadli, ICC-12434, JG-62 and ICC-12429. The RAPD marker A07C_{417}, linked to H_2 locus, was present in all genotypes except in ICC-12249, WR 315 and ICC-12236. The absence of both the markers, CS-27_{700} and A07C_{417} ($h_1h_1h_2h_2$) indicated the resistant reaction in the genotypes ICC-12249 and WR-315. While presence of both the markers ($H_1H_1H_2H_2$) in Karikadli, ICC-12434, JG-62 and ICC-12429 indicated the early wilting reaction and presence of either of the markers ($H_1\_h_2h_2$ or $h_1h_1H_2\_$) in other genotypes (A-1, ICCV-2, ICC-96030, ICC-4958, ICC-96029, BG-256, K-850, ICC-12237, ICC-12252, ICC-12236) indicated late wilting reaction. Thus, these established molecular markers at DNA level facilitated easy and early identification of susceptible or resistant lines. Utilization of these markers in plant breeding will be complementary to the conventional breeding which can hasten up its progress.

1. Introduction

Chick pea (*Cicer arietinum* L.) with an area of 10 mha under cultivation, is second only to common bean (*Phaseolus vulgaris*) and third in production among the legumes. In spite of wide spread all over the world, its yield levels are being hampered to a great extent by wilt disease. Chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris* is a serious problem in almost all chickpea growing areas. The average annual losses to wilt have been estimated to the tune of 10 to 90% (Jimenez-Diaz et al., 1993; Cortes et al., 2000) under disease favourable conditions. The pathogen can survive in the soil years together even without its host (Haware et al., 1996). Because of the diverse, widespread, soil borne nature and capacity to survive in soil for many seasons without host made the pathogen very difficult to handle leading to heavy yield losses in chickpea. Hence the best strategy to manage this disease is to use the resistant cultivars (Sharma et al., 2005).

Development of wilt resistant varieties require phenotyping of large number of germplasm and breeding lines against specific races of pathogen at field level which is laborious, expensive and time consuming. Further such phenotyping using sick plots is likely to encounter problems like uneven distribution of inoculum and presence of other soil borne fungi. Therefore characterisation of wilt resistance using established DNA marker linked to wilt resistance genes is the best means of screening large number of genotypes. Two major loci H_1 and H_2 are reported to control the *Fusarium* wilt disease reaction (Upadhyaya et al., 1983; Brinda and Ravikumar, 2005). In the present study two primers CS-27_{700} and A07C_{417} linked to the two major loci H_1 and H_2 respectively were used to characterize the wilt resistance in diverse chickpea genotypes.

2. Materials and Methods

2.1. Plant material

Sixteen diverse genotypes viz., JG-62, WR-315, K-850, BG-256, Karikadli, ICCV-2, A-1, ICC-4958, ICC-12236, ICC-12237, ICC-96030, ICC-12234, ICC-12249, ICC-12429, ICC-96029 and ICC-12252 were used for characterisation of *Fusarium* wilt resistance using the established molecular markers.

2.2. DNA extraction

The sixteen genotypes were grown in small plastic pots separately and DNA was extracted from the growing bud of each genotype by following rapid method of isolation (Edwards et al., 1991) with some modifications. The quality and concentration of the extracted DNA was assessed by gel electrophoresis using 0.8% agarose.

2.3. Polymerase chain reaction

Purified genomic DNA extracts (30 ng) of the sixteen genotypes were used as template DNA. The two primers viz., CS-27_{700} an Allele Specific Associated Primer (ASAP) and an RAPD primer A07C_{417} (both custom synthesized at sigma, Bangalore) were used to amplify the specific fragments of DNA of the 16 diverse genotypes (Table 1). Polymerase chain reaction was carried out using Thermal Cycler Eppendorf Master cycler gradient. The amplification of the DNA fragment linked to ASAP primer was done by the method proposed by Mayer et al. (1997) and the conditions were as follows: Initial Denaturation at 95ºC for 5 minutes followed by 40 cycles of Denaturation, Annealing and Extension at 94°C for 20 seconds, 62ºC for 1 minute and 72ºC for 1 minute, respectively. The final extension was calibrated for 8 minutes at 72ºC. While DNA fragment linked to RAPD primer was amplified following the slightly modified conditions provided by Williams et al. (1990) where, Initial Denaturation was done for 5 minutes at 95ºC followed by 40 cycles of Denaturation, Annealing and Extension at 94ºC for 1 minute, 36ºC for 1 minute and 72ºC for 2 minutes, respectively with final Extension at 72ºC for 8 minutes. The amplified PCR products of RAPD and ASAP markers were separated on 1.2 and 2% gels, respectively. The gels were photographed using the gel documentation system (Uvitech, Cambridge, England).

