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ORIGINAL PAPER

QTL analysis for capsaicinoid content in *Capsicum*

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Abstract Pungency or "heat" found in *Capsicum* fruit results from the biosynthesis and accumulation of alkaloid compounds known as capsaicinoids in the dissepiment, placental tissue adjacent to the seeds. Pepper cultivars differ with respect to their level of pungency because of quantitative and qualitative variation in capsaicinoid content. We analyzed the segregation of three capsaicinoids: capsaicin, dihydrocapsaicin and nordihydrocapsaicin in an inter-specific cross between a mildly pungent *Capsicum annuum* 'NuMex RNaky' and the wild, highly pungent *C. frutescens* accession BG2814-6. F_3 families were analyzed in three trials in California and in Israel and a dense molecular map was constructed comprised mostly of loci defined by simple sequence repeat (SSR) markers. Six QTL controlling capsaicinoid content were detected on three chromosomes. One gene from the capsaicinoid biosynthetic pathway, BCAT, and one random fruit EST, 3A2, co-localized with QTL detected in this study on chromosomes 3 and 4. Because one confounding factor in quantitative determination of capsaicinoid is fruit size, fruit weight measurements were taken in two trials. Two QTL controlling fruit weight were detected, however, they did not co-localize with QTL detected for capsaicinoid content. The major contribution to the phenotypic variation of capsaicinoid content (24–42% of the total variation) was attributed to a digenic interaction between a main-effect QTL, *cap7.1*, and a marker located on chromosome 2 that did not have a main effect on the trait. A second QTL, *cap7.2* is likely to correspond to the QTL, *cap*, identified in a previous study as having pronounced influence on capsaicinoid content.

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Introduction

The biosynthesis of capsaicinoids, the compounds responsible for the sensation of pungency or heat in hot pepper, is a trait unique to the *Capsicum* genus. Our longterm interest is to understand the specific genetic shifts in the evolution of this genus that account for the acquisition of this trait, therefore it is important to identify the genes that contribute to the presence, amount and profile of these compounds in pepper. Capsaicinoid analogs are characterized by a vanillylamine "head" derived from the phenylpropanoid pathway coupled with a fatty acid "tail." It is thought that these compounds evolved to deter mammalian herbivory thereby promoting seed dispersal by birds that lack crushing molars and harsh digestive systems, and travel greater distances, roosting in trees under which light conditions are especially favorable for wild peppers (Tewksbury and Nabhan 2001).

The capsaicinoid molecule is formed as the result of an acyl transfer reaction between medium chain (C = 9–11) branched fatty acyl CoA components and vanillylamine resulting in at least 10 and as many as 25

capsaicinoid analogues that differ with respect to acyl chain length, branching and degree of saturation (Reilly et al. 2001; Maillard et al. 1997). In general, capsaicin and dihydrocapsaicin, both C10, are typically the most abundant of these compounds in domesticated hot pepper varieties (Govindarajan et al. 1987). These two capsaicinoids are distinguished only by the presence of a double bond between carbons 6 and 7 in capsaicin that is absent in dihydrocapsaicin.

Despite the sensation of pain when consumed, humans domesticated several species of this New World genus nearly 10,000 years ago and have carried pepper as spice, vegetable and medicine all over the world. The characteristic pain perceived when capsaicinoids contact tissue is a consequence of a characterized interaction between the capsaicinoid molecule and the pain receptor, TRPV1 also known as VR1 (Caterina et al. 1997). The intensity of the heat sensation is positively correlated with capsaicinoid amount (Krajewska and Powers 1988). Capsaicinoid type also influences pungency, in terms of potency of specific analogs and their different sensory effects (Krajewska and Powers 1988; Todd et al. 1977).

Capsaicinoids are synthesized and accumulate in the dissepiment, the epidermal layer of the placenta of pepper fruit, typically showing onset of synthesis at 15–25 days post-anthesis (Stewart et al. 2005; Iwai et al. 1979). Capsaicinoid biosynthesis continues throughout fruit development until the end of the growth phase. During the ripening stage of fruit development, however, some decrease in capsaicinoid content may occur (Iwai et al. 1979; Contreras-Padilla and Yahia 1998; Estrada et al. 2000), possibly because of degradation caused by enzymatic oxidation. Peroxidases may be involved in this process because expression and activity of a peroxidase enzyme is positively correlated with capsaicinoid degradation (Diaz et al. 2004).

