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Research Article

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Validation of Molecular Markers Linked to *Fusarium* Wilt Resistance in Chickpea Genotypes

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ABSTRACT

Chickpea (Cicer arietinum L.) is third most important legume crop called as poor men's meat due to good source of protein. However, the productivity of chickpea is limited due to fungal disease wilt, caused by Fusarium oxysporum f. sp. ciceris. The resistance breeding aimed to developed disease resistance variety and Marker Assisted Selection can gear up conventional breeding approaches. The present research work was appointed for validation of different markers viz., CS-27, UBC-170, CS-27A, OP-U17-1, UBC-825, TA-59, TA-96 and TR-19 previously reported to be linked to wilt disease either susceptibility or resistance in chickpea. Among susceptibility linked markers CS-27$_{700}$ and UBC-825$_{1200}$ validated for all susceptible genotypes, whereas CS-27A$_{700}$ was not validated only in susceptible genotypes, GG-4 while OP-U17-1$_{1014}$ was not validated in GG-4. The resistance linked markers UBC-170$_{500}$ was validated in all resistant genotypes. The markers TA-59$_{258}$ and TA-96$_{265}$ did not give specific amplicon in resistance genotypes GG-1, GG-2 and annigeri, GG-1, respectively while TR-19$_{227}$ did not amplify specific product in resistance genotypes annigeri, GG-1 and GG-2. Out of eight markers studied, three markers CS-27$_{700}$, UBC-825$_{1200}$ and UBC-170$_{500}$ were validated in fifteen diverse chickpea genotypes and found to be consistence for marker assisted characterization. These markers could be utilized in future for maker assisted breeding for wilt resistance in chickpea.

Key words: *Cicer arietinum L., Fusarium wilt, Molecular markers, Resistance, Susceptibility.*

INTRODUCTION

Chickpea is the third most important pulse crop in the world, after dry bean and pea and ranks first in Indian subcontinent[1]. It is a good source of protein in developing countries and known as poor man's meat[2], though its production is hampered by abiotic and biotic stresses. Major biotic stress which limits chickpea production worldwide is the *Fusarium* wilt caused by fungus *Fusarium oxysporum* f. sp. *ciceris*.

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The wilt disease was first reported from India by Butler in 1918[3] which cause yield loss up to 90 % annually under epidemic condition[4]. The pathogen penetrates the vascular bundles of roots and reduces water uptake and ultimately plant is wilted to die[5,6]. Disease control through crop rotation is not effective because pathogen can survive without host in soil up to several years, further use of fungicide leads to environmental pollution[7]. Biocontrol agents and cultivation of resistant cultivars can effectively provide eco-friendly control of the disease[8]. Vertical resistance gene of resistant genotypes could be used for pyramiding in single cultivar to develop horizontal resistance that may give durable resistance[9]. The development of wilt resistant variety through breeding is a major objective for breeders in present days. But, screening through conventional methods has limitations of time and poor in consistency[10].

Linkage analysis of wilt resistance genes was done by different researchers. They showed that the markers and the resistance genes were clustered on the same linkage group *i.e.* linkage group 2. The validation of different linked molecular markers *viz.,* random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), sequence characterized amplified regions (SCAR) and simple sequence repeats (SSR) was done on mapping population only. This information could be helpful for the identification of resistant genotypes and for effective utilization in marker-assisted selection (MAS) if it is validated on different genetic background. RAPD marker $CS\text{-}27_{700}$ was located 7.2, 5.2 and 9.2 cM away from resistance gene *foc-1*, *foc-4* and *foc-5*, respectively[16]. The SCAR marker $CS\text{-}27A_{700}$ linked to 4.9, 1.5, 0.5, 3.3 and 2.9 cM away from *foc-1*, *foc-2*, *foc-3*, *foc-4* and *foc-5*, respectively, which was developed from RAPD marker CS-27[16]. Benko-Issepon[15] reported susceptibility linked DAF marker $OP\text{-}U17\text{-}1_{1014}$ which was linked 4.1 cM to *foc-4*. The $UBC\text{-}825_{1200}$ primer of ISSR marker was developed by modifying 3' anchoring region of UBC-855 was located 5.0 cM from *foc-4*[11,12]. RAPD primer $UBC\text{-}170_{550}$ was located 7.0, 9.0 and 2.5 cM from *foc-1*, *foc-4* and *foc-5* resistance gene, respectively[17]. Winter[13] characterized and mapped 120 STMS marker on the chickpea genome map. They mapped resistance linked markers TA-59, TA-96 and TR-19 on same linkage group on which gene for *Foc-4* resistance was located. The specific amplicon size of TA-59, TA-96 and TR-19 associated with disease resistance was 258, 265 and 227 bp, respectively. Previously reported different markers are available, but have not been validated in different genetic background. So, in present study different eight molecular markers reported to be linked to wilt resistance were validated on fifteen diverse chickpea genotypes.

