Turk J Agric For
28 (2004) 183-187

QTL Analysis of Ascochyta Blight Resistance in Chickpea

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Received: 13.08.2003

Abstract: Recent advances in quantitative trait loci (QTL) analysis have facilitated studies on Ascochyta blight, caused by *Ascochyta rabiei* Pass (Lab.), resistance in chickpea (*Cicer arietinum* L.). Using a recombinant inbred line (RIL) population derived from an interspecific cross between *C. arietinum* and *C. reticulatum*, the same 2 QTLs conferring resistance to Ascochyta blight were identified at 2 locations by interval mapping. Genotype X environment (G x E) interaction was significant both between years at the same location and between locations. The effect of QTL-1 on linkage group 8 (LG-8) was greater than that of QTL-2 on LG-4 at Pullman while the effect of QTL-2 was higher than that of QTL-1 at Eskişehir. Dissection of QTLs with molecular markers provides a better understanding of resistance to Ascochyta blight in chickpea. Validation of both QTLs in a second environment promises the application of marker-assisted selection (MAS) for this trait. Changes in magnitudes of the QTL's effect in 2 locations indicate possible differences in pathogen populations and environmental interactions.

Key Words: Chickpea, Ascochyta blight, quantitative trait locus, recombinant inbred line, molecular markers

Nohutta Antraknoza Dayanıklılığın Kantitatif Karakter Analizi

Özet: Son yıllarda kantitatif karakterleri kontrol eden lokusların (QTL) analizi için geliştirilen yeni uygulamalar sayesinde nohutta (*Cicer arietinum* L.), *Ascochyta rabiei* Pass (Lab.) adlı fungusun neden olduğu antraknoz hastalığına dayanıklılık çalışmaları da yeni bir yön kazanmıştır. *C. arietinum* ve *C. reticulatum* kullanılarak yapılan türler arası melezden geliştirilen bir rekombinant kendilenmiş hat (RIL) populasyonunda antraknoza dayanıklılığı kontrol eden iki kantitatif karakter lokusu bulunmuştur (QTL-1 ve QTL-2). İnterval haritalama yöntemi kullanılarak iki farklı lokasyonda da aynı kantitatif karakter lokuslarının etkin olduğu belirlenmiştir. Genotip x çevre interaksiyonu hem lokasyonlar hem de aynı lokasyonda yıllar arasında önemli çıkmıştır. Bağlılık grubu 8 (LG-8) üzerinde bulunan QTL-1 dayanıklılığın kontrolünde Pullman'da daha etkin bulunurken, LG-4 üzerinde bulunan QTL-2 Eskişehir'de daha etkin olmuştur. Kantitatif karakter lokuslarının moleküler markörler kullanılarak incelenmesi, nohutta antraknoza dayanıklılığın daha iyi anlaşılmasını sağlayacaktır. Aynı kantitatif karakter lokuslarının ikinci bir lokasyonda da etkin olduğunun bulunması, bu karakter için markör destekli seleksiyonun ümitvar olduğunu göstermektedir. İki lokasyondaki QTL etkilerinin farklı olması, buralardaki patojen populasyonlarının olası farklılığı ve çevre interaksiyonundan kaynaklanmaktadır.

Anahtar Sözcükler: Nohut, antraknoz, kantitatif karakter lokusu, rekombinant kendilenmiş hat, moleküler markörler

Introduction

Chickpea is the most important food legume in the Mediterranean basin, the Indian subcontinent, West Asia and North Africa. Among the biotic stresses that affect chickpea, Ascochyta blight causes extensive crop losses in most regions of the world (Jimenez-Diaz et al., 1993). Sources of resistance to Ascochyta blight have been well documented (Reddy and Singh, 1984) and varieties with resistance to the disease have been developed by international and national breeding programs (Acikgoz et al., 1994; Singh and Reddy, 1994; Muehlbauer et al., 1998). However, variations in disease reactions from one year and location to another have been the case for many lines (Singh et al., 1981). These variations were due to multigenic inheritance of resistance to Ascochyta blight (Muehlbauer and Singh, 1987; Tekeoglu et al., 2000).

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Application of molecular marker techniques has helped to better understand characters controlling by multiple genes. Using recombinant inbred lines (RILs) developed from an interspecific chickpea cross (*C. reticulatum* x *C. arietinum*) Santra et al. (2000) identified 2 QTLs conferring resistance to Ascochyta blight at Pullman, Washington, USA, in 1997-1998. They used the same population (CRIL-7: PI 599072 x FLIP 84-92C) that is used in this study. The objectives of this study were to validate the QTLs conferring resistance to Ascochyta blight and to possibly identify additional QTLs controlling resistance to the disease at a different location.