Early genetic studies identified a single dominant gene, *C*, now known as *Pun1*, that in the homozygous recessive condition results in absence of pungency regardless of genotype at other loci throughout the genome that affect pungency level or other aspects of this trait (Deshpande 1935; Webber 1911). This gene encodes AT3, a putative acyltransferase (Stewart et al. 2005) on chromosome 2 (Ben-Chaim et al. 2001; Blum et al. 2002; Lefebvre et al. 1995), however, the precise role of this enzyme in capsaicin biosynthesis has yet to be established. The only phenotypic variation ascribed to this locus to date, presence/absence of pungency, is a consequence of the loss-of-function allele known as *pun1*, a recessive allele for non-pungency that apparently results from a 2.5 kb deletion spanning the first exon and part of the promoter region thereby preventing expression of AT3 (Stewart et al. 2005).

The amount of capsaicinoid produced in hot peppers is a quantitatively inherited trait (Zewdie and Bosland 2000a, b). Varieties with different levels of pungency are well known (http://www.chilepepperinstitute.org/Pungency.htm), however, pungency level is also significantly affected by the environment (Harvell and Bosland 1997; Estrada et al. 1999; Zewdie and Bosland 2000b).

To date, limited information regarding the genetic control of quantitative variation of capsaicinoid content is available. Only two previous studies have focused on this aspect of the trait (Blum et al. 2003; Zewdie and Bosland 2000a), one of which revealed a major QTL for capsaicinoid content, termed *cap*, on chromosome 7 (Blum et al. 2003). In this study, *cap* was identified in an inter-specific cross between the pungent *C. frutescens* BG 2816 wild accession and the non-pungent *C. annuum* variety 'Maor' by screening for polymorphisms between high and low pungent F_2 bulks. This approach, although powerful in detecting major QTL (Wang and Paterson 1994), may not be sensitive enough to detect additional QTL with more minor effect. Because no co-localization was observed between a set of predicted structural genes from the capsaicinoid biosynthesis pathway and variation in capsaicinoid content (Blum et al. 2003), *cap* was postulated to be a regulator of the pathway or a yet unknown structural gene.

The goal of the present study was to extend our understanding of the genetic control of capsaicinoid biosynthesis in *Capsicum* by defining the genomic regions that control the presence and accumulation of three major capsaicinoid analogues, capsaicin, dihydrocapsaicin and nordihydrocapsaicin in a cross between high and low pungency pepper lines. This study provided confirmation of the existence of a QTL in the *cap* region. Through whole-genome QTL analysis in an appropriate population grown in two environments over 3 years, additional QTL affecting this important trait were elucidated.

Materials and methods

Plant material

Highly polymorphic inter-specific F_2 and F_3 populations were constructed by crossing *C. annuum* cv. 'NuMex RNaky', a New Mexico chile with low levels of capsaicinoids and large fruit (Nakayama and Matta 1985) with the *C. frutescens* accession BG 2814-6 (Grube et al. 2000) characterized by small berry-sized highly pungent fruit. An F_2 population consisting of 396 plants

originated from a single (RNaky × BG 2814-6) F_1 plant was grown in the greenhouse at Cornell University in 1999, harvested for leaf tissue for DNA extraction and self-pollinated to obtain F_3 seeds for QTL analysis.

Trait evaluation

The 2 parents, F_1 and 234 F_3 families were grown in the field in Gilroy California during the summer of 2001 and in Lahish, Israel during the summers of 2002 and 2003. The experiments were arranged in a randomized block design with two replications. Each replicate consisted of ten plants from each F_3 family, the parents and F_1. Three mature fruits from each plant were harvested about 90 days after planting and were bulked with fruits from other plants in the family. Tissue preparation and HPLC analysis was done according to Blum et al. (2003). Capsaicinoid content in ppm was calculated according to the following equation: peak area of capsaicinoid × (ppm standard/peak area of standard) × (ml acetone/gram sample). All samples were evaluated for six traits: (1) Capsaicin (cap) content in ppm; (2) Dihydrocapsaicin (dhc) content in ppm; (3) Nordihydrocapsaicin (ndhc) content in ppm; (4) Total capsaicinoid (total) content calculated by summing the ppm values of all three capsaicinoids; (5) Ratio of capsaicin to dihydrocapsaicin (cap/dhc); (6) Fruit weight (fw) in grams. Statistical analyses of family means and correlations among traits were done using JMP 4.0 statistical software (SAS 2000).

