

cTOF-21-J12, 43 cM, Chromosome 7 (A co-dominant SCAR marker for *I3* gene)

Ana Cristina Barillas and Douglas P. Maxwell, University of Wisconsin-Madison
September 3, 2008

Introduction

The goal for this research was to develop a co-dominant CAPS or SCAR marker for the *I3* gene, which conditions resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 3. PCR primers for markers in the chromosomal region between the molecular markers TG183 (42 cM) and TG639 (43.3 cM) (Hemming et al., 2004) were evaluated on homozygous susceptible, and homozygous resistant tomato inbred lines as well as heterozygous F1 hybrids obtained from J. W. Scott, University of Florida, R. Gardner, North Carolina State University or commercial hybrids.

Reference:

Hemming, M. N., S. Basuki, D. J. McGrath, B. J. Carroll, and D. A. Jones. 2004. Fine mapping of the tomato *I-3* gene for Fusarium wilt resistance and elimination of a co-segregating resistance gene analogue as a candidate for *I-3*. *Theor. Appl. Gen.* 109:409-418.

Primers

The sequence of cTOF-21-J12 was compared with sequences at GenBank. Putative exons were identified by the match with *Vitis vinifera*, AM427259, and forward primer, P7-43DF1, and reverse primer, P7-43DR1, were designed from the putative exon regions to amplify a genomic region, which should include at least one intron. Two long fragments of approximately 1,060 bp and 1,270 bp resulted for the susceptible and resistant genotypes, respectively. Forward primer P7-43DF3 was designed from the sequences to give shorter PCR fragments of 650 bp for the susceptible and 875 bp for the resistant genotype.

Table 1. PCR primers on chromosome 7

Primer Name	Primer Sequence (5'-3')
P7-43DF1	GGTAAAGAGATGCGATGATTATGTGGAG
P7-43DF3	CACGGGATATGTTRTTGATAAGCATGT
P7-43DR1	GTCTTTACCACAGGAACCTTATCACC

*PCR annealing temperature at 53°C (TGEN53).

Sequence

M82 (Partial Sequence), (i3/i3)

```

1      GGTAAAGAGA TCGGATGATT ATGTGGAGGT GATGTGTGGG TGTACAAGCC ATCGATATGG
61     TGATGCTGTT GCTAGACTTA GGGTTTTCTC GTCTGGTGAA CTGAAATCA CCTGTGAATG
121    TACTCCTGGA TGCCTGAAG GTTTTTTTTT TTAACAACT TTAGATCAA TGGGGGTTT
181    CGTCAAATGC ATGTTGTTTC TGGGTTTTAT TCTATCGTCG ACTCTGTGTT TGATTATGT
241    GTAACATTTT ATGTGTATGT AAGTTTGCCT CAGAGAGCAT AGTTTCTGCT TCTGTGGTAG
301    TGTGTGTTCT TTGGTGTGTT GTGTTGAAGT TCTTGATGTT TCTTTGGCAA TTTTGTGCGT
361    CTCTTGTCAA CCTTACCTTG CGTACATTCT ATGTCTCAAG ACTCCACTTT GTGAAATTAC

```

```

421   ACGGGATATG TTATTGATAA GCATGTCAAC AAAGGTAGAA ATACATTGTG TGTTCGTTAT
481   GTGATAATGG TTCTTTGAAG GCAGGTTTGTG TTGTTTAAAGA CTCTTGACCT TTGATGCAGA
541   GTTAGAGTTC TGTTTGATTT TTCTTCAAAG CATCACTTTT GATGGTATCA TCTTATGCTA
601   AACAACTCAT TGGTCTACC ATTTCTACAA CTTGGCCATG ACAACTTTTT GAAAACCATC
661   AACTCTTAAT TAATAAATGT TTATCGTGGG GCTTGTGCAG TGTTTTTGGT TGAATATTTA
721   GCTGTGATAT CAAAAGAAAT CTTATACTAT CACTCACCAG ATTCTCTCGA CAGCATTTGT
781   TGTGAGAGCA TATTTCTCTT TTTAATCATT CACTATGTAT CCTCCTGGTT TCCTCCTTTC
841   TTTATATTTT ACAATCATAA GCCACAAAGA ATCGATTTTG TACTTGATAA AATAACACCC
901   CACAGAAAGA ATTATCTGAT AAAGTAAAT AAGTCTTACC ACTTGTACTT GTGCTACTTA
961   ACAAGAGTCT CTTTTTTCAC TGTCTGGAAT GTAGATAGTC AGCTTCTTAG TTGTTAATAC
1021  TTCTTGTATG TCAATGGTGA TAAAGTTCCT GTGGTAAAGA C

