

Figure S1. Calibration curves for the quantifications of A in mRNA. The amounts of A ranged 0.1 – 2.5 μmol.

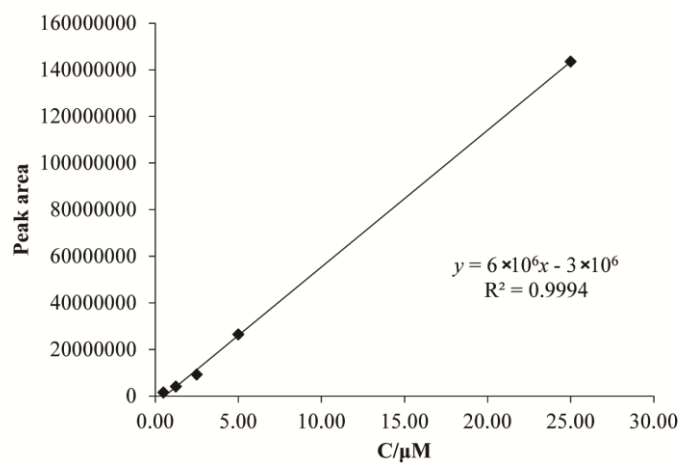


Figure S2. Calibration curves for the quantifications of C in mRNA. The amounts of C ranged 0.5 – 25 μmol.

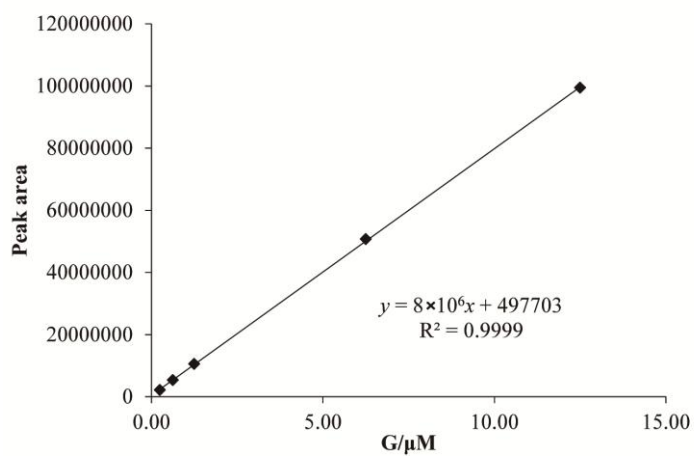


Figure S3. Calibration curves for the quantifications of G in mRNA. The amounts of G ranged 0.25 – 12.5 μmol.

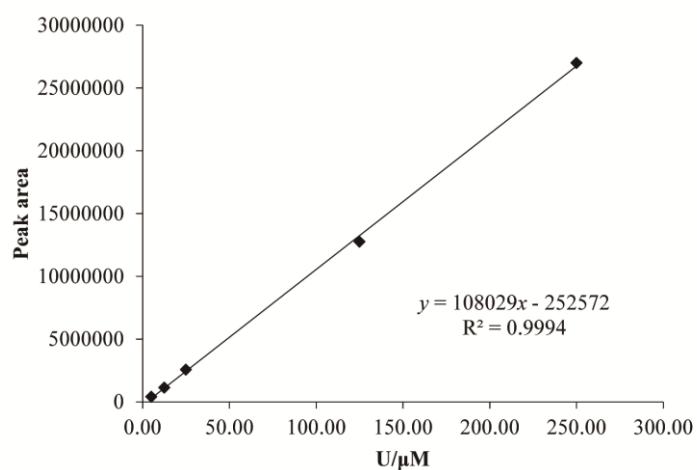


Figure S4. Calibration curves for the quantifications of U in mRNA. The amounts of U ranged 5 – 250 μmol.