Marker analysis and map construction

A subset of 100 individuals from the F_2 population was used to map 728 markers including SSR, AFLP and RFLP loci. The complete map, termed FA03, is posted at The Solanaceae Genome Network web site, http://www.sgn.cornell.edu. Tomato cDNA and genomic clones were chosen for RFLP analysis to allow assignment of the linkage groups to chromosomes based on previously published pepper maps (Livingstone et al. 1999; Paran et al. 2004). Most SSR loci were defined by proprietary markers kindly provided to us by Syngenta, DNA Landmarks and Seminis. Additional publicly available SSR sequences were provided by Byung-Dong Kim, Seoul National University, Korea (Lee et al. 2004), Istvan Nagy and Gyorgy Kiss, Agricultural Biotechnology Center, Hungary and Umesh Reddy, Alcorn State University, MS, USA. The markers were amplified using PCR, based on the procedure described by Thomson et al. (2003). Amplified PCR products were run on a 4% polyacrylamide gel followed by silver staining as described by Panaud et al. (1996). AFLP markers were generated by KeyGene N. V. Wageningen, The Netherlands and by Sunseeds as described by Vos et al. (1995). *Eco*RI (E)/*Mse*I (M) primers combinations were used, with the following selective nucleotides for the AFLP primers: E32-AAC, E33-AAG, E34-AAT, E36-ACC, E37-ACG, E40-AGC, E41-AGG, M47-CAA, M48-CAC, M49-CAG, M51-CCA, M53-CCG, M54-CCT, M55-CGA, M60-CTC.

In addition to anonymous markers selected for thorough genome coverage, branched-chain amino acid aminotransferase (*Bcat*) GenBank accession no. AY034379, and *3A2* (GenBank accession no. CF269943 were used as probes for mapping).

Mapping was performed using JoinMap 3.0 (Van Ooijen and Voorrips 2001). Markers were grouped at minimum LOD 4.0. Order within linkage groups was inferred for markers that had a maximum recombination frequency of 0.3 and LOD value larger than 2.0. Threshold for removal of loci based on goodness-of-fit tests was set at 5.0. Map distances were calculated using the Kosambi function.

QTL analysis

Initial QTL mapping was performed with the complete set of markers in the FA03 population. To simplify presentation of results, however, a subset of framework markers distributed along the chromosomes was chosen for the QTL analysis presented in this study. Additional markers with significance for the traits based on the analysis with the complete set of markers were also included. QTL mapping was performed by interval analysis using a LOD score of 3.0 as a minimum significance level for QTL detection. This threshold was derived by 1,000 permutation tests at a significance level of $p < 0.05$. All marker analyses were performed using QGene v.3.04 software (Nelson 1997). Estimates of percent phenotypic variation explained by individual and multiple QTL (R^2), additive (a) and dominance (d) effects were determined for markers with the highest F value within a given QTL interval. Two-way ANOVA procedures were performed using Visual Basic in Excel and were used in a comprehensive assessment of digenic interactions. Only interactions with a minimum of five individuals in each of the allelic combinations that were significant in at least two environments ($P < 0.005$) were reported.

Results

Linkage map construction

A total of 728 molecular markers including 489 SSR loci, 195 AFLP, 8 specific PCR markers and 36 RFLP

markers were used to construct the linkage map including the candidate genes, *pAMT*, *COMT*, and *Bcat* drawn from the proposed model for capsaicinoid biosynthesis (Blum et al. 2003; Stewart et al. 2005). The map consists of 12 major and 4 small linkage groups with a total length of 1358.7 cM.

Phenotypic variation and correlations among traits

Mean phenotypic values, and standard errors for the parents, F_1 and F_3 generations for all traits are presented in Table 1. The content of the different capsaicinoids was 10–30× higher in BG 2814-6 than in RNaky. The capsaicinoid content in the F_1 generation was higher than the more pungent parent, BG 2814-6, indicating overdominance or heterosis for this trait. The overall mean total capsaicinoid content of the F_3 generation across the three experiments was slightly lower than BG 2814-6. Among the three capsaicinoid analogues, capsaicin was the most abundant in most experiments ranging from 38 to 64% of the total capsaicinoid detected in the parents. Nordihydrocapsaicin was the least abundant analogue, ≤17% of the parental means. Individual F_3 families showed transgressive segregation for all traits tested in this study except for fruit weight (data not shown). With respect to range of variation in fruit weight, BG 2814-6 has extremely small fruit, typically ≤0.5 g total weight, while RNaky fruit typically weigh about 50 g.

Genetic correlations between environments and all traits are presented in Table 2. Capsaicin content was highly correlated with dihydrocapsaicin and moderately correlated with nordihydrocapsaicin. Mean correlation coefficients across environments were $r = 0.71$ and 0.37 for dihydrocapsaicin and nordihydrocapsaicin, respectively. The ratio of capsaicin/dihydrocapsaicin was moderately correlated with capsaicin content ($r = 0.46$ cross environments) but not significantly correlated with dihydrocapsaicin. Fruit weight was moderately negatively correlated with capsaicinoid content ($r = 0.33$ with capsaicin content across environments). The highest correlations between environments were observed for capsaicin content ($r = 0.68$–0.86); the lowest correlations between environments were observed for nordihydrocapsaicin content ($r = 0.56$–0.68).

