INHERITANCE OF CBB RESISTANCE IN A RESISTANT INTER-CROSS POPULATION OF COMMON BEAN

Kelli Durham[1], Liz Lee[1], Kangfu Yu[2], K. Peter Pauls[1], and Alireza Navabi[1,2]

[1]Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada. [2]Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, ON, Canada.

Plant Agriculture

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Introduction

- Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a damaging widespread disease of common bean (*Phaseolus vulgaris L.*).
- Genetic resistance to CBB in common bean is limited, but has been introgressed through inter-specific crosses with tepary bean (*P. acutifolius*).
- In Canadian common bean germplasm, CBB resistance in navy bean has been introgressed from two distinct sources of *P. acutifolius* i.e., PI440795, from which OAC Rex (Michaels *et al.* 2006) was developed and PI319443, from which HR67 and HR45 (Park and Dhanvantari 1994) germplasm lines were developed.
- A major CBB resistance QTL, associated with the microsatellite marker pvCTT001, was mapped on linkage group B5 in OAC Rex, (Tar'an *et al*. 2001). However, recent results from Perry *et al.* (unpublished and the original microsattelite mapping study (Yu et al 2000b) positioned PVctt001 on chromosome B4.
- A major CBB resistance QTL, associated with the SCAR marker UBC420, was mapped on linkage group B6 in HR67 (Yu *et al.* 2000a).

Objectives

- **To study the segregation of CBB resistance response in a resistant intercross population between OAC Rex and HR45, and**
- **To study the effects of CBB QTL of chromosomes B4 and B6 and their interaction effects on CBB resistance.**

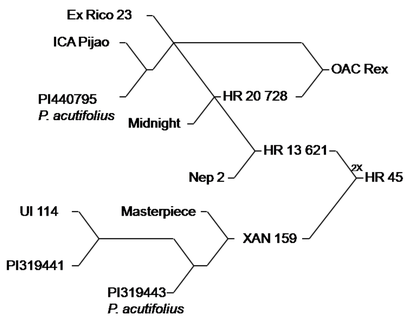
A demonstration of an additive interaction between the QTL would support the possibility of pyramiding the two QTL in a common background, to obtain higher levels and longer-lasting resistance to CBB.

Plant Material

An $F_{4:5}$ recombinant inbred line (RIL) population of reciprocal crosses between the resistant genotypes OAC Rex and HR45, known to carry different resistance QTL, was evaluated for resistance to CBB and genotyped with molecular markers associated with CBB QTL.

Parental lines of the RIL population

- OAC Rex is resistant to CBB and is a high yielding, white seeded variety.
- HR45 is highly resistant to CBB and has dull white seeds.
- Both parents carry SU91, but are polymorphic for QTL associated with UBC420 and PVctt001.



Phenotyping

Field Trials

- Field trials were planted at GPCRC-Harrow in 2009 and 2010 in a 15 by 15 unbalanced square lattice design with two replications. Plant material included: OAC Rex, HR45, Dresden (susceptible check) and 218 $F_{4:5}$ lines.
- Plots were artificially inoculated and multiple evaluations of CBB severity in the field were conducted with one week intervals using a 0-5 visual scale based on the percentage of plot leaf area with disease symptoms.
- The Area Under the Disease Progress Curve was estimated for each experimental unit as $AUDPC=\sum[(S_i+(S_{i+1})/2)(T_{i+1})-T_i)]$, where S is a measure of disease severity and T is days.

Growth Room Trials

- A growth room experiment was conducted at GPCRC-Harrow in 2010 in a RCBD with two replications. Plant material included OAC Rex, HR45, the susceptible check (cv. Dresden) and 53 RILs selected based on low, moderate and high AUDPC from the 2009 field trial.
- Plants were artifcially inoculated and evaluations of CBB severity were conducted on a 0-5 visual scale (Yu et al. 2000a) at 13 and 15 days after inoculation. Image analysis was performed using ASSESS 2.0 on electronic images taken from each of the infected leaves.

Artificial inoculation of field trial at GPCRC Harrow 2009

Severe common bacterial blight on susceptible check Dresden

Artificial inoculation of growth room experiment 2010

Genotyping

- Lines were genotyped with UBC420, PVctt001, and an additional 18 markers developed based on sequence information in the genomic regions where these markers are located, in addition to 1 SNP marker.
- In previous studies, PVctt001 accounted for 42% of phenotypic variation for CBB resistance in an OAC Seaforth/OAC Rex $F_{2:4}$ population (Tar'an *et al.* 2001).
- In previous studies, UBC420 accounted for 62% of phenotypic variation for CBB resistance in an HR67/Envoy F_5 population (Yu *et al.* 2000a).
- Gene3, Gene4, Gene9, 10a, 10b, 14, 15, SSR1, SSR2 and SSR6 were developed from a 70kb region surrounding UBC420 (Shi *et al.* 2010).
- P1, P2, P4 and P5 were developed from a 300kb of a sequenced CBB-associated genomic region in OAC Rex (Perry *et al. In publication).*

Result 1. Growth Room Trial

Table 1. Single marker QTL analysis preformed by one-way ANOVA in PROC GLM in SAS 9.2 (SAS Institute Inc.) for field 2009 and 2010 and average growth room (GR) ratings 13 and 15 days after inoculation (DA1). The percent infected leaf area as determined in image analysis (IA).

