

## C2\_At3g21820, 43 cM, Chromosome 7

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July 8, 2008

### Introduction

The goal for this research was to develop a co-dominant CAPS or SCAR marker for the *I3* gene, which corresponds to 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 C2\_At3g21820 (SGN-U327486, 660 nt) was compared with sequences at GenBank. Putative exons were identified by the match with *Vitis vinifera*, AM482072, and two forward and two reverse primers were designed from the putative exon regions to amplify a genomic region, which should include at least one intron. The primer pair listed below gave the strongest single PCR fragment.

Table 1. PCR primers on chromosome 7

Primer Name	Primer Sequence (5'-3')
P7-43F1	AGGGGTTTATGCTGAGACGGATTTCAAAGAAG
P7-43R1	TTTTGAAGGGCTAGAAGAACATTCTCC

\*PCR annealing temperature at 53°C (TGEN53), ~400-bp fragment.

### Sequence

Purple Russian Allele a, i3/i3 (Partial Sequence); Purple Russian is a heritage tomato (OP) from Seed Savers Exchange, Decorah, Iowa

GENBANK ACCESSION NUMBER: EU915399

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1      TAAGNTGTCT GAGGACAATG CTTGCTGGTG CCCAACATCC TTCAAATAAG GTATTGCTGA
61     TGGAACTACT GGCACAGTGT CATTTCCTTC CTCACAGTAA TCATCTGACC TTTTTTTTTT
121    TGTATTTTTT CAATTTCTC TTGGTGGACA TGCTTCACCT TGATCTTTTG CATGTGGCAG
181    GTAGACTGCT TGGTATGTAG TTATTGTTTC TGCTTTGTTG GGTCTATAGA GCTTCAAATT
241    GGGAGGAAGC TATATTTAGA ACAGCTGGGA GTCTCCCCTA TCGATGAGTG TCATATGCAA
301    AAAGATTGTT ATAACTCTGA TTCATCTGTT GGTGAAGATG ATTCTGATGT AGAAGTCGCG
361    C
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Purple Russian Allele b, i3/i3 (Partial Sequence)

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1      TAAGNTGTCT GAGGACAATG CTTGCTGGTG CCCAACATCC TTCAAATAAG GTATTGCTGA
61     TGGAACTACT GGCACAGTGT CATTCTCTTC CTCACAGTAA TCATCTGACC TTTTFTTTTT
121    TTGTATTTTT TCAATTTTCT CTTGGTGGAC ATGCTTCACC TTGATCTTTT GCATGTGGCA
181    GGTAGACTGC TTGGTATGTA GTTATTGTTT CTGCTTTGTT GGGTCTATAG AGCTTCAAAT
241    TGGGAGGAAG CTATATTAG AACAGCTGGG AGTCTCCCCT ATCGATGAGT GTCATATGCA
301    AAAAGATTGT TATAACTCTG ATTCATCTGT TGGTGAAGAT GATTCTGATG TAGAAGTCGC
361    GC
  
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NC EBR-8, I3/I3 (Partial Sequence), inbred from R. Gardner

GENBANK ACCESSION NUMBER: EU926655

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1      TTAGGGGTTT ATGCTGAGAC GGATTTCAA GAAGAGGACC TTGTCTTGAA GGATCAAATG
61     CTTGCTGGTG CCCAACATCC TTCAAATAAG GTATTGCTGA TGGAACTAGT GGCACAGTGT
121    CATTCTCTTC CTCGCAGTAA TCATCTGACT TTTTFTTTGT TGGTATTTTT TCAATTTTCT
181    CTTGGTGGAC ATGCTTCACC TTGATCTTTT GCTTGTGGCA GGTAGACTGC TTGGTATGTA
241    GTTATTGTTT CTGCTTTGTT GGGTCTATAG AGCTTCAAAT TGGGAGGAAG CTATATTAG
301    AACAGCTGGG TGTCTCCCCT AACGATGAGT GTCATATGCA AAAAGATTGT TATAACTCTG
361    ATTCATCTGT TGGTGAAGAT GATTCTGATG TAGAAGATCA GCAAGTATCT GGAGAATGTT
421    CTTCTAGCCC TTCAAAA
  
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Comparison of Purple Russian b (top sequence) with NC EBR-8 (bottom sequence)