QTL identification

A total of six different main effect QTL affecting capsaicinoid content were identified in this study that mapped to chromosomes 3, 4 and 7 (Table 3; Fig. 1). Two fruit weight QTL were also detected in chromosomes 2 and 3.

Table 1 Means and standard errors of capsaicinoid content (ppm) in the parents, F_1 and F_3 generations of the FA03 population

	BG2814-6			RNaky			F_1			F_3		
	2001	2002	2003	2001	2002	2003	2001	2002	2003	2001	2002	2003
Capsaicin	3,657	1,577 ± 79	1,560 ± 126	67	133 ± 7	79 ± 15	4,731	1,848 ± 36	1,389 ± 52	2,733 ± 134	1,093 ± 51	1,457 ± 88
% from total	60	59	54	38	64	44	60	53	47	53	50	55
Dihydrocapsaicin	1,796	731 ± 40	1,190 ± 81	80	75 ± 11	97 ± 20	2,606	1,375 ± 21	1,439 ± 63	1,827 ± 78	920 ± 34	1,010 ± 51
% from total	29	30	41	45	36	54	33	39	49	36	43	38
Nordihydrocapsaicin	647	309 ± 13	125 ± 11	30	ND	5 ± 3	579	288 ± 5	105 ± 4	563 ± 26	151 ± 8	197 ± 11
% from total	11	11	5	17	–	2	7	8	4	11	7	7
Total capsaicinoids	6,100	2,617 ± 129	2,875 ± 213	177	208 ± 18	181 ± 37	7,916	3,511 ± 53	2,933 ± 112	5,125 ± 224	2,165 ± 86	2,666 ± 144
Capsaicin/ Dihydrocapsaicin	2.0	2.1	1.3	0.8	1.8	0.8	1.8	0.7	1.0	1.5	1.2	1.4
Fruit weight (g)		0.3 ± 0.01	0.2 ± 0.01		50.5 ± 3.0	49 ± 2.6		2.5 ± 0.1	2.5 ± 0.1		18.4 ± 0.7	33.4 ± 1.8

Data in 2001 were obtained for bulked fruits for the parents and F_1

ND not detectable

Table 2 Pearson correlation coefficients ($P < 0.05$) between traits and environments for capsaicinoid accumulation in the FA03 population

Trait	cap 01	cap 02	cap 03	dhc 01	dhc 02	dhc 03	ndhc 01	ndhc 02	ndhc 03	total 01	total 02	total 03	c/d 01	c/d 02	c/d 03	fw 02	fw 03
Capsaicin (cap) 02	0.68																
Capsaicin (cap) 03	0.79	0.86															
Dihydrocapsaicin (dhc) 01	0.85	0.56	0.66														
Dihydrocapsaicin (dhc) 02	0.58	0.83	0.72	0.52													
Dihydrocapsaicin (dhc) 03	0.70	0.68	0.84	0.75	0.78												
Nordihydrocapsaicin (ndhc) 01	0.62	0.36	0.43	0.72	0.52	0.58											
Nordihydrocapsaicin (ndhc) 02	0.25	0.28	0.20	0.34	0.58	0.41	0.56										
Nordihydrocapsaicin (ndhc) 03	0.41	0.33	0.47	0.56	0.54	0.77	0.68	0.59									
Total capsaicinoids (total) 01	0.97	0.65	0.75	0.94	0.63	0.75	0.75	0.34	0.52								
Total capsaicinoids (total) 02	0.67	0.96	0.82	0.60	0.95	0.76	0.48	0.49	0.47	0.67							
Total capsaicinoids (total) 03	0.78	0.81	0.97	0.73	0.78	0.94	0.53	0.32	0.64	0.78	0.82						
Capsaicin/ Dihydrocapsaicin (c/d) 01	0.44	0.41	0.36	NS	NS	NS	NS	NS	NS	0.24	0.27	0.25					
Capsaicin/ Dihydrocapsaicin (c/d) 02	0.40	0.60	0.50	NS	NS	NS	NS	−0.32	−0.20	0.25	0.38	0.35	0.71				
Capsaicin/ Dihydrocapsaicin (c/d) 03	0.38	0.57	0.54	NS	NS	NS	NS	−0.21	−0.27	0.23	0.40	0.35	0.68	0.83			
Fruit weight (fw) 02	−0.37	−0.32	−0.35	−0.33	−0.34	−0.32	−0.30	−0.31	−0.27	−0.37	−0.36	−0.36	NS	NS	−0.29		
Fruit weight (fw) 03	−0.33	−0.28	−0.35	−0.33	−0.32	−0.35	NS	NS	NS	−0.34	−0.32	−0.36	NS	NS	NS	0.83	

