

Protocol for Sap Inoculation

David(Mingxiong) PANG and Jeanmarie Verchot

Texas A&M AgriLife Research and Extension-The Dallas Center

October 17th 2018

Part I

Natural RRV infection study

Procedure for SAP inoculation



RRV-Infected Julia Child branches (from California), PCR-verified and delivered from “College Station” by Maddi Shires.

Instantly collected the tissues separately and stored in -80°C (Green-Leaves, Red-Leaves, Stem-Thorns, and flowers and buds for later to extract total RNAs and prepare Sap for inoculations.

Manual Inoculation procedure: (Conducted on Feb. 12, 2018)

Step 1: Grind stored mixture ($\sim 0.5\text{g}$) of RRV-infected rose tissues 20 mL in 0.05 phosphate (pH 7.0) supplemental with 1 unit of RNase Inhibitor;

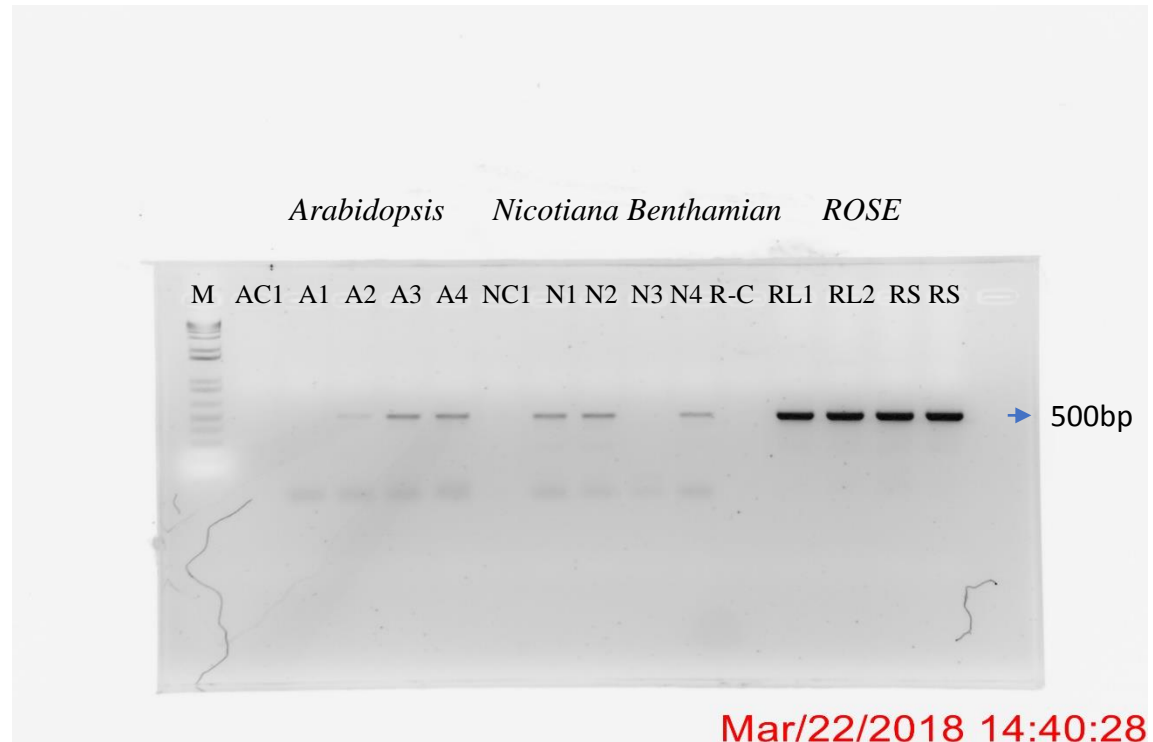
Step 2: Rub *Arabidopsis* (6-leaf-stage), *Nicotiana Benthamian* (6-leaf stage), and Rose leaves gently by carborundum;

Step 3: Sprinkle the SAP solutions onto the leaves using an airbrush;

Step 4: Pierce the leaves by syringe needles by imitating mite infection;
Cover the inoculated plants with a hood overnight or one more day.



