

DETECTION OF QTLs FOR BLIGHT RESISTANCE IN CHICKPEA GENOTYPES WITH DNA BASED MARKERS

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Abstract

Chickpea blight caused by *Ascochyta rabiei* is one of the major diseases in Pakistan and other chickpea growing regions of the world. Different QTLs for resistance against the fungus have been identified in both inter and intraspecific crosses and are located on Linkage Group (LG) 2, 4a, 4b and 6. The objective of the present study was to screen local genotypes for the QTLs involved in resistance against blight. For this screening SSR, SCAR, ISSR and RAPD techniques have been tried to detect the reported QTLs in 21 mutants/local genotypes. The screening for *Ascochyta* blight of these genotypes has been carried out in the *Ascochyta* blight screening nursery for three years at NIAB. The results revealed that QTL linked with STMS, RAPD and ISSR markers on LG2, 4a and 6 are not involved in conferring resistance in local genotypes. Another important QTL on LG 4b is saturated with RAPD, SCAR and STMS markers and our studies of local genotypes showed strong linkage of STMS and SCAR markers with blight resistance on this linkage group.

Introduction

Ascochyta blight, caused by *Ascochyta rabiei* [Pass] Labr. is a widespread foliar disease that causes extensive crop losses in most regions of the world where the crop is commonly grown. Occurrence and severity of *Ascochyta* blight is weather dependent and environmental conditions favorable to the chickpea crop (>350 mm annual rainfall, 23-25°C) also favor the disease. *Ascochyta* blight infections may cause 100% yield loss (Nene & Reddy, 1987; Jimenez-Diaz *et al.*, 1993; Acikgoz *et al.*, 1994). The average yield (550-650kg/ha) of chickpea is much lower in Pakistan, due to various abiotic and biotic stresses (Shah *et al.*, 2005). The blight epidemics of 1979-80, 1980-81, and 1981-82 reduced chickpea production by 48, 46, and 46%, respectively (Malik & Bashir, 1984; Malik *et al.*, 1991).

The disease affects stems, leaflets, pods and seeds causing necrotic lesions and breakage of stems and petioles. Several pathotypes of the fungus have been described (Udupa *et al.*, 1998) and pathogenicity or aggressiveness within the same isolate can vary according to environmental conditions (Porta-Puglia, 1992). Pathogenic and genetic diversity in *Ascochyta rabiei* populations in Pakistan were evaluated (Jamil *et al.*, 2000). Biological pathotyping of 130 *A. rabiei* isolates showed that 4 isolates belonged to pathotype I (least aggressive), 79 isolates to pathotype II (medium aggressive) and 47 isolates to pathotype-III (highly aggressive). Most of the aggressive isolates (pathotype III) occurred in Northern Punjab and in the North Western Frontier Province. The use of

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resistant or tolerant cultivars is often practiced in many breeding programmes in order to control *Ascochyta* blight of chickpea.

Marker-assisted selection (MAS) is helpful in identifying qualitatively and quantitatively inherited agronomically desirable traits and biotic and abiotic stress resistance. The molecular marker studies have been reported by Malik *et al.*, (2007) and Ahmad *et al.*, (2007) in wheat and cotton respectively by MAS. Many blight QTLs have been tagged with molecular markers and mapped on the chickpea linkage groups. Santra *et al.*, (2000) identified two major quantitative trait loci, QTL-1 and QTL-2 for blight resistance in Recombinant Inbred Line (RIL) population from an interspecific cross using isozyme, RAPD, and inter-specific-sequence-repeat (ISSR) markers. Later, using the same RIL population, sequence-tagged microsatellite sites (STMS) and DNA amplification fingerprinting (DAF) markers were incorporated into the chickpea map region where the above mentioned QTLs were situated (Tekeoglu *et al.*, 2002; Rakshit *et al.*, 2003).

