

Identification and characterization of molecular marker(s) associated with resistance to TSWV and leaf spots in peanuts

Baozhu Guo, Corley Holbrook, Guohao He*,
Peggy Ozias-Akins, Albert Culbreath, and Craig Kvien

USDA-ARS and the University of Georgia, Costal Plain Experiment Station, Tifton, GA
*Tuskegee University, AL

SUMMARY

In peanut production areas in the southeastern U.S., tomato spotted wilt virus disease caused by tomato spotted wilt tospovirus (TSWV) has become more prevalent and more severe. TSWV has become a major limiting factor for many peanut producers and control methods are limited. Both early (*Cercospora arachidicola*) and late (*Cercosporidium personatum*) leaf spot diseases are also among the worst foliar diseases of the cultivated peanut. Our strategy is to develop peanut cultivars with resistance to these diseases by using marker-assistant breeding and selection. We have been characterizing and developing DNA polymorphic markers associated with the resistant traits in peanut lines resistant or susceptible to TSWV and/or leaf spots, and generating a segregating population to map/clone the resistant loci/gene(s). RILs (recombinant inbred lines) are derived from Tifrunner, Runner type and resistant to TSWV and leaf spots, and GT-C20, Spanish type and susceptible to TSWV and leaf spots but resistant to *Aspergillus/aflatoxin*, bacteria wilt and rust. We also screened peanut lines, 0013 and 448A (resistant) and GK7 and Coan (susceptible) for polymorphic DNA markers.

The progress has been made (2006):

1. One segregating population has been made from the cross between Tifrunner and GT-20 (a Spanish type), and 180 F4/5 have been reached.
2. Total 450 soybean SSR (simple sequence repeats) markers from soybean have been translated to peanut. Total 87 SSRs have been able to amplify peanut genomic DNA.
3. EST-based SSRs have been developed from peanut ESTs from 8 cDNA libraries. Over 2,000 ESTs have been sequenced from 2 cDNA libraries, Tifrunner leaf tissues and 013 immature seeds. Total 1345 EST sequences have been deposited in GenBank (accession number CD037499 to CD038843), and 400 unigenes have been generated from these ESTs and used for microarray analysis. Over 40 EST-derived SSRs have been developed.
4. Total 278 SSRs have been developed from about 5,000 sequences from SSR enriched genomic libraries, and deposited into GenBank (accession number AY526357 to AY526456, and AY731521 to AY731698).
5. Total 22,944 EST clones have been attempted for sequencing and 20,048 quality ESTs have been achieved from 6 cDNA libraries of Tifrunner and GT-C20 with 4,100 and 2,700 unigenes, respectively. The preliminary data show that there are over 600 SSRs and SNPs.

Table 1. Summary of peanut seed ESTs. Libraries of cDNA were constructed from Tifrunner (TF) and GT-C20 (C20) seed tissues of R5, R6, and R7, respectively.

| | TFR5 | TFR6 | TFR7 | C20R5 | C20R6 | C20R7 | Total Sequences |
|----------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| | 003-06 | 001-05 | 001-02 | | 001-02 | 001-02 | |
| Plates Attempted | 2 | 0 | 4 | 001-055 | 4 | 6 | |
| Singlet After Trimming | 5,100 | 4,230 | 2,046 | 4,678 | 1,977 | 2,017 | 20,048 |
| Contigs and Singlets | 2,695 | 1,769 | 622 | 1,825 | 681 | 685 | |
| Contigs | 559 | 302 | 141 | 390 | 101 | 138 | |
| Sinnglets | 2,136 | 1,467 | 481 | 1,435 | 580 | 547 | |
| % Redundancy at 90% (50bp) | 47.2% | 58.2% | 69.6% | 61% | 65.6% | 66% | |
| Total sequences attempted | 5,760 | 4,800 | 2,304 | 5,280 | 2,304 | 2,496 | 22,944 |
| Total unigenes | 4,186 | | | 2,701 | | | 5,812 |
| Total SSRs | 468 | | | 338 | | | |

Fig. 1. SNPs between TR-Tifrunner and C20:

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Contig[0857a]_TF      AGGTCACGTGCTTGTGGTGCCACAGAACTTCGCCGTGGCTGGAAAGTCCCAGAGCGAGAA 1431
Contig[1574a]_C20    AGGTCACGTGCTTGTGGTGCCACAGAACTTCGCCGTGGCTGGAAAGTCCCAGAGCGAGAA 1426
Contig[1594a]_C20    AGGTCACGTGCTTGTGGTGCCACAGAACTTCGCCGTGGCTGGAAAGTCCCAGAGCGAGAA 1426
Contig[0001b]_TF     AGGTCACGTGCTTGTGGTGCCACAGAACTTCGCCGTGGCTGGAAAGTCCCAGAGCGAGAA 1429
Contig[0001c]_TF     GGGTCACGTGCTTGTGGTGCCACAGAACTTCGCCGTGGCTGGAAAGTCCCAGAGCGAGAA 1405
Contig[0001e]_TF     AGGTCACGTGCTTGTGGTGCCACAGAACTTCGCCGTGGCTGGAAAGTCCCAGAGCGAGAA 1259
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Contig[0857a]_TF      CTTTGAATACGTGGCATTCAAGACAGACTCAAGGCCAGCATAGCCAACCTAGCCGGTGA 1491
Contig[1574a]_C20    CTTTGAATACGTGGCATTCAAGACAGACTCAAGGCCAGCATAGCCAACCTAGCCGGTGA 1486
Contig[1594a]_C20    CTTTGAATACGTGGCATTCAAGACAGACTCAAGGCCAGCATAGCCAACCTAGCCGGTGA 1486
Contig[0001b]_TF     CTTTGAATACGTGGCATTCAAGACAGACTCAAGGCCAGCATAGCCAACCTAGCCGGTGA 1489
Contig[0001c]_TF     CTTTGAATACGTGGCATTCAAGACAGACTCAAGGCCAGCATAGCCAACCTAGCCGGTGA 1465
Contig[0001e]_TF     CTTTGAATACGTGGCATTCAAGACAGACTCAAGGCCAGCATAGCCAACCTAGCCGGTGA 1319
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Contig[0857a]_TF      AAATCCTTCATAGATAACTTGCCGGAGGAGGTGGTTGCAAATTCATATGGCCTCCCAAG 1551
Contig[1574a]_C20    AAATCCTTCATAGATAACTTGCCGGAGGAGGTGGTTGCAAATTCATATGGCCTCCCAAG 1546
Contig[1594a]_C20    AAATCCTTCATAGATAACTTGCCGGAGGAGGTGGTTGCAAATTCATATGGCCTCCCAAG 1546
Contig[0001b]_TF     AAATCCTTCATAGATAACTTGCCGGAGGAGGTGGTTGCAAATTCATATGGCCTCCCAAG 1549
Contig[0001c]_TF     AAATCCTTCATAGATAACTTGCCGGAGGAGGTGGTTGCAAATTCATATGGCCTCCCAAG 1525
Contig[0001e]_TF     AAATCCTTCATAGATAACTTGCCGGAGGAGGTGGTTGCAAATTCATATGGCCTCCCAAG 1379
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Contig[0857a]_TF      GGAGCAGGCAAGGCAGCTTAAGAACAACAACCCCTTCAAGTTCCTCGTTCACCGTCTGA 1611
Contig[1574a]_C20    GGAGCAGGCAAGGCAGCTTAAGAACAACAACCCCTTCAAGTTCCTCGTTCACCGTCTGA 1606
Contig[1594a]_C20    GGAGCAGGCAAGGCAGCTTAAGAACAACAACCCCTTCAAGTTCCTCGTTCACCGTCTGA 1606
Contig[0001b]_TF     GGAGCAGGCAAGGCAGCTTAAGAACAACAACCCCTTCAAGTTCCTCGTTCACCGTCTGA 1609
Contig[0001c]_TF     GGAGCAGGCAAGGCAGCTTAAGAACAACAACCCCTTCAAGTTCCTCGTTCACCGTCTCA 1585
Contig[0001e]_TF     GGAGCAGGCAAGGCAGCTTAAGAACAACAACCCCTTCAAGTTCCTCGTTCACCGTCTGA 1439
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Contig[0857a]_TF      ACAGTCTCTGAGGGCTGTGGCTTAAAAACAAGCGTGACATGTATGTGTGTATCCACTAC 1671
Contig[1574a]_C20    ACAGTCTCTGAGGGCTGTGGCTTAAAAACAAGCGTGACATGTATGTGTGTATCCACTAC 1666
Contig[1594a]_C20    ACAGTCTCTGAGGGCTGTGGCTTAAAAACAAGCGTGACATGTATGTGTGTATCCACTAC 1666
Contig[0001b]_TF     ACAGTCTCTGAGGGCTGTGGCTTAAAAACAAGCGTGACATGTATGTGTGTATCCACTAC 1669
Contig[0001c]_TF     ACAGTCTCTGAGGGCTGTGGCTTAAAAACAAGCGTGACATGTATGTGTGTATCCACTAC 1645
Contig[0001e]_TF     ACAGTCTCTGAGGGCTGTGGCTTAAAAACAAGCGTGACATGTATGTGTGTATCCACTAC 1499
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