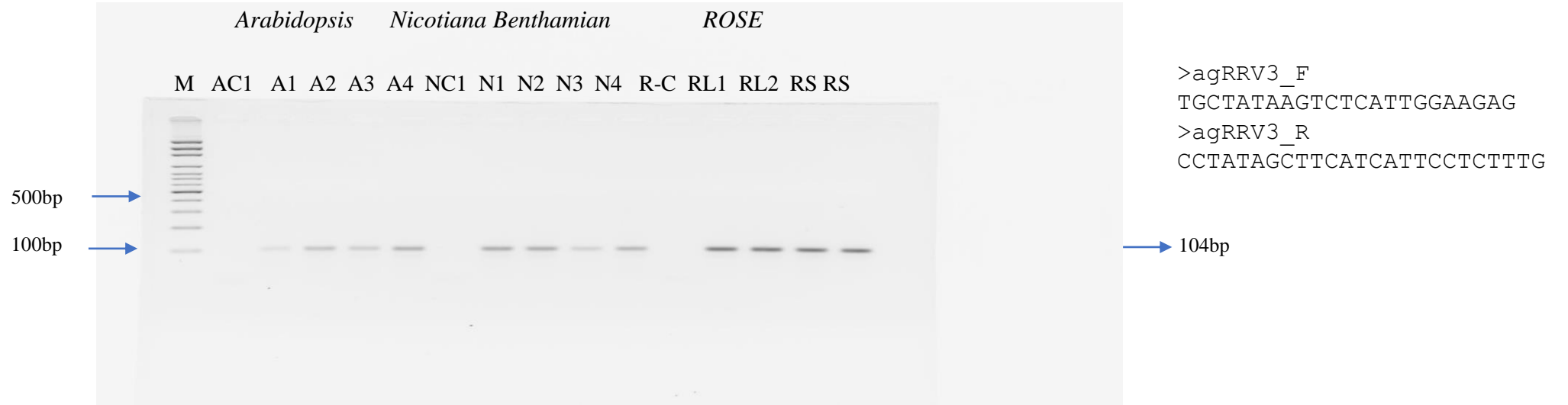
After inoculation of 6 days (Conducted Feb. 20, 2018), extracted total RNA, and prepared cDNAs for PCR verification of the infections.



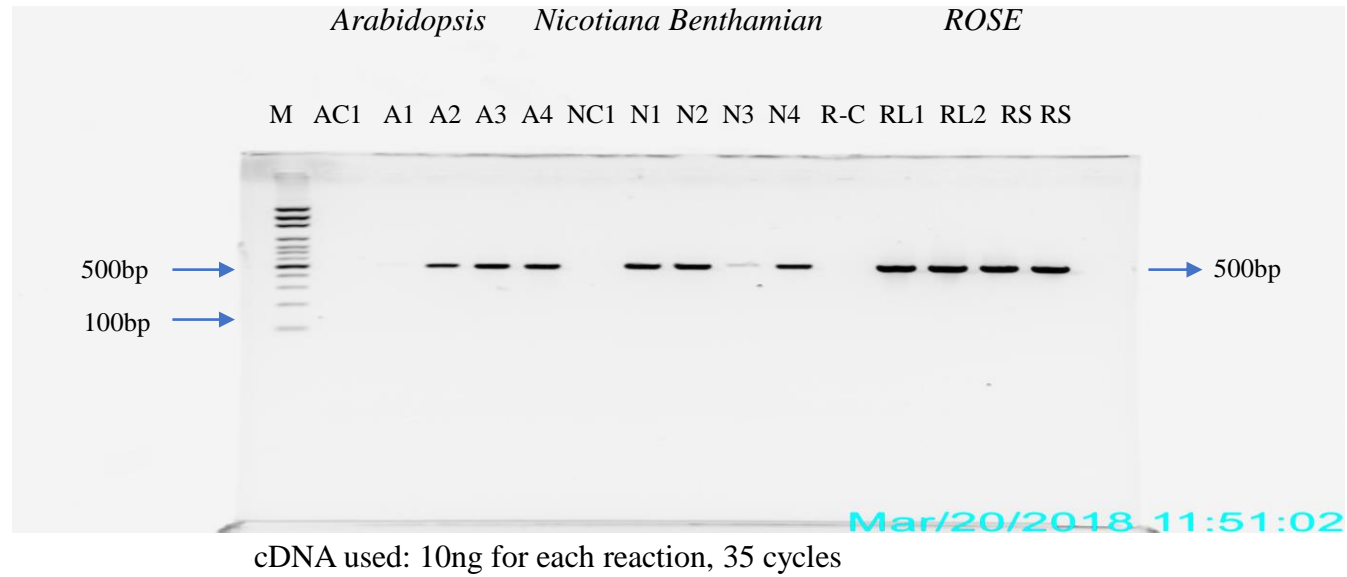
```
>agRRV1-F1
TCACACCCACTGAGAGGTATC
>agRRV1-R1
ATTGGACACACTCACACAGATT
```