Udupa & Baum (2003) identified a major gene conferring resistance to pathotype I of *A. rabiei* and two independent QTLs conferring resistance to pathotype II. Further QTLs for resistance to pathotypes I and II were added by Cho *et al.*, (2004) using a different RIL population. One of those QTLs could be the same as QTL-1 reported by Santra *et al.*, (2000) in an interspecific cross because of the presence of the common STMS GAA47 (Tekeoglu *et al.* 2002). Flandez-Galvez *et al.*, (2003) reported three QTLs using F₂ progeny derived from a cross between desi x kabuli genotypes in a genomic region coincident with LG4 in the interspecific crosses. These reports demonstrated that the resistance to pathotype I is located on LG2 and pathotype II is located on LG4.

This study was conducted for the identification of QTLs involved in conferring resistance in induced mutants/local genotypes. These efforts would make it possible to deploy resistance genes more efficiently and effectively in chickpea breeding programmes. Marker assisted selection (MAS) for blight resistance would greatly accelerate the development of new chickpea cultivars.

Materials and Methods

Seed source: Molecular markers used to detect resistant loci in twenty one mutants/local genotypes are mentioned in Table 1 & 2. The genotypes included five local, five mutants, seven approved varieties and four high yielding advanced lines produced by chickpea breeding group. These genotypes have been screened for *Ascochyta* blight in *Ascochyta* blight screening nursery for three years at NIAB (Table 1). The blight screening method was used as described by Shah *et al.*, (2005).

Nine RAPD decamer primers, one ISSR sequence primers (prefix UBC), three SCAR primers and six STMS markers were selected for the analysis of these genotypes as they had been associated with QTLs for blight resistance previously reported by several authors (Santra *et al.*, 2000; Tekeoglu *et al.*, 2002; Collard *et al.*, 2003; Flandez-Galvez *et al.*, 2003 and Udupa & Baum, 2003).

PCR analysis: For DNA extraction, about 100 mg of young leaf tissue was harvested from 10 days old seedlings of 21 chickpea genotypes. DNA was extracted using the CTAB method (Khan *et al.*, 2004). Optimal reaction conditions for RAPD analysis was established according to Williams *et al.*, (1990). Amplification was carried out in 20 μ l

reactions containing: 100 ng of plant genomic DNA, buffer 10mM Tris HCl pH 8.8, 50mM KCl, 0.08% Nonidet P 40, 0.2 mM dNTPs, 2.5 mM MgCl₂, 0.4 uM of primer, 1 U of Taq DNA polymerase (Fermentas). Amplification was achieved in a Gradient Thermal Cycler (My Gene), programmed for 40 cycles with the following temperature profile: 1 min at 94°C, 2 min at 35°C and 2°C min at 72°C. Cycling was concluded with a final extension at 72°C for 8 min.

Genomic DNA of these genotypes was also used as template for SCAR primers. Each PCR reaction contained the same buffer MgCl₂ and Taq as in case of RAPD except Primer i.e 0.4uM of each forward and reverse primer. The thermal profile for PCR was an initial denaturation at 94 °C for 4 min followed by 40 cycles of 94°C min for 20 sec, the annealing at 58 °C for 1 min, and the elongation at 72 °C for 1 min with a final extension at 72 °C for 8 min.

ISSR analysis was performed following the protocol and sequences developed by Ratnaparkhe *et al.*, (1998). The 20 µl reaction volumes had similar conditions as described for RAPD and SCAR. The Thermal Cycler was programmed for 35 cycles of

Table 1: Disease reaction in field and molecular marker data of local genotypes

Genotypes	Disease reaction	Type	Breeding Method	SSR		SCAR	
				TA 146	SCY 603	SCM 590	SCAE 19
CM 32/96	S	Kabuli	Mutant	S	S	S	S
CM477/97	R	Desi	Mutant	R	R	R	R
CM325/97	S	Desi	Mutant	S	S	S	S
6153	S	Desi	Local line	S	S	S	S
CM72	R	Desi	Approved mutant variety	R	R	R	R
CM 2100/96	S	Desi	Mutant	S	S	S	S
C 727	S	Desi	Local line	S	S	S	S
CM 88	R	Desi	Approved mutant variety	R	R	R	R
K850	S	Desi	Local line	S	S	S	S
CM98	R	Desi	Approved mutant variety	R	R	R	R
Pb 2000	R	Desi	Approved variety	R	R	R	R
Bittle 98	S	Desi	Approved variety	S	S	S	S
B8/02	S	Desi	Advance line	S	S	S	S
B8/03	R	Desi	Advance line	R	R	R	R
AUG480	S	Black seeded	Local line	S	S	S	S
Aug 424	S	Desi	Local line	S	S	S	S
CMC 44	S	Desi	Advance line	S	S	S	S
Pb 91	S	Desi	Approved variety	S	S	S	S
Paidar 91	R	Desi	Approved variety	R	R	R	R
96052	S	Desi	Advance line	S	S	S	S
C 44	S	Desi	Approved variety	S	S	S	S