Materials and Methods

The 206 RILs from the interspecific cross of *C. arietinum* (FLIP 84-92C) x *C. reticulatum* (PI 599072) along with parental lines were used to conduct the experiment. The RILs and parents were planted in a randomized complete block design with 2 replications in the Ascochyta blight screening nursery at the Anadolu Agricultural Research Institute, Eskişehir, Turkey, in 1999. Creation of disease epidemics using chickpea debris infected by the virulent pathogen in this region and disease scoring followed the methods described by Tekeoglu et al. (2000). The disease data collected at Eskişehir were combined with the data collected at Pullman the previous 2 years (1997 and 1998) and used for variance analysis. QTL analysis was performed using the markers on the map reported by Santra et al. (2000) (Figure 1) and Tekeoglu et al. (2002). Mean disease scores of RILs with alternative alleles within a single QTL were compared using two-tailed t-tests, whereas mean disease scores of allele combinations at both QTLs were compared using least significant difference (LSD) tests. The QTL analysis was performed using "QGene" (Nelson, 1997).

Results and Discussion

G x E interaction was significant between years at the same location (Pullman) and between locations (Pullman and Eskişehir) (Table 1). Variations observed in disease development in the 2 years of this study (1997 and 1998) at the same location could be due to variation in inoculum density, differences in inoculation time or changes in temperature and wetness period in these years. For the different locations it is more likely that the variability is due to differences in the pathogen population. Significant G x E interactions is expected since the chickpea's response to ascochyta blight is highly affected by environment (Lichtenzveig et al., 2002). Environmental instability and involvement of minor genes in resistance response explain the quantitative nature of Ascochyta blight resistance in chickpea.

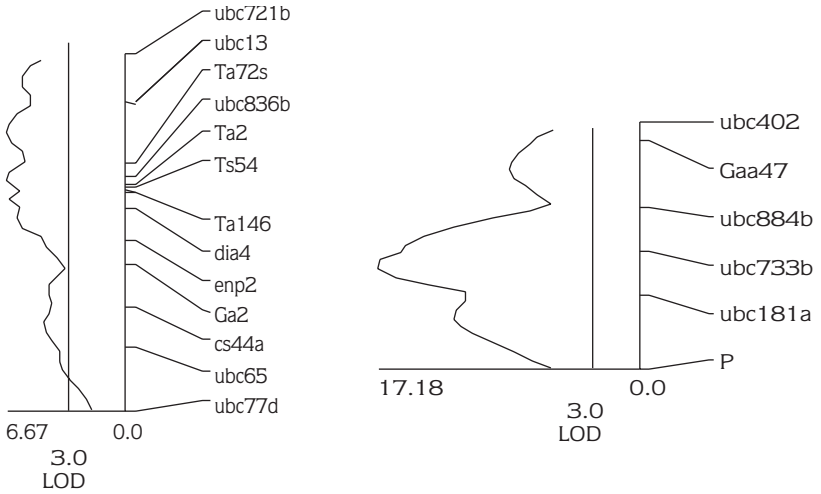

Figure 1. QTL-1 and QTL-2 conferring resistance to Ascochyta blight on linkage groups 8 and 4 of the *Cicer* genome, respectively, detected at Pullman.

Table 1. Analysis of variance of Ascochyta blight disease scores for the CRIL-7 population at Pullman, Washington, USA (1997 and 1998) and Eskişehir, Turkey (1999).

Source	df	Mean square	F value	P > F
Location	1	95.46	308.84**	0.0001
Year	1	0.51	7.64	0.05
Block (Year)	5	0.83	0.97	0.435
RIL	220	17.48	20.58**	0.0001
Location x RIL	170	4.04	1.59**	0.0005
Year x RIL	175	2.41	2.84**	0.0001
Error	242	0.85		

CV = 15.41

The same 2 QTLs conferring resistance to Ascochyta blight at Pullman were identified by interval mapping on linkage groups 4 and 8 at Eskişehir (Figure 2). No additional QTL has been detected. It was the case for some studies that a QTL affecting a quantitative trait in one environment may not be detected in other environments (Tanksley, 1993; Ullrich et al., 1997; Brouwer et al., 2000). However, the detection of the same QTLs in the 2 locations reported here confirmed their major effects (48 and 51% in Pullman and Eskişehir, respectively) on resistance (Table 2). Therefore, these QTLs promise to be useful for MAS. Flanking markers of the QTLs and proportion of phenotypic variation (R^2) explained by each marker were ubc733 (R^2: 0.41), ubc181a (R^2: 0.32) on QTL-1 and ubc836b (R^2: 0.20), dia4 (R^2: 0.18) on QTL-2 at Pullman; ubc733 (R^2: 0.20), Gaa47 (R^2: 0.17) on QTL-1 and Ta2 (R^2: 0.47), ubc836b (R^2: 0.46) on QTL-2 at Eskişehir.

The effect of QTL-1 on linkage group 8 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 Eskişehir (Table 2). This observation was confirmed by comparing mean disease scores of RILs carrying alternative loci for the 2 QTLs. Austin and Lee (1998) reported similar changes in magnitudes of QTL effects due to environmental interactions in maize.