Ara_RRV_R1	GGTTTTGACTATAACAACCAATCATGAAGAGTTGACATTTGATGTAAGACTCAGGACTTATATAGATGA
Nb_RRV_R1	GGTTTTGACTATAACAACCAATCATGAAGAGTTGACATTTGATGTAAGACTCAGGACTTATATAGATGA
RoSe_RRV_R1	GGTTTNGACTATAACAACCAATCATGAAGAGTTGACATTTGATGTAAGACTCAGGACTTATATAGATNA
cons	*****
Ara_RRV_R1	TGAGTATTACTTAAACTGTTTAAATAGACAATTTACACATAGAACGTAATTATATATTGGGTTATCTATG
Nb_RRV_R1	TGAGTATTACTTAAACTGTTTAAATAGACAATTTACACATAGAACGTAATTATATATTGGGTTATCTATG
RoSe_RRV_R1	TGAGTATTACTTAAACTGTTTAAATAGACAATTTACACATAGAACGTAATTATATATTGGGTTATCTATG
cons	*****
Ara_RRV_R1	TGAAACTAATCTTTTTTCATAAATACCCTGATTATTTGAAGCCTGTACTAATAGATCTTGGACCTAACCA
Nb_RRV_R1	TGAAACTAATCTTTTTTCATAAATACCCTGATTATTTGAAGCCTGTACTAATAGATCTTGGACCTAACCA
RoSe_RRV_R1	TGAAACTAATCTTTTTTCATAAATACCCTGATTATTTGAAGCCTGTACTAATAGATCTTGGACCTAACCA
cons	*****
Ara_RRV_R1	GCTAATATCATTAAATGACTGGTTACACTGAATTTGATATTGTCACCTGAAAGAATCAATCCACAGAAGTT
Nb_RRV_R1	GCTAATATCATTAAATGACTGGTTACACTGAATTTGATATTGTCACCTGAAAGAATCAATCCACAGAAGTT
RoSe_RRV_R1	GCTAATATCATTAAATGACTGGTTACACTGAATTTGATATTGTCACCTGAAAGAATCAATCCACAGAAGTT
cons	*****
Ara_RRV_R1	TGGCAATTTGATCTTTTTTAGCAATTATTTCCATGAGCATGGTAATCTTATATGGGCAATCTGTGTGA
Nb_RRV_R1	TGGCAATTTGATCTTTTTTAGCAATTATTTCCATGAGCATGGTAATCTTATATGGGCAATCTGTGTGA
RoSe_RRV_R1	TGGCAATTTGATCTTTTTTAGCAATTATTTCCATGAGCATGGTAATCTTATATGGGCAATCTGTGNMNN
cons	*****