Table 2: List of molecular markers that were not polymorphic in local genotypes

SSR		RAPD	ISSR
TR 20	UBC181a	OPAF16	UBC836b
TR19	UBC681a	OPA109	
GA 16	UBC733b	OPAC12	
GAA47	UBC881	OPM02	
	OPX04		

the following temperature profile: initial denaturation at 94°C 5 min, 94°C for 1 min, 50°C for 1 min and 72°C for 2 min followed by a final extension at 72°C for 8 min.

For STMS, PCR amplification reaction contained 15 µl consisting of 100mM Tris HCl pH 8.8, 50mM KCl, 0.08% Nonidet P 40, 0.2 mM dNTPs, 2.5mM MgCl₂, 0.35µM of each primer, 0.8 U of Taq DNA polymerase and 50ng of genomic DNA. STMS primer sequences and optimal amplification conditions were achieved according to the protocol of Winter *et al.*, (1999) in a MyGene (MG 96G) thermocycler. After denaturing the DNA for 2 min at 94°C the reaction mixture was subjected to 35 cycles of the following temperature profile: 94°C for 20 s, 55°C for 50 s and 60°C for 50 s.

Amplification products from the RAPD and ISSR protocols were electrophoresed in gels composed of 1.5% agarose in 1 X TBE. STMS and SCAR were analysed in 5.5% non denaturing polyacrylamide gels also in 1 X TBE buffer. Agarose gels were stained in ethidium bromide and Polyacrylamide gels were stained using a silver-stain protocol (Halima *et al.*, 2006) and photographed using UVi pro Platinum System. Analysis of the gels and calculations of molecular weights of the amplified products were done by UVi BandMap and UVi pro platinum 1.1 software.

Results and Discussion

The present studies showed that no polymorphism was detected in chickpea genotypes with RAPD primers used by Santra *et al.*, (2000) and Cobos *et al.*, (2006). These genotypes were also characterized for SSR markers GAA47 (LG 4a), TA 146, TA 72 (LG4b), TR 20, TR19 (LG6), GA 16 (LG 2). These markers were already reported to be linked with blight resistance (Udupa & Baum, 2003, Cho *et al.*, 2004). Only one SSR marker TA 146 was found to be associated with resistance in genotypes as depicted in Figure 2. Contrary to other studies no linkage was detected between other SSR markers and resistance to blight in any genotype.

Four RAPD markers located on LG4b were developed into sequence characterized amplified regions (SCARs) markers by Iruela *et al.*, (2006) and added in the linkage group reported by Millan *et al.* (2003). Among these SCAR markers employed in this study, only three SCAR markers were polymorphic associated with resistance. STMS and RAPD markers on this linkage group covered the distance of about 38.1 cM with a tight cluster of markers in the middle of this group (Fig 1). Our findings showed that one STMS marker (TA 146) and three SCAR markers (SCAE19₃₃₆, SCK13₆₀₃, SCY17₅₉₀) covering the distance of 0.5 cM on this linkage group were linked with resistance in genotypes (Fig 3 and 4).

The region on LG 4b contained STMS marker TA 146 and TA 72, SCAR and RAPD markers. The upper region of LG4b containing RAPD and one SCAR marker covering a distance of 16.7 cM. Based on these results it is clearly indicated that none of the QTLs (already reported) located on LG 2, 4a, and 6 were involved in conferring resistance in genotypes. A specific region on LG 4b involving one STMS and 3 SCAR markers is associated with resistance in genotypes.