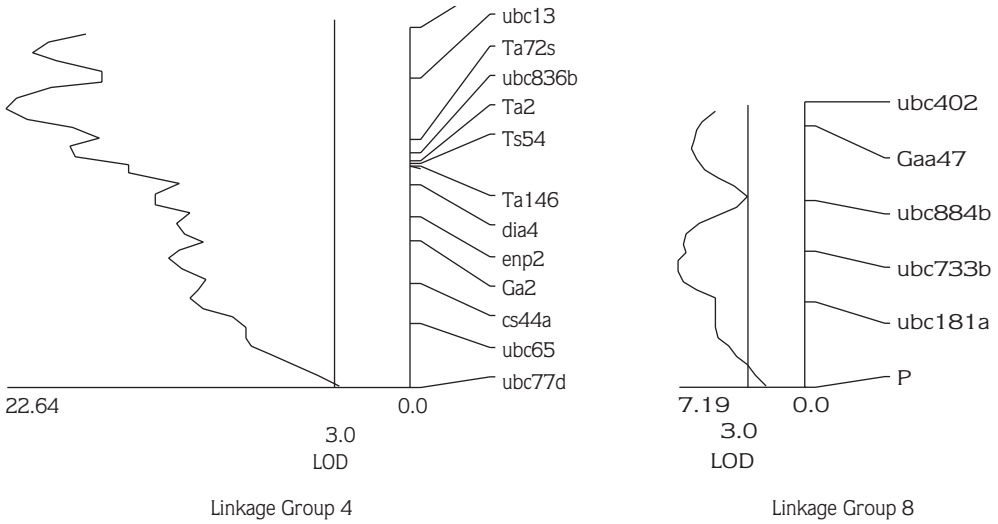

Figure 2. QTL-1 and QTL-2 conferring resistance to Ascochyta blight on linkage groups 8 and 4 of the *Cicer* genome, respectively, detected at Eskişehir.

Table 2. Mean disease scores of RILs carrying alternatif allele markers at QTL-1 and QTL-2 and their interactions in the CRIL-7 population at Pullman, Washington, USA and Eskişehir, Turkey.

Location	R^2 (%)			Mean Disease Scores							
	QTL-1	QTL-2	QTL-1 + QTL-2	QTL-1		QTL-2		QTL-1 + QTL-2			
				RR†	SS	RR	SS	RRRR‡	RRSS	SSRR	SSSS
Pullman	45	21	48	4.78	8.69**	5.85	8.28**	4.49a§	6.03b	7.63c	8.91c
Eskişehir	23	47	51	4.17	6.52**	3.78	7.05**	3.31a	6.22b	4.59a	7.48b

**Significant at P <0.001.

†RR- Resistant parent (Flip84-92C) alleles, SS- Susceptible parent (PI 599072) alleles.

‡RRRR- Resistant parent alleles, the first 2 letters for QTL-1, and the second 2 for QTL-2.

§F-protected LSD. Means in the same row followed by the same letter are not significantly different (P<0.05).

Allelic differences within each QTL were also examined using RILs carrying alternative alleles for each locus. Differences between disease scores of RILs carrying FLIP 84-92C (resistant parent) alleles for flanking markers and those of carrying PI 599072 (susceptible parent) alleles were highly significant (Table 2). Lower mean disease scores in RILs carrying resistant parent alleles at either QTL confirmed that the 2 QTLs conferring resistance to Ascochyta blight come from FLIP 84-92C. The mean disease scores of RILs carrying flanking markers for QTL-1 at Pullman were lower than those of carrying flanking markers for QTL-2, indicating that QTL-1 was more effective than QTL-2 at Pullman. However, the situation was opposite at Eskişehir, which was consistent with QTL analysis results presented in Figures 1 and 2.

The presence of flanking markers for both QTLs resulted in lower mean disease scores than for individual QTLs, indicating additive action of QTLs without epistasis. QTL analysis, based on the data collected from 2 locations in 3 years, indicated that 2 major QTLs confer resistance to Ascochyta blight in chickpea and that the effects of individual QTLs vary depending on the environment. These QTLs can be transferred to susceptible backgrounds by MAS.

Conclusions

The presence of QTLs for Ascochyta blight resistance can be verified by finding the same association between resistance and the markers in other populations. This was not possible before because the flanking markers found in the interspecific cross were not polymorphic in other RIL populations that were evaluated for blight resistance. However, the availability of STMS markers located in the vicinity of the QTLs and their high R^2 values provides a means of testing this prospect. Further confirmation of QTLs can be shown by making selections for resistance across available germplasm using flanking markers described here and determining the degree of resistance of the lines in disease nurseries. In addition, RILs carrying individual or both QTLs can be backcrossed to a susceptible background via MAS and individual effects of the QTLs can be determined in a different genetic background.

Additional screening of the RILs at Eskişehir, Turkey, and other locations would provide additional confidence in determining the effects of environment and possibly differing pathotypes on Ascochyta blight disease development.

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