Table 1: Sequence details of the ASAP and RAPD markers used in the study

Primer		Sequence (5'-3')
CS-27	(F)	5'AGCTGGTCGCGGGTCAGAGGAAGA3'
CS-27	(R)	5'AGTGGTCGCGATGGGGCCATGGTG3'
A07C		5'GAAACGGGTGC3'

3. Results and Discussion

Many high yielding varieties were developed in chickpea which are performing well all over the world but, there are very few varieties which are resistant to *Fusarium* wilt with high yield potential. One of the reasons for this draw back could be the unexploitation of resistant sources in the available germplasm and breeding lines because of the difficulties in phenotyping them against different wilt causing races of the pathogen at field level. Molecular markers can be utilised for such screening and development of resistant lines. Genetic studies (Upadhyaya et al., 1983; Brinda and Ravikumar, 2005), suggested that two independent loci govern resistance to race 1 of the *Fusarium* wilt causing pathogen. Dominant alleles at both loci ($H_1\_H_2\_$) result in early wilting reaction, homozygous recessive at either of the loci ($H_1\_h_1h_2$ *or* $h_1h_1H_2\_$) result in late wilting reaction while homozygous recessive at both loci ($h_1h_1h_2h_2$) result in complete resistance. Mayer et al. (1997) for the first time tagged the wilt resistance locus, H_1 with an Allele Specific Associated Primer (ASAP), CS-27_{700} which amplifies a DNA fragment of 700 base pairs linked to the susceptible reaction. Later, Soregaon et al. (2007) identified an RAPD marker, A07C linked to H_2 locus which amplifies 417 base pair DNA fragment linked to susceptible reaction. These two established DNA markers can be effectively utilized for characterization of *Fusarium* wilt reaction in any chickpea genotype.

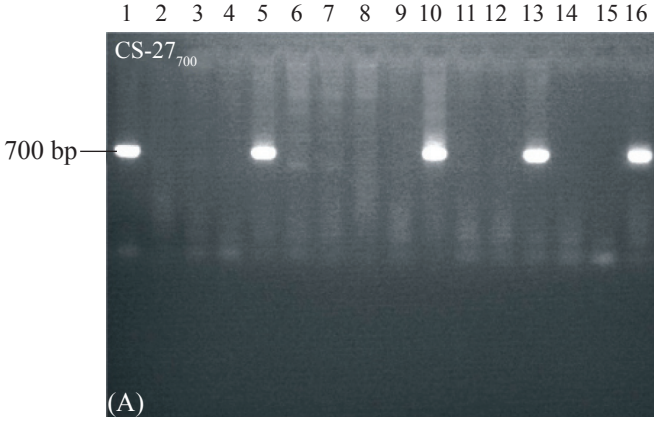
In the present study sixteen diverse genotypes which include both *kabuli* and *desi* types along with national check A-1 were genotyped using the two markers. The ASAP marker, CS-27_{700} linked to H_1 locus was present in five genotypes viz., ICC-12236, Karikadli, ICC-12434, JG-62 and ICC-12429 (Plate 1A). The RAPD marker, A07C_{417} linked to H_2 locus was present in all genotypes except in ICC-12249, WR-315 and ICC-12236 (Plate 1B). The absence of both the markers, CS-27_{700} and A07C_{417} ($h_1h_1h_2h_2$) indicated the complete resistance in the genotypes, ICC-12249 and WR-315, while presence of both the markers ($H_1H_1H_2H_2$) in Karikadli, ICC-12434, JG-62 and ICC-12429 indicated the early wilting reaction and presence of either of the markers ($H_1\_h_2h_2$ or $h_1h_1H_2\_$) in other genotypes (A-1, ICCV-2, ICC-96030, ICC-4958, ICC-96029, BG-256, K-850, ICC-12237, ICC-12252, ICC-12236) indicated late wilting reaction (Table 2).