```

NC123S, I3/I3 (Partial Sequence)

```

1     GGTAAGAGA TGCGATGATT ATGTGGAGGT GATGTGTGGG TGTACAAGCC ATCGATATGG
61    TGATGCTGTT GCTAGACTTA GGGTTTCTC GTCTGGTGAA CTTGAAATCA CCTGTGAATG
121   TACTCTGGA TGCCTGAAG GTTTCTTTT TTTAACAAAC TTTAGATCAA ATGGGGGTTT
181   GCGTCAAATG CATGTTGTTC GTGGGTTTTA TTCTATCGTC GACTCTGTGT TTGATTTATG
241   TGTAACATTT TATGTGTATG TAAGTTGTC TCAGAGAGCA TAGTTTCTGC TTCTGTGGTA
301   CTGTGTGTTT TTTGGTGTG TGTGTTGAAG GTCTTGATGT TTCTATGGCA ATTTTGTGCG
361   TCTCTGTCA ACCTTACCTT GCGTACATTC TATCATCTCC AGACTCCACT TGTGAGATTA
421   CACGGGATAT GTTGTGATA AGCATGTCAA ACAAAGGTAG AAATACATTG TGTGATAATG
481   GTTCTTTGAA GGCAGGTTTA GTTGTTTAAG ACTCTTGCTT GACCTTTGAT GCAGAGTTAG
541   AGTTCTGTTT GATTTTCTT CAAAGCATCA CTTTGTATGG TATCAACTTA TGCTAAACAA
601   CTCAATGGTT TACCATTTC TACAACCTGG CCATGGCAAC TTTTGAATA CTATCAACTC
661   TTAATTAAT AATGTTTATC GTGGGCTTG TGCAGTGTG TTGGTTGAAT ATTTAGCTGT
721   GATATCAAAA GAAATCTTAT ACTATCACTC ACCAGATTCT CTCGACAGCA TTTGTGTCA
781   GAGCATATTT CTCTTTTAA TCATTCACTA TGTATCCTCT TGGTTTCTC CTTCCTTTAT
841   ATTTTACAAT CATAAGCCAC AAAGAATCGA TTTGTACTT TGATAAAATA ACACCCACA
901   GAAAAAATTA TCTGATAAAG TAAATTAAGT CCTACCCTT GTACTTGTGC TACTTAACAA
961   GAGTCTCTTT TTTCTCTGTC TGGAATGTAG ATAGTCAGCT TCTTAGTGT AAATACTTCT
1021  TGTATGTGCT GTAATAATGA GTGGAGAGTT AATGGAACGT GTTATGTTGA ATCTGATGCT
1081  ATATTTTCTC CTCTTTTACA TTATTTTATA GTCTTGTGTG GCATTGTGAT CCTATTAAGA
1141  GTTTTGTGTT TTGTGAACAG ACAAGCTTAC TCCGTCTGCA TTTGAGAAGC ATTCTGGGAG
1201  AGAACTGCT AGGAAATGGA AAAATAATGT ATGGATCATT GTCAATGGTG ATAAAGTTCC
1261  TGTGGTAAAG AC

```

Comparison of M82 (top sequence) with NC EBR-8 (bottom sequence)

```

M82_P7-43DF1      GGTAAGAGATGCGATGATTATGTGGAGGTGATGTGTGGGTGTACAAGCCATCGA      60
CB5_P7-43DF1      GGTAAGAGATGCGATGATTATGTGGAGGTGATGTGTGGGTGTACAAGCCATCGA

M82_P7-43DF1      TATGGTGATGCTGTTGCTAGACTTAGGGTTTTCTCGTCTGGTGAACCTTGAATCACCTGT      120
CB5_P7-43DF1      TATGGTGATGCTGTTGCTAGACTTAGGGTTTTCTCGTCTGGTGAACCTTGAATCACCTGT

M82_P7-43DF1      GAATGTACTCCTGGATGCACTGAAGTTTT.TTTTTTAACTTTAGATCAAATGGG      180
CB5_P7-43DF1      GAATGTACTCCTGGATGCACTGAAGTTTTCTTTTTTAACTTTAGATCAAATGGG

M82_P7-43DF1      GGTTCGCTCAAATGCATGTTGTTTCGTGGGTTTTATTCTATCGTCGACTCTGTGTTTGAT      240
CB5_P7-43DF1      GGTTCGCTCAAATGCATGTTGTTTCGTGGGTTTTATTCTATCGTCGACTCTGTGTTTGAT

M82_P7-43DF1      TTATGTGTAACATTTTATGTGTATGTAAGTTTGCTCAGAGAGCATAGTTTCTGCTTCTG      300
CB5_P7-43DF1      TTATGTGTAACATTTTATGTGTATGTAAGTTTGCTCAGAGAGCATAGTTTCTGCTTCTG

M82_P7-43DF1      TGGTAGTGTGTGTTCTTTGGTGTGTGTGTTGAAGTCTTGTATGTTTCTTTGGCAATTTT      360
CB5_P7-43DF1      TGGTAGTGTGTGTTCTTTGGTGTGTGTGTTGAAGGTCTTGTATGTTTCTATGGCAATTTT

M82_P7-43DF1      GTGCGTCTCTGTCAACCTTACCTTGCCTACATTTCTATGCTCAAGACTCCACTTTGTGA      420
CB5_P7-43DF1      GTGCGTCTCTGTCAACCTTACCTTGCCTACATTTCTATCATCTCCAGACTCCACTTTGTGA

M82_P7-43DF1      AATTACACGGGATATGTTATTGATAAGCATGTC.AACAAAGGTAGAAATACATTGTGTGT      480
CB5_P7-43DF1      GATTACACGGGATATGTTATTGATAAGCATGTC AACAAAGGTAGAAATACATTGTGTGT.

M82_P7-43DF1      TCGTTATGTGATAATGGTTCCTTTGAAGCAGGTTTAGTTGTTTAAAGCT....CTTGACC      540