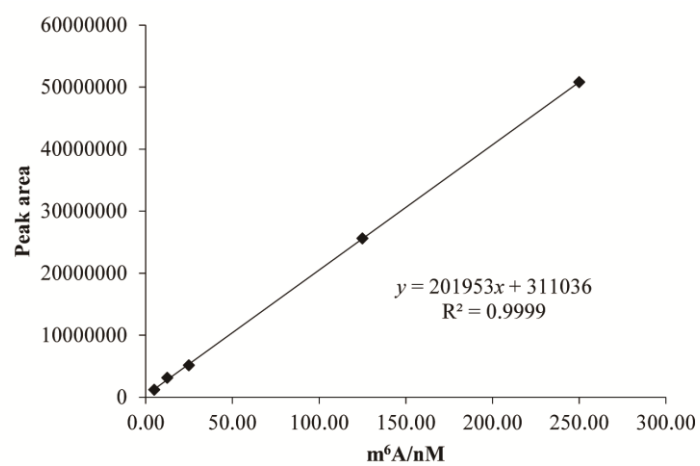


Figure S5. Calibration curves for the quantifications of m⁶A in mRNA. The amounts of m⁶A ranged 0.005 – 0.250 μmol.

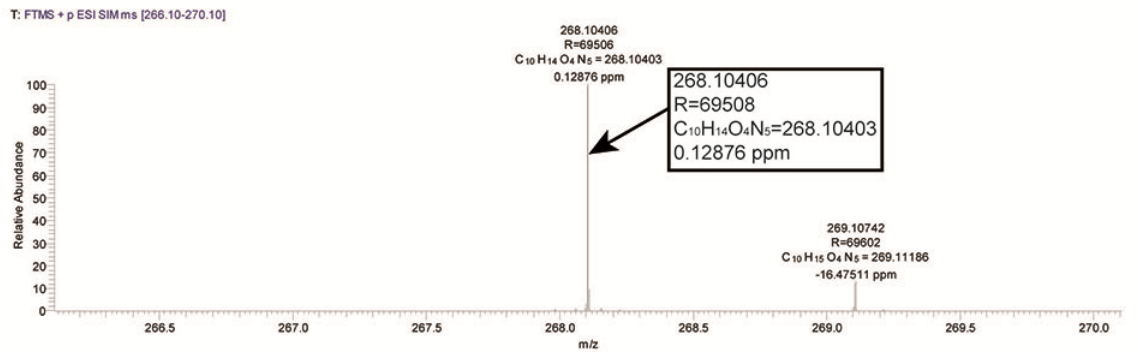
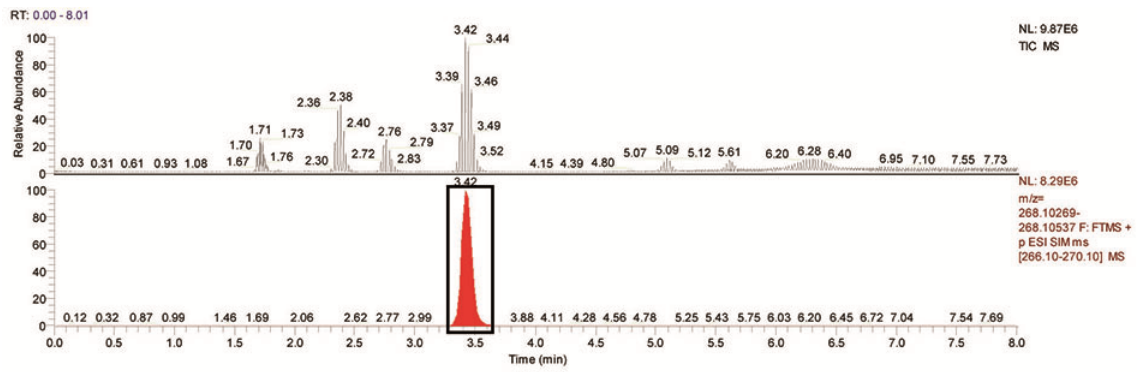


Figure S6. Representative UHPLC-HR-MS/MS results for the quantifications of A in *N. tabacum* K-326 with selected-ion chromatograms.