MATERIALS AND METHODS

Total DNA was extracted from the dry seed material of fifteen diverse chickpea genotypes (Table 1) using DNA extraction method described by Chakraborty[18] with minor modifications. Further qualitative and quantitative analysis was done using 0.8 per cent agarose gel electrophoresis and Nanodrop spectrophotometer, respectively. The PCR amplification for each molecular marker was done as per procedure given by respective researchers (Table 2). PCR amplified products of RAPD, SCAR, DAF and ISSR primers were separated on 1.5 per cent whereas PCR amplified product of STMS primers was separated on 3.0 per cent agarose gel containing 0.5 μg/ml Et-Br at 60 V with standard DNA marker. The separated bands were visualized under UV light and photographed under Bio-Rad Gel documentation system. The band size was determined by using AlphaEaseFC 4.0 software with reference to standard molecular marker. The presence of specific resistance or susceptible linked allele reported by earlier workers was used to validate molecular markers in chickpea genotypes against *Fusarium oxysporum* f. sp. *ciceris* resistance.

RESULT AND DISCUSSION

The advance molecular marker assisted characterization for disease resistance will boost up conventional plant breeding strategies of disease resistance. The RAPD marker CS-27 gave amplicon of 700bp in susceptible (cadu-54, L-550, C-104, shanho, JG-62 and GG-4) and intermediate/susceptible (chaffa) genotypes (Fig. 1A). But, specific band was absent in resistant (K-850, WR-315, BG-212, surutato-77 and JG74), tolerant (GG-1 and GG-2) and intermediate/resistant (annigeri) genotypes (Table 3). The SCAR marker CS-27A amplified a single band of 700bp in susceptible genotypes but absent in susceptible genotypes, GG-4 and intermediate/susceptible genotype (Table 3). The specific band was not observed in resistant, tolerant and intermediate/resistant genotypes. The DAF marker OP-U17-1 produced a fragment of 1014 bp in all susceptible genotypes except GG-4. The specific band was not found in resistant, tolerant and intermediate/resistant genotypes (Table 3). The ISSR marker UBC-825 amplified a fragment of 1200 bp in susceptible, tolerant (GG-1) and also in intermediate/susceptible genotypes (Fig. 1B). The specific band was not produced in resistant, tolerant (GG-2) and intermediate/resistant genotypes (Table 3). The RAPD marker UBC-170 resulted in 500 bp amplicon in resistant, tolerant and also in intermediate/resistant genotypes (Fig. 1C). The particular fragment was not seen in susceptible and intermediate/susceptible genotypes (Table 3). The STMS marker TA-59 amplified a total four alleles in studied genotypes, the size of alleles were 245, 258, 270 and 282 bp. The allele of 258 bp was observed in five resistant and also in intermediate/resistant genotypes. This allele was absent in susceptible, intermediate/susceptible and tolerant genotypes. The marker TA-96 amplified a total three alleles with size of 240, 250 and 265 bp (Fig. 1D). The allele of 265 bp was present in four resistant genotypes except BG-212. This allele was absent in susceptible, tolerant, intermediate/susceptible and intermediate/resistant genotypes. The marker TR-19 amplified five alleles of 215, 227, 245, 255 and 290 bp. The allele of 227 bp was found in five resistant genotypes, but absent in tolerant and intermediate/resistant genotypes. This allele was not observed in susceptible and in intermediate/susceptible genotypes, except L-550 (Table 3).