```

CB5_P7-43DF1ATAATGGTTCTTTGAAGGCAGGTTTAGTTGTTTAAAGACTCTTGCTTGACC	
M82_P7-43DF1	TTTGATGCAGAGTTAGAGTTCTGTTTGATTTTTCTCAAAGCATCACTTTTGATGGTATC	600
CB5_P7-43DF1	TTTGATGCAGAGTTAGAGTTCTGTTTGATTTTTCTCAAAGCATCACTTTTGATGGTATC	
M82_P7-43DF1	ATCTTATGCTAAACAACCTCATTGGTTCTACCATTTCTACAACCTGGCCATGCAACTTTT	660
CB5_P7-43DF1	AACCTTATGCTAAACAACCTCATTGGTTCTACCATTTCTACAACCTGGCCATGGCAACTTTT	
M82_P7-43DF1	TGAAAACCATCAACTCTTAATTAATAAATGTTTATCGTGGGGCTTGTGCAGTGTTTTTGG	720
CB5_P7-43DF1	TGAAAACCTATCAACTCTTAATTAATAAATGTTTATCGTGGGGCTTGTGCAGTGTTTTTGG	
M82_P7-43DF1	TTGAATATTTAGCTGTGATATCAAAGAAATCTTATACTATCACTCACCAGATTCTCTCG	780
CB5_P7-43DF1	TTGAATATTTAGCTGTGATATCAAAGAAATCTTATACTATCACTCACCAGATTCTCTCG	
M82_P7-43DF1	ACAGCATTGTTGTGCAGAGCATATTTCTCTTTTTAATCATTCACTATGTATCCTCCTGGT	840
CB5_P7-43DF1	ACAGCATTGTTGTGCAGAGCATATTTCTCTTTTTAATCATTCACTATGTATCCTCCTGGT	
M82_P7-43DF1	TTCTCTCTTTCTTTATATTTTACAATCATAAGCCACAAAGAATCGATTTTGATC.TTGAT	900
CB5_P7-43DF1	TTCTCTCTTTCTTTATATTTTACAATCATAAGCCACAAAGAATCGATTTTGATCTTGAT	
M82_P7-43DF1	AAAATAACACCCACAGAAA GAATTATCTGATAAAGTAAATTAAGTCCTACCCTTGATC	960
CB5_P7-43DF1	AAAATAACACCCACAGAAA AAATTATCTGATAAAGTAAATTAAGTCCTACCCTTGATC	
M82_P7-43DF1	TTGTGCTACTTAACAAGAGTCTCTTTTTTCACTGTCTGGAATGTAGATAGTCAGCTTCTT	1020
CB5_P7-43DF1	TTGTGCTACTTAACAAGAGTCTCTTTTTTCTCTGTCTGGAATGTAGATAGTCAGCTTCTT	
M82_P7-43DF1	AGTTGTTAATACTTCTTGTATGT.....	1080
CB5_P7-43DF1	AGTTGTAATACTTCTTGTATGTCTGTGTAATAAGAGTGGAGGTTAATGGACGTTTA	
M82_P7-43DF1	1140
CB5_P7-43DF1	TGTTGAATCTGATGCTATATTTTCTCTCTTTTACATTATTTTATAGTCTTGTGTCAT	
M82_P7-43DF1	1200
CB5_P7-43DF1	TGTGATCCTATTAAGAGTTTGTGTTTTGTGAACAGACAAGCTTACTCCGTCTGCATTG	
M82_P7-43DF1CA	1260
CB5_P7-43DF1	AGAAGCATTCTGGGAGAGAACTGCTAGGAAATGGAAAAATAATGTATGGATCATTGTCA	
M82_P7-43DF1	ATGGTGATAAAGTTCCTGTGGTAAAGAC	1288
CB5_P7-43DF1	ATGGTGATAAAGTTCCTGTGGTAAAGAC	

Comments

Partial sequences were obtained for four tomato samples. M82 (susceptible) and NC123S (resistant) sequences had differences of approximately 28 SNPs and 6 indels, one of which was 215 bp. The M820 sequence was the same as the other susceptible line, GMh6330; and the NC123S sequence was the same as the other resistant line sequence, NC EBR-8.

The sequence of M82 had 100% nt identity with the chromosome 7 BAC clone C07HBa0045O10 at the SGN site and matches nt 114,068-115,118. The sequence of NC123S matched the same clone with a 97% nt identity.

This primer pair can be used as a co-dominant SCAR marker for the detection of the I3 gene in tomato breeding programs.