RRV-R3

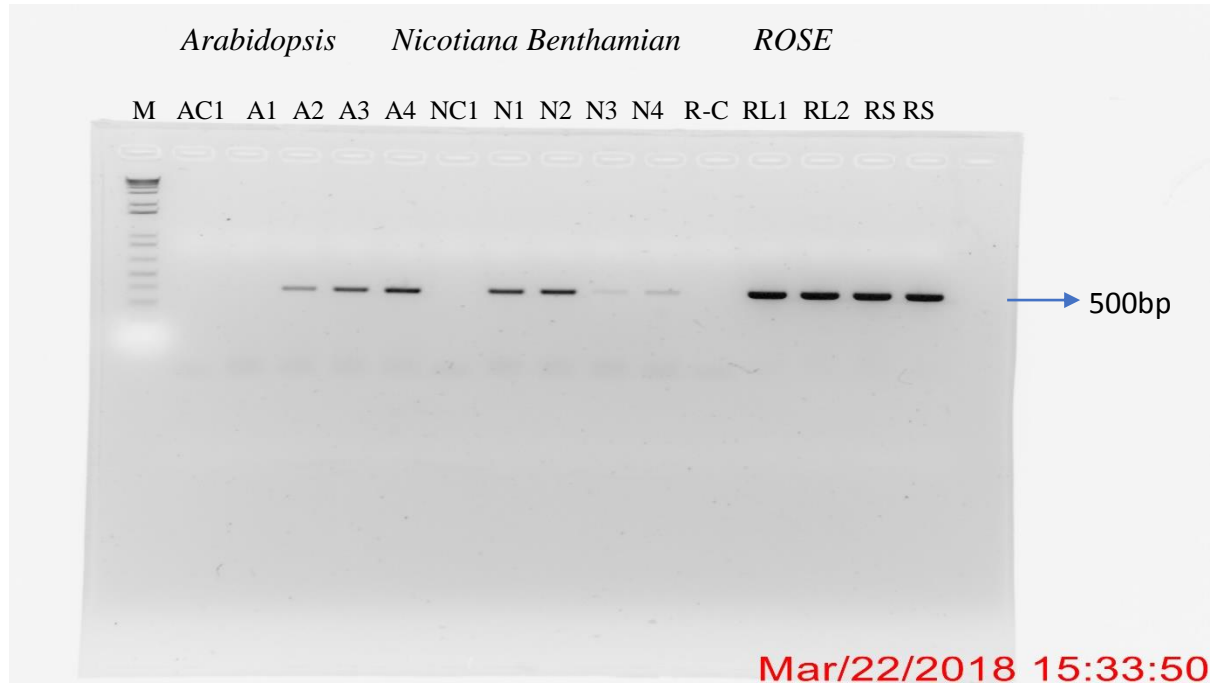


RRV-R4



RRV-R4 Primers from Maddi

RRV-R5



```

Ara-RRV-R5  TTAATCACTGTTTCTCAGTTTATATAAGTTGTAACCTCTTTTGTAACTAGATTGTAATGGGTAAT
Nb-RRV-R5  TTAATCACTGTTTCTCAGTTTATATAAGTTGTAACCTCTTTTGTAACTAGATTGTAATGGGTAAT
Rose-RRV-R5 TTAATCACTGTTTCTCAGTTTATATAAGTTGTAACCTCTTTTGTAACTAGATTGTAATGGGTAAT
cons
Ara-RRV-R5  TAGGTATGTAATCTATGTCATCAATTTTGTATCATCTGATATATCTTTTCATGGAGCTATTGATAT
Nb-RRV-R5  TAGGTATGTAATCTATGTCATCAATTTTGTATCATCTGATATATCTTTTCATGGAGCTATTGATAT
Rose-RRV-R5 TAGGTATGTAATCTATGTCATCAATTTTGTATCATCTGATATATCTTTTCATGGAGCTATTGATAT
cons