These markers have been validated by different groups of scientists in susceptible and resistant genotypes. Mayer[16] developed a set of F_6 recombinant inbred lines (RILs) of C-104 (susceptible) × WR-315 (resistant). They screened the RAPD primers for *Fusarium* wilt resistance and found that CS-27 marker associated with *Fusarium* wilt resistance. Padaliya[19] also validated the CS-27_{700} in susceptible genotypes (JG-62 and GG-4) with fragment size 700 bp. RAPD marker CS-27 also gave the amplification of 700 bp in susceptible genotypes used in present research. Mayer[16] further cloned and sequenced DNA fragment of CS-27 and developed SCAR marker CS-27A. This primer amplified a 700 bp fragment linked to the allele for susceptibility of *Fusarium* wilt. Rani[20] used CS-27A for validation and observed the amplification of 700bp in susceptible genotype (PKG024). In present study also the 700bp amplicon was also observed in susceptible genotypes through SCAR marker CS-27A. Benko-Issepon[15] used OP-U17-1_{1014} for characterization of F_7 derived F_8 RILs. They reported OP-U17-1 gave amplification of 1014 bp band in susceptible RILs. DAF markers OP-U17-1 was also reported in correlation with amplification of 1014 bp in susceptible genotypes used in this study. Padaliya[19] reported that UBC-825_{1200} amplified in susceptible (JG-62 and GG-4) and intermediate/susceptible (chaffa) genotypes. Marker UBC-825 also gave amplification of 1200 bp in susceptible genotype PKG024[20]. ISSR marker UBC-825 having 1200 bp amplification was also validated using susceptible/resistant genotypes in present research. UBC-170 marker gave the amplification of 500 bp in resistant genotypes[19], the same result is obtained in present study also. Three STMS markers *viz.,*

TA-59, TA-96 and TR-19 were validated by Padaliya[19] and observed that TA-59, TA-96 and TR-19 amplified 258 bp, 265 bp and 227 bp allele respectively in resistant genotypes (WR-315 and ICCV-2). These TA-59, TA-96 and TR-19 STMS markers were also validated on different fifteen chickpea genotypes.

Table 1: Chickpea (*Cicer arietinum* L.) genotypes used for validation of wilt linked molecular marker

S. No.	Genotypes	Reaction to wilt disease	Alternative identifiers/Pedigree
1	Cadu-54	Susceptible	ICC 7528
2	L-550	Susceptible	C104×NP12
3	C-104	Susceptible	ICC 6331
4	Shanho	Susceptible	ICC 7545
5	JG-62	Susceptible	ICC 4951
6	Chaffa	Intermediate/Susceptible	ICC 4934
7	Annigeri	Intermediate/Resistant	ICC 4918
8	K-850	Resistant	ICC 5003
9	WR-315	Resistant	ICC 8933
10	BG-212	Resistant	ICC 11088
11	Surutato-77	Resistant	(L-186×Macarena)×Macarena
12	JG-74	Resistant	ICC 6098
13	GG-1	Tolerant	GCP 2×ICCV 2
14	GG-2	Tolerant	JG 1258×BDN 9-3
15	GG-4	Susceptible	ICCL 84224×Annigeri

Table 2: The *Foc* linked markers used for validation

S.No.	Primer name	Expected fragment size	Linked to	Primer sequence (5'→ 3')	Source
1	CS-27	700 bp	S	AGT GGT CGC G (10)	Mayer *et al.* (1997)
2	CS-27A	700 bp	S	(f) ACC TGG TCG CGG GTC AGA GGA AGA (24) (r) AGT GGT CGC GAT GGG GCC ATG GTG (24)	Mayer *et al.* (1997)
3	OP-U17-1	1014 bp	S	ACC TGG GGA G (10)	Benko-Iseppon *et al.* (2003)
4	UBC-825	1200 bp	S	ACA CAC ACA CAC ACT (15)	Ratnaparkhe *et al.* (1998b)
5	UBC-170	500 bp	R	ATC TCT CCT G (10)	Tullu *et al.* (1998)
6	TA- 59	258 bp	R	(f) ATC TAA AGA GAA ATC AAA ATT GTC GAA (27) (r) GCA AAT GTG AAG CAT GTA TAG ATA AAG (27)	Winter *et al.* (1999)
7	TA- 96	265 bp	R	(f) TGT TTT GGA GAA GAG TGA TTC (21) (r) TGT GCA TGC AAA TTC TTA CT (20)	
8	TR-19	227 bp	R	(f) TCA GTA TCA CGT GTA ATT CGT (21) (r) CAT GAA CAT CAA GTT CTC CA (20)	