NS not significant

Capsaicin content Five QTL were detected on chromosomes 3 (*cap3.1*), 4 (*cap4.1* and *cap4.2*) and 7 (*cap7.1* and *cap7.2*). For all QTL, the allele from the highly pungent parent contributed to increased capsaicin content. Four QTL (*cap3.1*, *cap4.2, cap7.1* and *cap7.2*) were detected in two locations; one (*cap4.1*) was detected in only one location. LOD scores for all QTL in non-significant locations were elevated but slightly below the significance threshold value (data not shown). Gene action at *cap3.1* and *cap4.1* was additive while the BG 2814-6 allele at *cap7.1* was partially dominant. The percent variation explained by each QTL was similar, although *cap7.2* was the only QTL with an effect larger than 20% of the explained variation. The proportion of the total phenotypic variation explained by all QTL calculated by multiple regression analysis was 24, 19 and 37% in 2001, 2002 and 2003, respectively.

In addition to the main effect QTL, one digenic interaction was detected between *cap7.1* and a marker in chromosome 2 that did not have a detectable main effect on the trait (Table 4; Fig. 1). The presence of BG 2814-6 alleles at both positions was correlated with the largest increase of capsaicin content (Fig. 2). This interaction had the largest effect on capsaicinoid accumulation observed in this study, explaining 37–42% of the total phenotypic variation compared to 16–17% explained by *cap7.1* alone.

Dihydrocapsaicin content Four out of the five QTL that were detected for capsaicin content were also identified for dihydrocapsaicin content (Table 3; Fig. 1). For all the QTL, the alleles from BG 2814-6 contributed to the increase of dihydrocapsaicin content. The gene action, magnitude and direction of effects were similar to

Table 3 QTL for capsaicin, dihydrocapsaicin and nordihydrocapsaicin accumulation in pepper fruit detected in the FA03 population

Trait	Location	QTL[a]	Interval[b]	Trait means[c]			Direction	LOD	R^2 (%)	*d*/*a*
				AA	AB	BB				
Capsaicin (ppm)	CA 2001	*cap3.1*	3A2-AA840692	2428.3	–	3519.6*	BG2814-6	3.9	16	–
	IS 2003		AA840692-3A2	1122.3	1516.6	1952.2	BG2814-6	3.2	13	−0.13
	IS 2003	*cap4.1*	Bcat-NP1015	1159.7	1533.4	2087.9	BG2814-6	3.3	14	−0.19
	CA 2001	*cap4.2*	GP1042-GP20039c	2435.4	–	3628.8*	BG2814-6	3.8	14	–
	IS 2003		GP1042-GP20039c	1363.8	–	2206.7*	BG2814-6	4.2	19	–
	IS 2002	*cap7.1*	NP2382-NP1005	811.8	1203.5	1297	BG2814-6	5.7	17	0.55
	IS 2003		NP2382-NP1005	1110.9	1727	1853.7	BG2814-6	4.8	16	0.61
	IS 2002	*cap7.2*	KG-E32/M49-334-NP2510	665.6*	–	1239.7	BG2814-6	4.0	25	–
	IS 2003		KG-E32/M49-334-NP2510	932.7*	–	1779.1	BG2814-6	4.0	21	–
Dihydrocapsaicin (ppm)	CA 2001	*dhc4.1*	Bcat-NP1015	1511.1	1842.4	2290.9	BG2814-6	3.2	12	−0.15
	IS 2003		Bcat-NP1015	826.9	1055.6	1399.7	BG2814-6	4.5	20	−0.20
	CA 2001	*dhc4.2*	GP1042-GP20039c	1646.7	–	2271.2*	BG2814-6	3.9	12	–
	IS 2003		GP1042-GP20039c	989.1	–	1364.5*	BG2814-6	3.1	12	–
	IS 2002	*dhc7.1*	NP2382-NP1005	749.5	992.7	1031.2	BG2814-6	4.5	14	0.64
	IS 2003		NP2382-NP1005	902.3	1130.7	1217.6	BG2814-6	3.2	9	0.45
	IS 2002	*dhc7.2*	KG-E32/M49-334-NP2510	664.2*	–	1009.7	BG2814-6	3.1	19	–
Nordihydrocapsaicin (ppm)	CA 2001	*ndhc7a.1*	TG199-NP2431	484.2	508.8	837.2	BG2814-6	5.0	22	−0.79
	IS 2003		TG199-NP2431	186.1	192.2	299.6	BG2814-6	3.2	15	−0.91
Total capsaicinoids (ppm)	CA 2001	*total3.1*	3A2-AA840692	4672.1	–	6290.8*	BG2814-6	3.1	12	–
	CA 2001	*total4.1*	Bcat-NP1015	4170.6	5126.7	6374.6	BG2814-6	3.5	12	−0.13
	IS 2002		Bcat-NP1015	1699.4	2156	2687.1	BG2814-6	3.2	15	−0.07
	IS 2003		Bcat-NP1015	2139.1	2796.2	3747.3	BG2814-6	4.2	18	−0.19
	CA 2001	*total4.2*	GP1042-GP20039c	4618.5*	–	6530.4	BG2814-6	3.9	13	–
	IS 2003		GP1042-GP20039c	2556.9*	–	3801	BG2814-6	4.0	16	–
	IS 2002	*total7.1*	NP2382-NP1005	1704.9	2362.7	2457.8	BG2814-6	5.2	14	0.55
	IS 2003		NP2382-NP1005	2228.8	3065.3	3271.6	BG2814-6	4.2	11	0.61
	IS 2002	*total7.2*	KG-E32/M49-334-NP2510	1,472	–	2405.1*	BG2814-6	3.8	23	–
	IS 2003		KG-E32/M49-334-NP2510	1,963	–	3167.2*	BG2814-6	3.5	17	–
Fruit weight (gr)	IS 2003	*fw2.1*	BD076366-NP2292	4.50	3.09	1.75	RNaky	3.3	26	0.02
	IS 2002	*fw3.1*	BM061910-NP1530	2.84	1.76	1.31	RNaky	4.1	15	0.4
	IS 2003		BM061910-NP1530	6.47	2.99	2.4	RNaky	6.9	30	0.72