Ara-RRV-R5  TGTGACAACCTGTTTAACTACCAGGTAAGCCATAATAAACATATCTTCAATTCATTATCACCAG
Nb-RRV-R5  TGTGACAACCTGTTTAACTACCAGGTAAGCCATAATAAACATATCTTCAATTCATTATCACCAG
Rose-RRV-R5 TGTGACAACCTGTTTAACTACCAGGTAAGCCATAATAAACATATCTTCAATTCATTATCACCAG
cons
Ara-RRV-R5  GCAAGTACTTGATCTTTTCATCAACAGCTTTATTCAACGCTATATCATTAAAGCANNNGTTGGCNT
Nb-RRV-R5  GCAAGTACTTGATCTTTTCATCAACAGCTTTATTCAACGCTTTATTC-----NNNNN-----
Rose-RRV-R5 GCAAGTACTTGATCTTTTCATCAACAGCTTTATTCAACGCTTTATTCAACGCTTTATTCAACGCTTT
cons

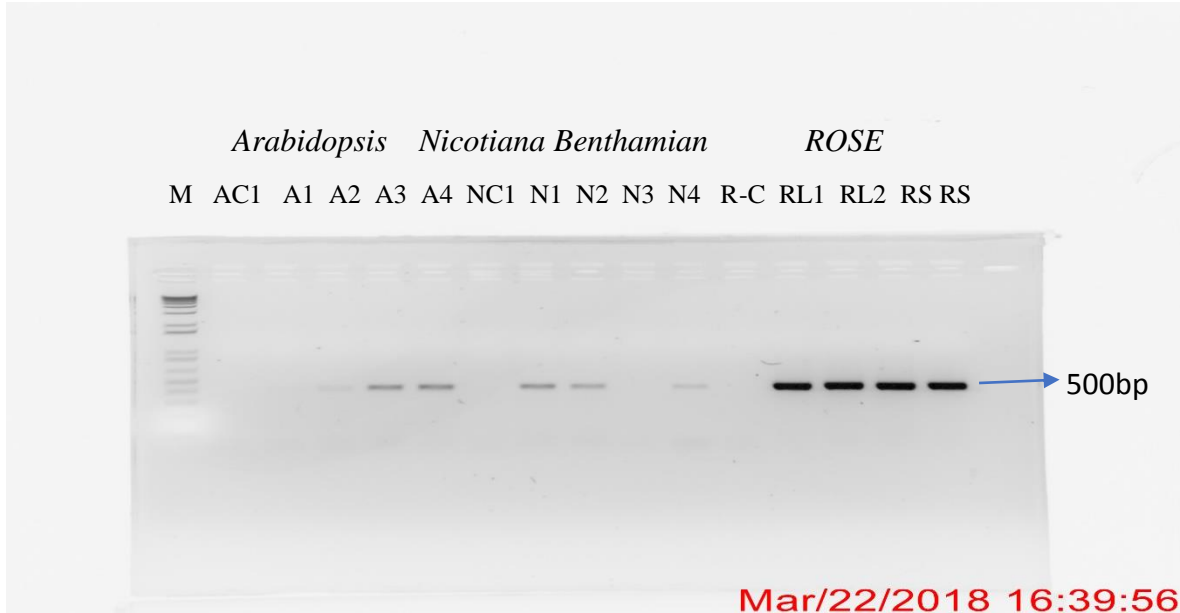
```

```

>agRRV5-F
AGTCTGTTTCTTAAGTGACTGACATT
>agRRV5-R
AATGATATAGCGTTGAATAAAGCTGT