PURPLE RUSSIAN A	.....TAAGNTGTCT..GAGGAC..AATG	20
PURPLE RUSSIAN B	.....TAAGNTGTCT..GAGGAC..AATG	20
NC EBR-8	TTAGGGGTTTATGCTGAGACGGATTCAAAGAAGAGGACCTTGTCTTGAAGGATCAAATG	60
CONSENSUS	A TGTCT AGGA AATG	
PURPLE RUSSIAN A	CTTGCTGGTGCCCAACATCCTTCAAATAAGGTATTGCTGATGGAAC <b>T</b> GGCACAGTGT	80
PURPLE RUSSIAN B	CTTGCTGGTGCCCAACATCCTTCAAATAAGGTATTGCTGATGGAAC <b>T</b> GGCACAGTGT	80
NC EBR-8	CTTGCTGGTGCCCAACATCCTTCAAATAAGGTATTGCTGATGGAAC <b>T</b> GGCACAGTGT	120
CONSENSUS	CTTGCTGGTGCCCAACATCCTTCAAATAAGGTATTGCTGATGGAAC <b>T</b> GGCACAGTGT	
PURPLE RUSSIAN A	CATTTCTTCCTC <b>A</b> CAGTAATCATCTGAC <b>C</b> TTTTTTTT <b>T</b> .GTATTTTTTCAATTTTCT	139
PURPLE RUSSIAN B	CATTTCTTCCTC <b>A</b> CAGTAATCATCTGAC <b>C</b> TTTTTTTT <b>T</b> <b>T</b> GTATTTTTTCAATTTTCT	140
NC EBR-8	CATTTCTTCCTC <b>G</b> CAGTAATCATCTGAC <b>T</b> TTTTTTTT <b>G</b> <b>T</b> GTATTTTTTCAATTTTCT	180
CONSENSUS	CATTTCTTCCTC CAGTAATCATCTGAC TTTTTTTT TT GTATTTTTTCAATTTTCT	
PURPLE RUSSIAN A	CTTGGTGGACATGCTTCACCTTGATCTTTTGC <b>A</b> TGTGGCAGGTAGACTGCTTGGTATGTA	199
PURPLE RUSSIAN B	CTTGGTGGACATGCTTCACCTTGATCTTTTGC <b>A</b> TGTGGCAGGTAGACTGCTTGGTATGTA	200
NC EBR-8	CTTGGTGGACATGCTTCACCTTGATCTTTTGC <b>T</b> TGTGGCAGGTAGACTGCTTGGTATGTA	240
CONSENSUS	CTTGGTGGACATGCTTCACCTTGATCTTTTGC TGTGGCAGGTAGACTGCTTGGTATGTA	
PURPLE RUSSIAN A	GTTATTGTTTCTGCTTTGTTGGGTCTATAGAGCTTCAAATGGGAGGAAGCTATATTAG	259
PURPLE RUSSIAN B	GTTATTGTTTCTGCTTTGTTGGGTCTATAGAGCTTCAAATGGGAGGAAGCTATATTAG	260
NC EBR-8	GTTATTGTTTCTGCTTTGTTGGGTCTATAGAGCTTCAAATGGGAGGAAGCTATATTAG	300
CONSENSUS	GTTATTGTTTCTGCTTTGTTGGGTCTATAGAGCTTCAAATGGGAGGAAGCTATATTAG	
PURPLE RUSSIAN A	AACAGCTGGG <b>A</b> GTCTCCCCT <b>A</b> TCGATGAGTGTATATGCAAAAAGATTGTTATAACTCTG	319
PURPLE RUSSIAN B	AACAGCTGGG <b>A</b> GTCTCCCCT <b>A</b> TCGATGAGTGTATATGCAAAAAGATTGTTATAACTCTG	320
NC EBR-8	AACAGCTGGG <b>T</b> GTCTCCCCT <b>A</b> TCGATGAGTGTATATGCAAAAAGATTGTTATAACTCTG	360
CONSENSUS	AACAGCTGGG GTCTCCCCTA CGATGAGTGTATATGCAAAAAGATTGTTATAACTCTG	
PURPLE RUSSIAN A	ATTCATCTGTTGGTGAAGATGATTCTGATGTAGAAGTCGCGC.....	360
PURPLE RUSSIAN B	ATTCATCTGTTGGTGAAGATGATTCTGATGTAGAAGTCGCGC.....	361
NC EBR-8	ATTCATCTGTTGGTGAAGATGATTCTGATGTAGAAGATCAGCAAGTATCTGGAGAATGTT	420
CONSENSUS	ATTCATCTGTTGGTGAAGATGATTCTGATGTAGAAG GC	

## **Comments**

Partial sequences were obtained for the 13 tested samples. Purple Russian (susceptible) and NC EBR-8 (resistant) sequences had differences of approximately 8 SNPs. From the sequences of the forward and reverse primers for Purple Russian, it was evident that Purple Russian was heterozygous at this locus and the sequences for the two alleles were assembled (allele a and allele b), which differed by a single-nucleotide indel. The Purple Russian sequence was the same as the other susceptible line, L40, an F2 plant from Llanero; and the NC EBR-8 sequence was the same as the sequences from the other resistant line, FLA7547 and NC123S. The heterozygous germplasm, NC07196, had the expected differences for the SNPs between the susceptible and resistant lines. The sequence for allele b for Purple Russian did not match any BAC or fosmid clone sequences at SGN site.