Both the markers were used simultaneously to study the inheritance pattern of *Fusarium* wilt reaction in Back Cross populations (Ravikumar et al., 2007) and Recombinant Inbred Lines (Ratna Babu and Ravikumar, 2010) of cross JG-62 (highly susceptible)×WR-315 (completely resistant). Both the markers are linked to susceptible reaction which is

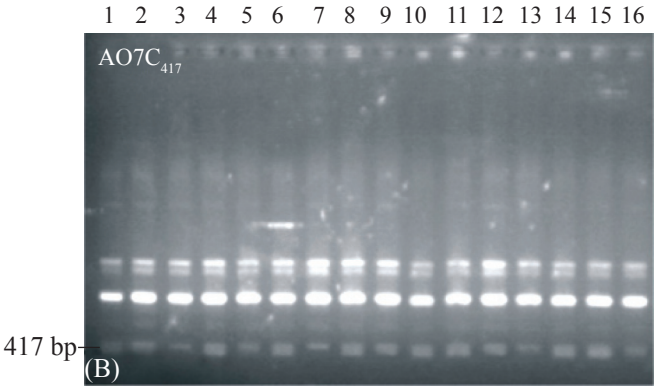
Table 2: Molecular marker reaction in diverse genotypes

Sl. no.	Genotype	CS-27$_{700}$	A07C$_{417}$	Disease reaction
1.	ICC-12249	-	-	Completely
2.	WR-315	-	-	resistant
3.	A-1	-	+	Late wilting
4.	ICCV-2	-	+	
5.	ICC-96030	-	+	
6.	ICC-4958	-	+	
7.	ICC-96029	-	+	
8.	BG-256	-	+	
9.	K-850	-	+	
10.	ICC-12237	-	+	
11.	ICC-12252	-	+	
12.	ICC-12236	+	-	
13.	Karikadle	+	+	Early wilting
14.	ICC-12434	+	+	
15.	JG-62	+	+	
16.	ICC-12429	+	+	

dominant over resistant disease reaction. Thus the above two established markers facilitate in identifying and differentiating the susceptible and resistant lines at seedling stage itself. The lines which show the amplification for both or either of the markers are susceptible to the wilt and can be screened out (Plate 1). While the genotypes which could not amplify both the fragments linked to these two markers are completely resistant and can be utilized in developing *Fusarium* wilt resistant varieties. Further, these markers can also be utilized in other programmes like gene pyramiding and other molecular breeding programmes.



1. JG-62 2. WR-315 3. ICC-96029 4. ICC-12252
5. ICC-12429 6. A-1 7. K-850 8. ICC-12237
9. ICC-96030 10. Karikadle 11. ICCV-2 12. BG-256
13. ICC-12236 14. ICC-4958 15. ICC-12249 16. ICC-12434



1. JG-62 2. Karikadle 3. WR-315 4. ICC-12252
5. ICC-12429 6. A-1 7. ICC-12249 8. K-850
9. ICC-12237 10. ICCV-2 11. ICC-12434 12. BG-256
13. ICC-12236 14. ICC-96030 15. ICC-96029 16. ICC-4958

Plate 1: Amplification of DNA markers linked to H_1(CS-27$_{700}$) and H_2 (A07C) loci of wilt resistance in chickpea genotypes

4. Conclusion

The difficulties in phenotyping of different genotypes, germplasm and breeding lines for *Fusarium* wilt at field level using sick plots can be avoided by using the established DNA markers at seedling stage itself. Further the marker based selection of line is highly useful in breeding wilt resistant varieties and will be complementary to the conventional breeding programmes.

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