[a] QTLs are names using an abbreviation of the trait followed by chromosome number and QTL number in the chromosome

[b] The most significant marker is underlined

[c] *AA* homozygous RNaky, *AB* heterozygous, *BB* homozygous BG 2814-6

*Denotes dominant allele

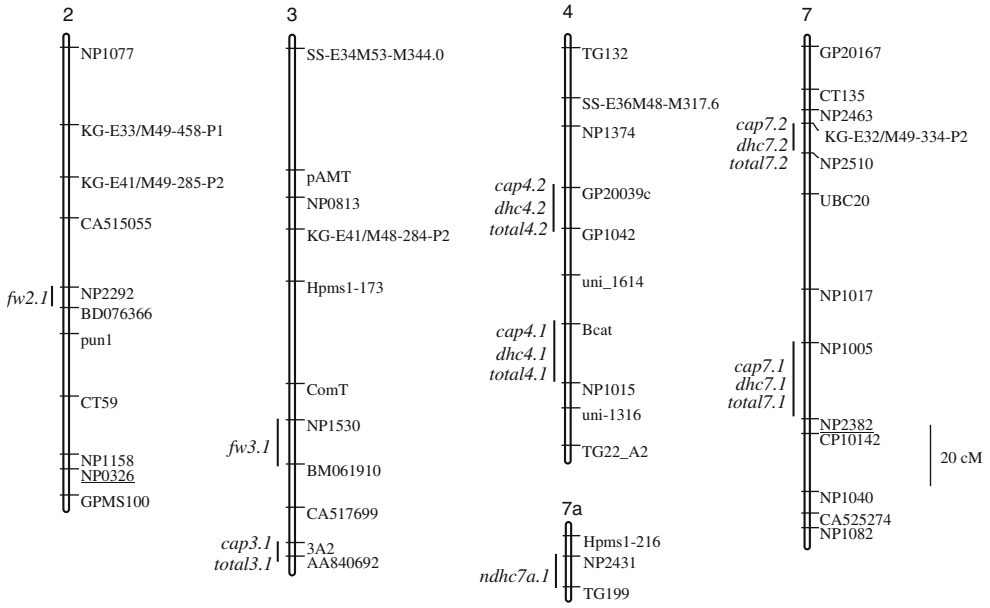

Fig. 1 Linkage map of the FA03 population illustrating QTL detected in this study. Only chromosomes containing QTL detected in this study are presented. The map contains limited number of framework markers. The complete map is presented at The Solanaceae Genome Network web site http://www.sgn.cornell.edu. Marker names and QTL designations are located to the right and left, respectively, of each linkage group. QTL intervals are presented as *solids bars*. *Underlined markers* participate in a digenic interaction

Table 4 Interactions between molecular markers associated with capsaicinoid content in the FA03 population