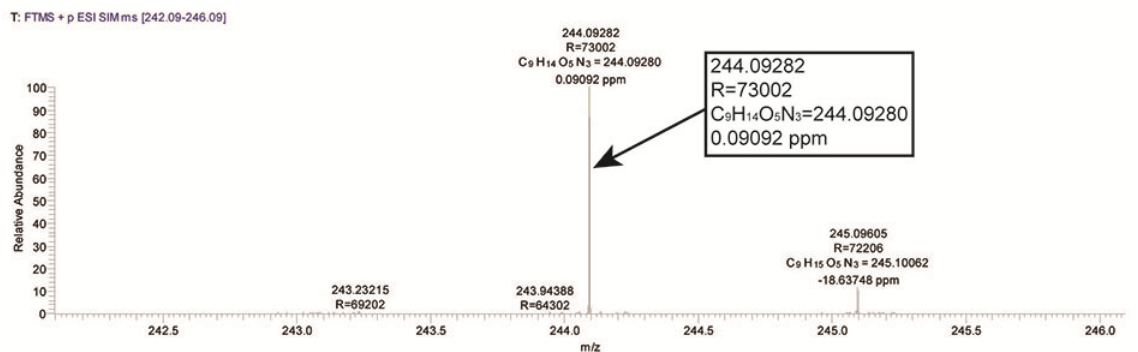
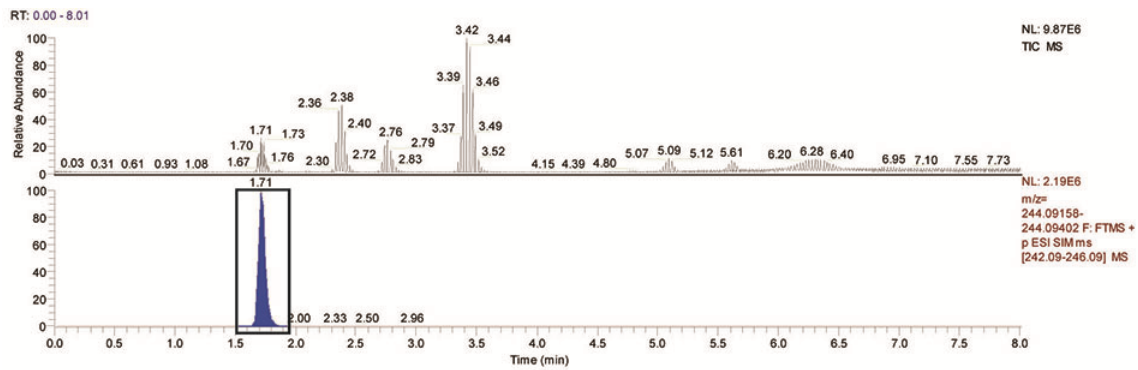


Figure S7. Representative UHPLC-HR-MS/MS results for the quantifications of C in *N. tabacum* K-326 with selected-ion chromatograms.