So far the genetics of *Ascochyta* blight resistance in chickpea strongly suggests polygenic inheritance of the trait. Different genes conferring resistance against blight are environment and pathotype specific that is why uptill now many QTLs have been identified in chickpea genotypes in different regions of the world. A collaborative study

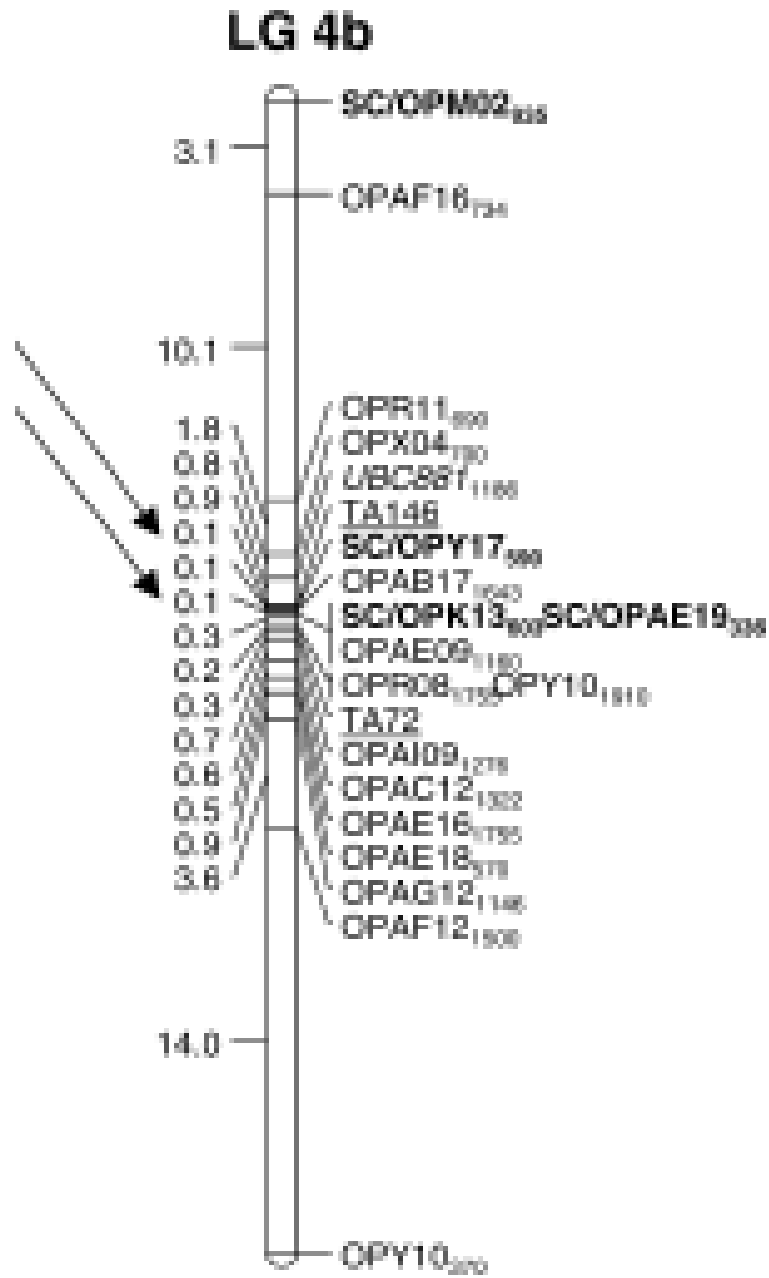


Fig 1: Linkage group 4b showing SCAR and STMS markers (Iruela *et al.*, 2006)