Trait	Location	Marker 1	LG	Marker 2	LG	*P*	R^2 (%)
Capsaicin	CA 2001	NP0326	2	NP2382	7	<0.0001	37
	IS 2002	NP0326	2	NP2382	7	<0.0001	37
	IS 2003	NP0326	2	NP2382	7	<0.0001	42
Dihydrocapsaicin	CA 2001	NP0326	2	NP2382	7	0.005	24
	IS 2002	NP0326	2	NP2382	7	0.005	28
	IS 2003	NP0326	2	NP2382	7	0.002	27

the QTL affecting capsaicin content consistent with the hypothesis that these positions are influencing the accumulation of both analogues. The proportion of the total phenotypic variation explained by all QTL for dihydrocapsaicin was 18, 13 and 25% in 2001, 2002 and 2003, respectively. The same digenic interaction detected for capsaicin content was also identified for dihydrocapsaicin content (Fig. 2). Again, this digenic interaction between *dhc7.1* and a position on chromosome 2 had the largest effect on the trait, explaining 24–28% of the total phenotypic variation compared to 9–14% explained by *dhc7.1* alone.

Nordihydrocapsaicin content Only one QTL, *ndhc7a.1*, that did not co-localize with QTL controlling the content of other capsaicinoids was detected. Again, the allele from the more pungent parent, BG 2814-6, increased capsaicinoid content and was almost completely recessive.

Total capsaicinoid content Five QTL that were identical in their position to the capsaicin content QTL were detected as significant for total capsaicinoid accumulation. The effects of the QTL ranged from 12 to 23% of the total explained phenotypic variation. The QTL with the largest effect was *total7.2*. The proportion of the total phenotypic variation explained by all QTL was 23, 27 and 31% in 2001, 2002 and 2003, respectively.

Capsaicin/Dihydrocapsaicin ratio No significant QTL were detected.

Fruit weight This trait was measured only in Israel 2002 and 2003. Two QTL were detected in chromosomes 2 (*fw2.1*) and 3 (*fw3.1*). As expected, the large-fruited RNaky alleles at both loci increased fruit size. Gene action was additive and overdominant at *fw2.1* and *fw3.1*, respectively.

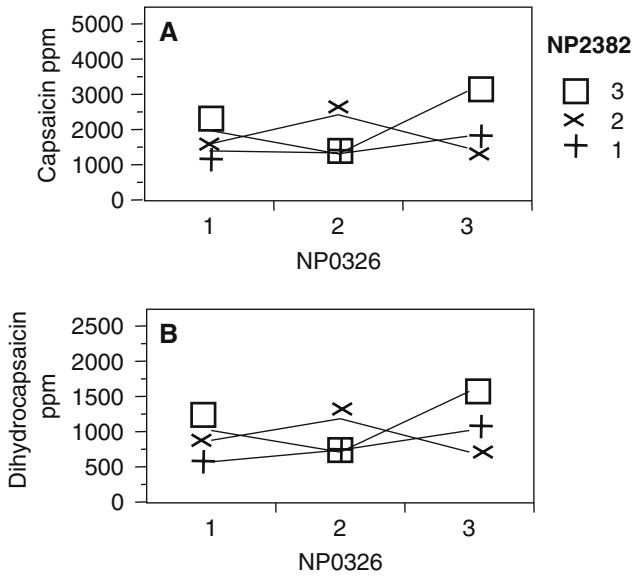

Fig. 2 Interaction plots of QTL detected in the FA03 that control capsaicinoid content. **a** Capsaicin content. **b** Dihydrocapsaicin content. The numbers 1, 2, and 3 represent the three genotypic classes at each marker: *1* homozygous RNaky, *2* heterozygous, *3* homozygous BG 2814-6

Co-segregation of candidate genes with QTL for capsaicinoid accumulation

Two candidate genes related to valine catabolism (3A2 and BCAT) appeared to co-localize with QTL for capsaicinoid accumulation, *cap3.1* and *cap4.1*, respectively. The candidate gene 3A2, based on an EST from a fruit-specific subtraction library (Liu et al. 2005) has motifs of hydroxyisobutyrate dehydrogenase, therefore a predicted role in the catabolism of valine, the building block of capsaicin and dihydrocapsaicin. BCAT is an enzyme known to function in the catabolism of branched-chain amino acids such as valine, leucine and isoleucine (Graham and Eastmond 2002). Because not all bands were mapped and only one segregating population has been sampled in this study, we cannot rule out the possibility that additional associations exist between candidate structural genes in this pathway and QTL that affect capsaicinoid content of fruit.