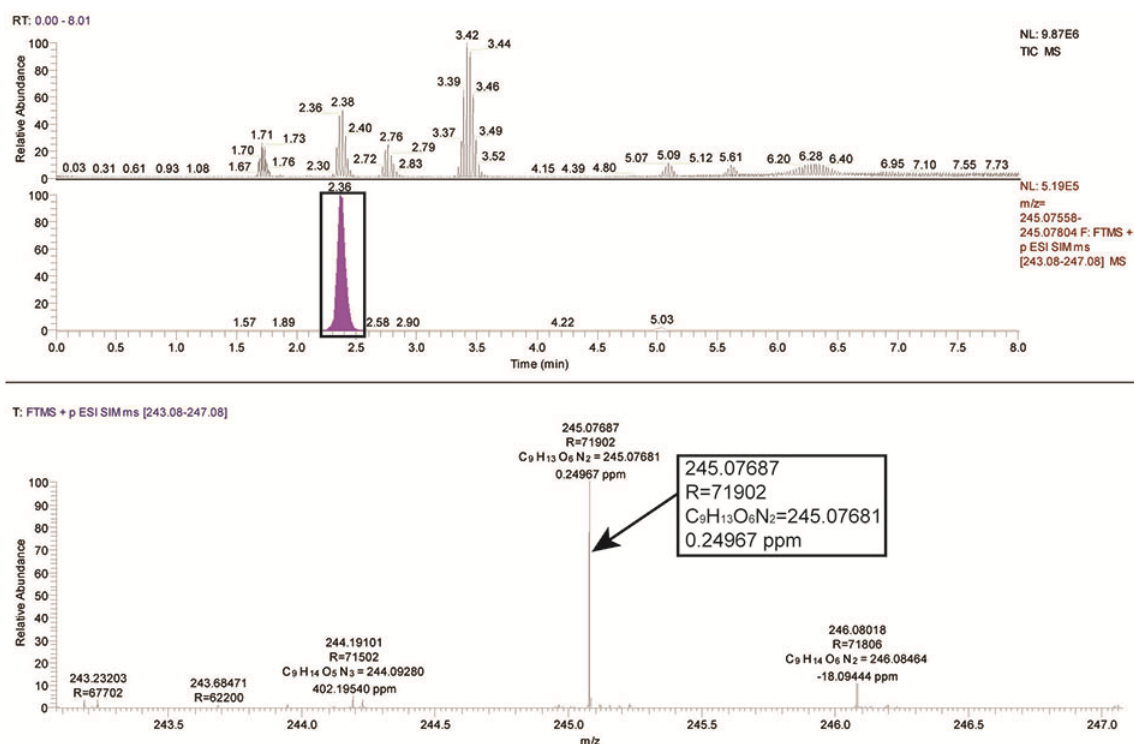


Figure S8. Representative UHPLC-HR-MS/MS results for the quantifications of U in *N. tabacum* K-326 with selected-ion chromatograms.

Table S1. Primer sequences for RT-qPCR analysis

| Gene name | Primer sequences (5'-3') | Amplicon length (bp) |
|----------------|-------------------------------------------------------|----------------------|
| XM_009766347 | F: ACGCTCATTTTGCTTCCAGT R: ATCCCCTTCCCAGTCGTCAT | 171 |
| XM_009766348 | F: TTGGCCGTAGAAGGCTTGAG R: GTGTAGGCCTGAACTCAGCA | 114 |
| XM_009775897 | F: GGCAAACGGAAGATGAAGCC R: CTGGTGCTGGTGTGATGGAT | 172 |
| XM_009770927 | F: TAGGCCGACACGTACTIONGAGA R: CGGCGGCATATTGAAACAGG | 163 |
| XM_009801708 | F: AGGTTTATGTCCCCACTGCG R: TCTGCAGAACTTTTGTGACATT | 114 |
| XM_009801663 | F: GTTCCGCCACTTTTGTGCGAG R: AGATGGCACCTCTGGGCATA | 148 |
| β -actin | F: GACATGAAGGAGGAGCTTGC R: ATCATGGATGGCTGGAAGAG | 178 |

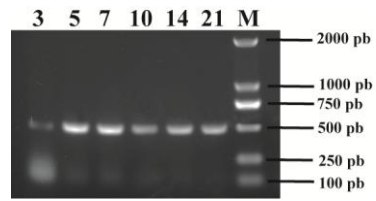


Figure S9. TMV detection by the PCR method. M: DL2000 maker, Lanes 3, 5, 7, 10, 14 and 21: The TMV coat protein gene in *N. tabacum* K326 at 3, 5, 7, 10, 14 and 21 days post infection TMV.