			GR 13-DAI		GR 15-DAI		Image Analysis 15-DAI	
Marker	Chr	Pos	Add. Effect	R^2	Add. Effect	R^2	Add. Effect	R^2
10a	6	17.18	0.42**	0.31	0.64**	0.39	0.88**	0.26
SSR1	6	18.99	0.49**	0.42	0.74**	0.54	0.93**	0.30
10b	6	19.55	0.51**	0.45	0.78**	0.59	0.99**	0.34
SSR2	6	20.13	0.48**	0.41	0.74**	0.53	0.98**	0.33
SSR6	6	20.30	0.46**	0.37	0.68**	0.44	0.84**	0.24
15	6	20.57	0.46**	0.37	0.71**	0.48	0.92**	0.29
Gene3	6	22.05	0.47**	0.38	0.68**	0.44	0.98**	0.32
UBC420	6	23.84	0.51**	0.45	0.74**	0.54	0.90**	0.30
Gene9	6	24.85	0.48**	0.41	0.71**	0.49	0.84**	0.26
g2467	4	4.90	NS	-	NS	-	-0.60*	0.18
PVctt001	4	9.60	NS	-	NS	-	-0.40**	0.15

Result 2. QTL Analysis of Field Data

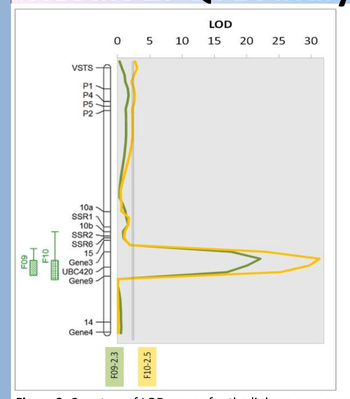


Figure 3. Counters of LOD scores for the linkage map representing the genomic region on chromosome 6 carrying a major CBB QTL. Linkage map was constructed using JoinMap and composite interval mapping was performed in MAPQTL. Phenotypic values from the field (AUDPC) in 2009 (F09) and 2010 (F10) were used in the analysis.

Table 2. Percentage of phenotypic variation explained by each locus for CBB severity (AUDPC) in the field in 2009 and 2010

Field 2009			
Group	Locus	% Explained	Additive Effect
1	UBC420	35.5	11.34
1	Gene3	31.9	9.76
2	PVctt001	1.2	-2.15
Field 2010			
Group	Locus	% Explained	Additive Effect
1	UBC420	46.6	10.67
1	Gene3	38.3	9.75
2	PVctt001	0.8	-1.53

Result 3. Interaction Effects

Table 3. Inter-marker epistatic interaction effects were computed for pairs of loci from the two linkage groups by two-way ANOVA using PROC GLM in SAS (SAS Institute Inc.). Only selected significant interactions between markers are reported. Image analysis was conducted 15 days after inoculation (DAI).

			PVctt001 (9.9cM)											g2468 (4.9cM)								
			Field 09		Field 10		GR 13-DAI		GR 15-DAI		Image Analysis					Field 10		Image Analysis				
Chr.	Marker	cM	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	Chr.	Marker	cM	R^2	p	R^2	p			
6	SSR1	18.96	0.35	0.030*	0.37	0.037*	NS		0.72	0.015*	0.64	0.002**	6	10a	17.18	0.37	0.014*	NS				
	SSR6	20.30	NS		NS		0.62	0.048*	0.72	0.030*	0.57	0.016*		SSR6	20.30	0.46	0.015*	NS				
	UBC420	23.84	0.38	0.029*	NS		NS		0.79	0.026*	0.57	0.024*		15	20.57	0.44	0.021*	0.52	0.021*			
	Gene9	24.85	0.38	0.014*	NS		NS		0.79	0.026*	0.57	0.024*		Gene3	22.05	NS		0.51	0.035*			

Conclusions

- The QTL associated with UBC420 was the major contributor to CBB resistance, accounting for between 35.5% to 46.6% of variation in AUDPC in the field trials and between 32% to 45% of variation in CBB ratings in the growth room trials.
- The most significant epistatic interactions for marker pairs on chromosomes 4 and 6 were observed between UBC420 and PVctt001 (F09) and g2467 and 15 (IA). This has caused deviation from additive interaction between CBB QTL on B6 and B4.
- Makers P1, P2, P4 and P5 are likely to be from a shared region of introgressed DNA found in both OAC Rex and HR45 and map to chromosome 6 in this population.
- QTL analysis based on image analysis of the infected leaves, generally, resulted in lower estimates of the QTL effects compared to visual rating. However, image analysis provided a more sensitive assay for detecting QTL with small effects.

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Acknowledgments

Technical assistance of Jan Brazolot, Terry Rupert, Tom Smith, Weilong Xie, Greg Perry and Marcus Shi is gratefully acknowledged.
This work is funded by :

Ontario White Bean Producers | Coloured Bean Growers | Agricultural Adaptation Council