Discussion

For many years, the genetic control of capsaicinoid content in pepper was considered extremely complex, strongly influenced by the environment, difficult to assess in large-scale studies and possible to assay only at the end of the plant life cycle. In recent years, however, integration of resources and approaches drawn from molecular biology and genomics into plant genetics has allowed key advances in our understanding of capsaicinoid biosynthesis. Phenylpropanoid biosynthesis from which the alkaloid moiety in capsaicinoids is derived, is now one of the best characterized secondary metabolic pathways in plants (Winkel-Shirley 2001). A number of structural and regulatory genes have also been implicated in capsaicin biosynthesis directly, based on differential up-regulation in pungent genotypes relative to non-pungent genotypes (Aluru et al. 2003; Curry et al. 1999; Kim et al. 2001; Stewart et al. 2005). The controlling transcription factors, however, remain unknown. It is possible that the QTL controlling capsaicinoid content identified in the present study may represent one or more of these missing elements in the capsaicinoid biosynthetic pathway.

While the absolute level of pungency is clearly important with respect to the perception of heat in a pepper, there is evidence also that different capsaicinoid analogues affect location, duration and intensity of the pungent sensation when a pepper is consumed (Krajewska and Powers 1988). In this study, we detected two QTL that affected one analogue without an influence on the other. One QTL, *cap3.1* was detected that influenced capsaicin and total capsaicinoid content, while *ndhc7a.1* affected only nordihydrocapsaicin (Fig. 1). Our relative inability to detect QTL (except *ndhc7a.1*) controlling nordihydrocapsaicin content can likely be attributed to its low levels in this mapping population. Although *cap3.1* and *ndhc7a.1* were detected as significant when each capsaicinoid was evaluated individually, their effects on overall ratios of capsaicinoids were not judged to be significant.

Our aim in searching for QTL that control the relative ratio of capsaicinoid analogues was to locate regions in the genome that might independently affect a single analogue. In theory, such a gene might control, for example, the degree of saturation of the fatty acids, or might determine relative ratios of substrates as illustrated by *Fgr* which modulates the ratio of fructose to glucose in mature tomato fruit (Levin et al. 2000). In fact, the identification of overlapping QTL that affect accumulation of each capsaicinoid analogue, with the exception of *cap3.1* and *ndhc7a.1* suggests the existence of a common genetic mechanism that accounts for accumulation of multiple capsaicinoid analogues. This is not unexpected given the relative stability of capsaicin analogue ratios in a wide array of domesticated pepper varieties. The QTL, *cap3.1*, may exert an effect that could overlie the common mechanism, resulting in a shift of one analogue relative to the others, although the extent of this influence did not result in a statistically significant QTL for capsaicinoid ratio at this position.

Because of the possible confounding effect of fruit size on a metabolic pathway affected by substrate levels and flux through the pathway, fruit weight data were collected in 2 years. Two fruit weight QTL were detected on chromosomes 2 and 3, however, neither co-localized with capsaicinoid QTL. This observation suggests that fruit weight, per se, does not have a significant effect on capsaicinoid accumulation, all other things equal, however a moderately significant negative correlation was detected (Table 2). Because capsaicinoids are synthesized only in the placenta, the inclusion of the pericarp tissue and seeds in samples collected for analysis likely diluted capsaicinoids as a function of fruit size and pericarp thickness. This is the probable basis for the observed negative correlation between capsaicinoid content and fruit weight. Based on their positions, the two fruit weight QTL detected in this study are likely orthologous to those detected in previous studies in pepper (Ben-Chaim et al. 2001; Rao et al. 2003).

The QTL detected in this study for capsasicin content were roughly similar in the magnitude of their effect. The QTL, *cap7.2*, had slightly higher effect than the other QTL and is likely orthologous to a major capsaicinoid content QTL, *cap*, previously identified in a population derived from *C. annuum* × *C. frutescens* cross by Blum et al. (2003). This conclusion is based on the location of the common marker UBC20, linked to this QTL in both studies. The largest effect on capsaicinoid content detected in the present study, however, was attributed to the interaction of *cap7.1*, a QTL not detected by Blum et al. (2003), and the marker NP0326 located on chromosome 2. An important distinction between the two crosses is that the *C. annuum* parent used by Blum et al. (2003) was a non-pungent genotype, while the *C. annuum* parent used in the present study was pungent, albeit at low levels. In the earlier study, the *pun1* allele was segregating and therefore present in the homozygous state in one-fourth of the progeny. Although only pungent plants were analyzed, 25% of the population was excluded due to the epistatic effect of *pun1/pun1*.

The type of digenic interaction observed in this study where one or both positions involved in the interaction has little or no detectable phenotypic effect alone can cause considerable frustration in a conventional plant-breeding program. The favorable configuration can come and go during crosses made to improve type or transfer other traits and confusing segregation is observed, where it is evident at all. Knowledge of the importance of this particular interaction will facilitate further genetic analysis of this complex genetic trait in pepper. The utilization of a QTL in breeding programs depends on its stability across environments and genetic backgrounds. Most QTL identified in the present study were detected consistently, indicating these are likely alleles with relatively stable effects that may be useful in breeding programs related to pungency in pepper.

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