```

RRV-R6



```

Ara-RRV-R6  ATTCTTTCACCTCTCACAAGATACCAAAATATGTGAAATCATGATGGATGCTTTCAGGCAATACACTT
Nb-RRV-R6  ATTCTTTCACCTCTCACAAGATACCAAAATATGTGAAATCATGATGGATGCTTTCAGGCAATACACTT
Rose-RRV-R6 ATTCTTTCACCTCTCACAAGATACCAAAATATGTGAAATCATGATGGATGCTTTCAGGCAATACACTT
cons
Ara-RRV-R6  GTCAATCCAAACAAGAACATGTGATTTGTAATACCTAAAAAATTTCTAAGTGATTTCTTTGTATC
Nb-RRV-R6  GTCAATCCAAACAAGAACATGTGATTTGTAATACCTAAAAAATTTCTAAGTGATTTCTTTGTATC
Rose-RRV-R6 GTNAATCCNAACAAGAACATGTGATTTGTAANACCTAAAAAATTTNTAAGNGATTNNTTCNGTATC
cons
Ara-RRV-R6  TTCATCCCGCTTATTTCAATTTGCTGTGTTTGTAGTTGATCTCGGAAAATTCAGCTGATTTTTTAGA
Nb-RRV-R6  TTCATCCCGCTTATTTCAATTTGCTGTGTTTGTAGTTGATCTCGGAAAATTCAGCTGATTTTTTAGA
Rose-RRV-R6 TTCATCCCGCTTATTTCAATTTGCTGTGTTTGTAGTTGATCTCGGAAAATTCAGCTGATTTTTTAGA
cons
Ara-RRV-R6  TATGCATCATGTAGAACAATCACCTACTATACTCTTTCATCGTTGCTAGGATCTCAATCATTGAGTTG
Nb-RRV-R6  TATGCATCATGTAGAACAATCACCTACTATACTCTTTCATCGTTGCTAGGATCTCAATCATTGAGTTG
Rose-RRV-R6 TATGCATCATGTAGAACAATCACCTACTATACTCTTTCATCGTTGCTAGGATCTCAATCATTGAGTTG
cons
Ara-RRV-R6  AAAATAGCAGTCTCCAATTCACAGAAATGATCANNNAATTTGTT-A
Nb-RRV-R6  AAAATAGCAGTCTCCAATTCACAGAAATGATCANNN-ATTTGTAN
Rose-RRV-R6 AAAATAGCAGTCTCCAATTCACAGAA-T-----N-A-----TAN
cons

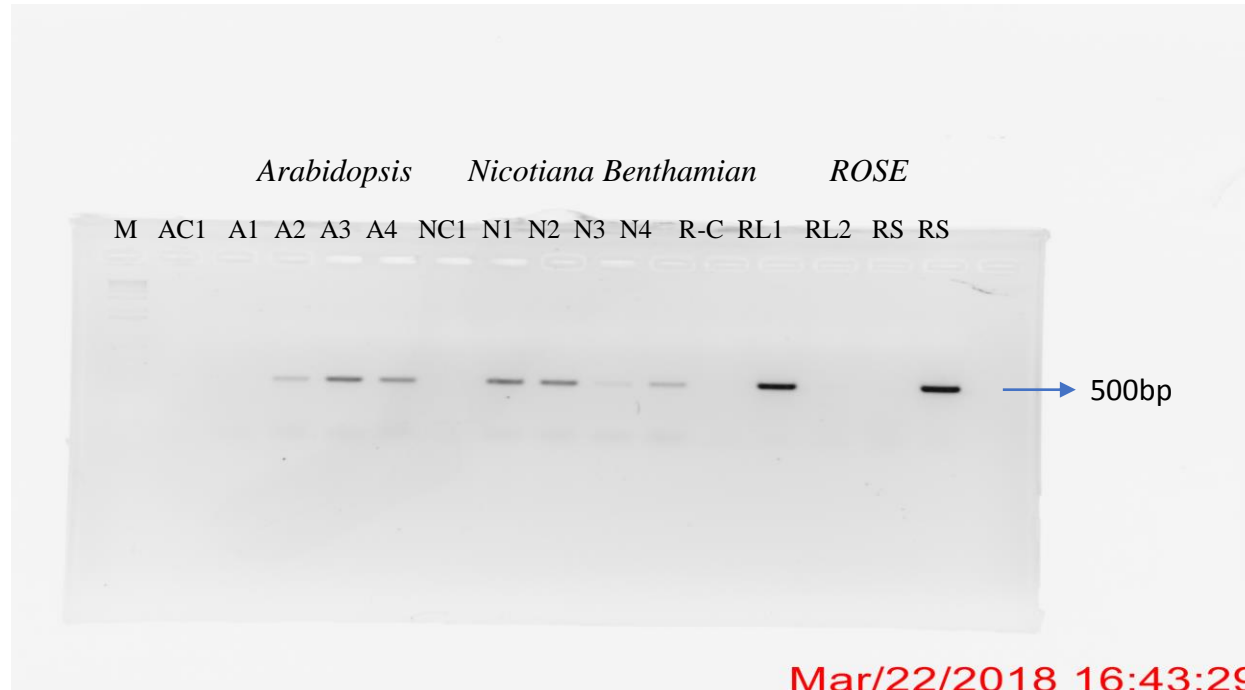
```

```

>agRRV6-F
CTTCTGGATCATAACCTAGGACCA
>agRRV6-R
AACAAATTCTCTGATCATTCTGTGAA