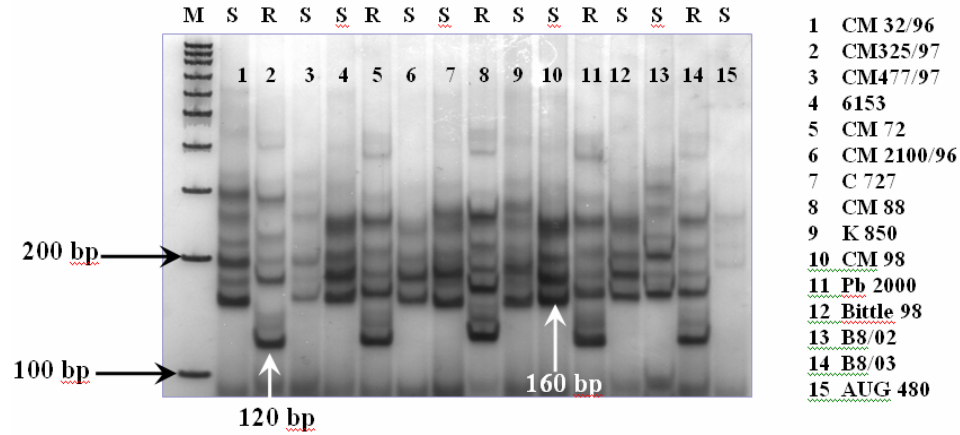


Fig 2: Sequence Tagged Microsatellite Sites (STMS) TA-146

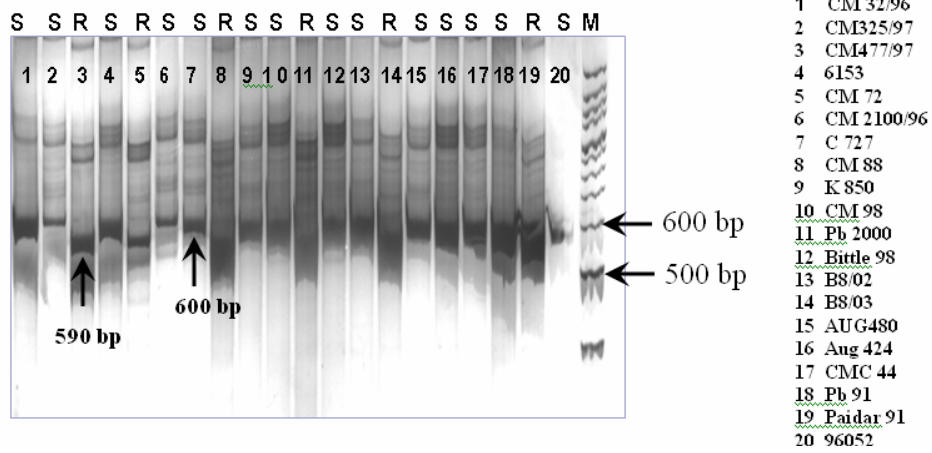


Fig 3: Sequence Characterized Amplified Region (SCAR) SCY17 590

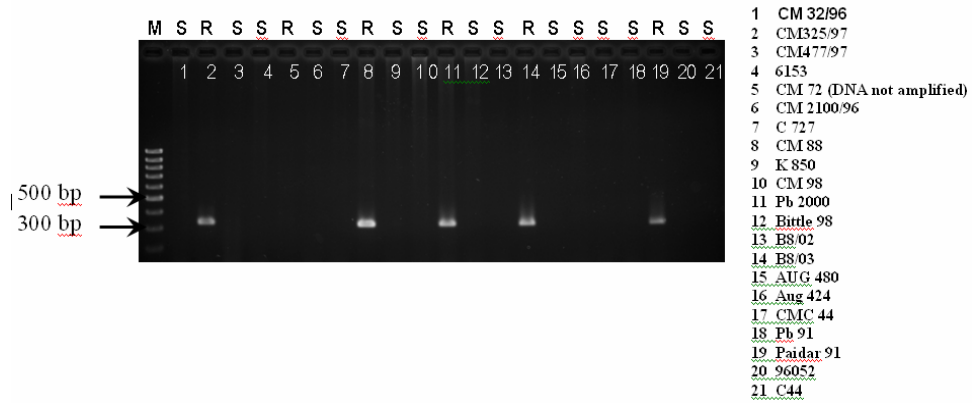


Fig 4: Sequence Characterized Amplified Region (SCAR) SCAE19 336

was conducted on QTLs for blight in Turkey and USA involving an interspecific RIL population (Mucella *et al.*, 2004). The effect of QTL-1 was greater than that of QTL-2 on linkage group 4 at Pullman, whereas the effect of QTL-2 was greater than that of QTL-1 at Eskisehir. Changes in magnitudes of the QTLs effect in two locations indicate possible differences in pathogen populations and environmental interactions. There is a need to study the inheritance of the region conferring resistance in local genotypes to confirm it as a QTL or a single recessive gene.

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