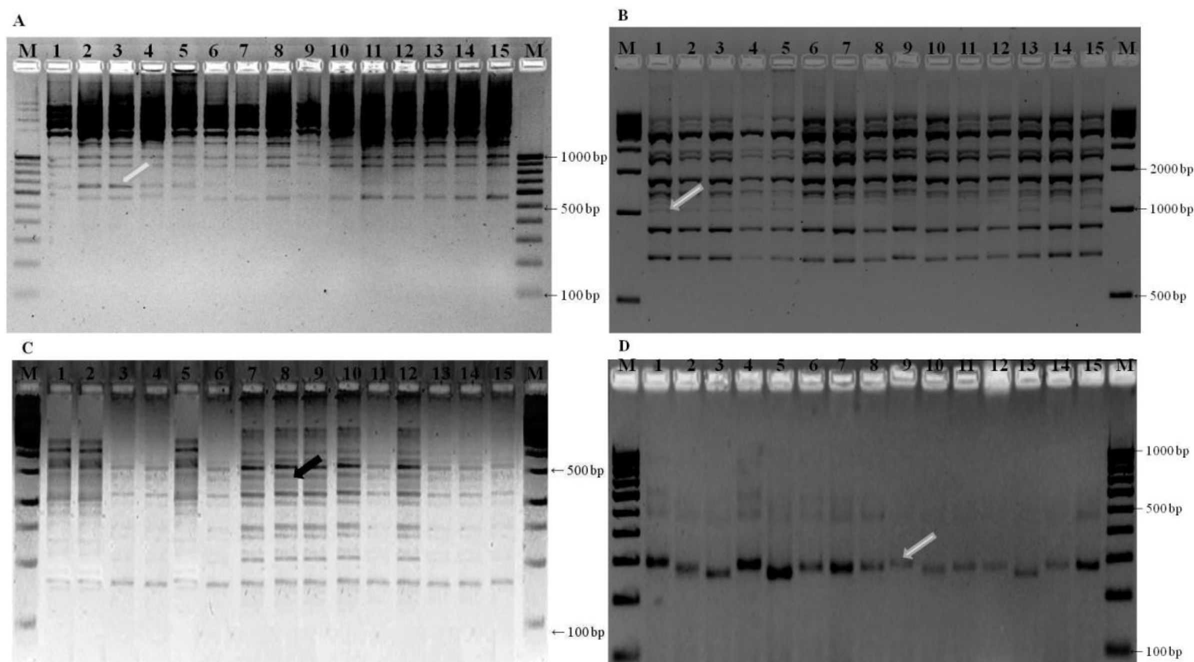
S= Susceptible, R=Resistant

Table 3: Reaction of fifteen diverse chickpea genotypes with eight molecular markers reported to be linked to wilt disease (*Foc*)

S. No.	Primers	Genotypes														
		Susceptible						Intermediate	Resistant						Tolerant	
		Cadu-54	L-550	C-104	Shanho	JG-62	GG-4	Chaffa (S)	Annigeri (R)	K-850	WR-315	BG-212	Surutato-77	JG-74	GG-1	GG-2
1	CS-27	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
2	UBC-170	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
3	CS-27A	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
4	OP-U17-1	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-
5	UBC-825	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-
6	TA-59	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-
7	TA-96	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+
8	TR-19	-	+	-	-	+	-	-	-	+	+	+	+	+	-	-

+/- represent present/absent of reported amplicon

Fig. 1



Amplification of alleles by marker in diverse chickpea genotypes. **A:** CS-27; **B:** UBC-825; **C:** UBC-170 and **D:** TA-96

Lanes: M: DNA ladder (100bp), 1:Cadu-54, 2:L-550, 3:C-104, 4:Shanho, 5:JG-62, 6:Chaffa, 7:Annigeri, 8:K-850, 9:WR-315, 10:BG-212, 11:Surutato-77, 12:JG-74, 13:GG-1, 14:GG-2, 15:GG-4.

(The linked fragment has been marked with arrow)

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CONCLUSION

The validated wilt linked markers could effectively be utilized in disease resistance breeding to enhance the selection strategy of parents and progeny. The phenotypic selection for the resistant gene can be done only in homozygous condition, because of its recessive nature, but using molecular markers, it can be identified in heterozygous condition also. This would reduce time in recessive gene backcrossing. Efficiency of MAS, however, depends on the linkage of marker to specific gene. Three markers CS-27, UBC-825 and UBC-170 were effectively validated on diverse fifteen chickpea genotypes having different genetic background. This marker will effectively be utilized in marker assisted breeding for wilt resistance in future.

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