```

RRV-R7



```

Ara-RRV-R7 GGTATATAGTCTATATTGTTTATATCATCTACATCATGAATATTTTTTCCAAAGCATCTTCTAGATGA
Nb-RRV-R7 GGTATATAGTCTATATTGTTTATATCATCTACATCATGAATATTTTTTCCAAAGCATCTTCTAGATGA
Rose-RRV-R7 GGTATATAGTCTATATTGTTTATATCATCTACATCATGAATATTTTTTCCAAAGCATCTTCTAGATGA
cons *****

Ara-RRV-R7 TCAATCACAGTTTCTATTGTATCTGGAATACCATAGTAAAAGGTATCCTCGATTTTCATCACCTGGT
Nb-RRV-R7 TCAATCACAGTTTCTATTGTATCTGGAATACCATAGTAAAAGGTATCCTCGATTTTCATCACCTGGT
Rose-RRV-R7 TCAATCACAGTTTCTATTGTATCTGGAATACCATAGTAAAAGGTATCCTCGATTTTCATCACCTGGT
cons *****

Ara-RRV-R7 TTATATGAGACTGTCTCATCAGTATGTTTCCAAAAAGTAACTTTGTTTGAACAACATACATCATCTACA
Nb-RRV-R7 TTATATGAGACTGTCTCATCAGTATGCTTCCAAAAAGTAACTTTGTTTGAACAACATACATCATCTACA
Rose-RRV-R7 TTATATGAGACTGTCTCATCAGTATGTTTCCAAAAAGTAACTTTGTTTGAACAACATACATCATCTACA
cons *****

Ara-RRV-R7 CAATCAATTATTGGTTTCTTCAATACATCTTCTTCACTTCTATTGACTTTTACTTCTTCTAGTTTAGCT
Nb-RRV-R7 CAATCAATTATTGGTTTCTTCAATACATCTTCTTCACTTCTATTGACTTTTACTTCTTCTAGTTTAGCT
Rose-RRV-R7 CAATCAATTATTGGTTTCTTCAATACATCTTCTTCACTTCTATTGACTTTTACTTCTTCTAGTTTAGCT
cons *****

Ara-RRV-R7 GTCACATCAATATGAATAGCTGCTAAGACNNNNCTGGA-----
Nb-RRV-R7 GTCACATCAATATGAATAGCTGCTAAGACNNNNCTGGAANNANTAGATGAAATGTGGTGTCTTCTG
Rose-RRV-R7 GTCACATCAATATGAATAGCTGCTAAGACNNNAGCTGGA-----
cons *****

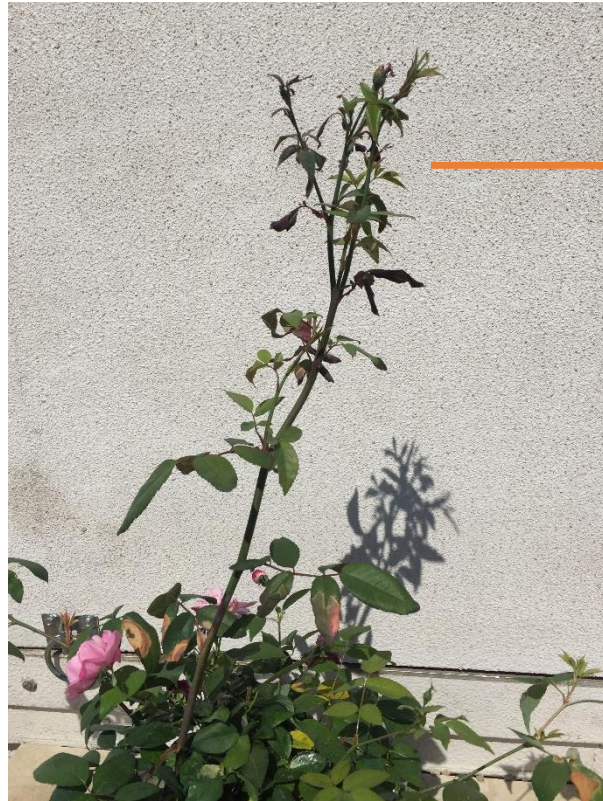
```

```

>agRRV7-F
AATGAAGAGTCAGATGTGAGACTTTAAC
>agRRV7-R
TTCCAGCTGATGTCTTAGCAG

```

After inoculation of 54 days (Conducted Mar. 26, 2018), extracted total RNA, and prepared cDNAs for PCR verification of the infections.



Potential 'witch broom', the phenotype after inoculated SAPs from "Julia Child", the infected plants from California. When I did the inoculation, this branch was not there. It is a new growth. So, collected leave samples from this branch to detect RRV systemic infections.