

## **SUPPLEMENTARY MATERIALS**

A geminivirus betasatellite damages structural and functional integrity of chloroplasts leading to symptom formation and inhibition of photosynthesis

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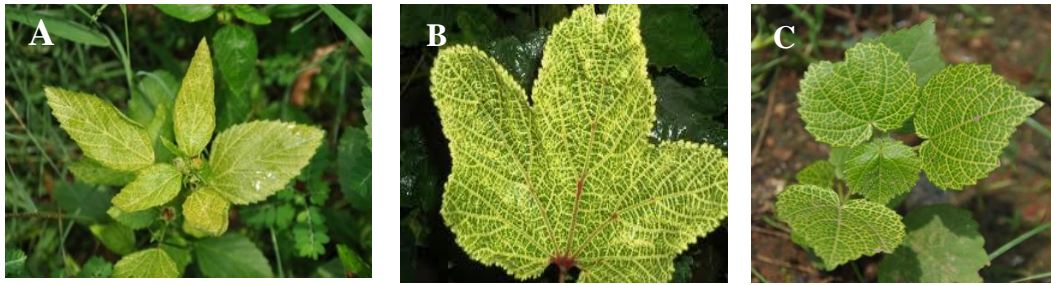
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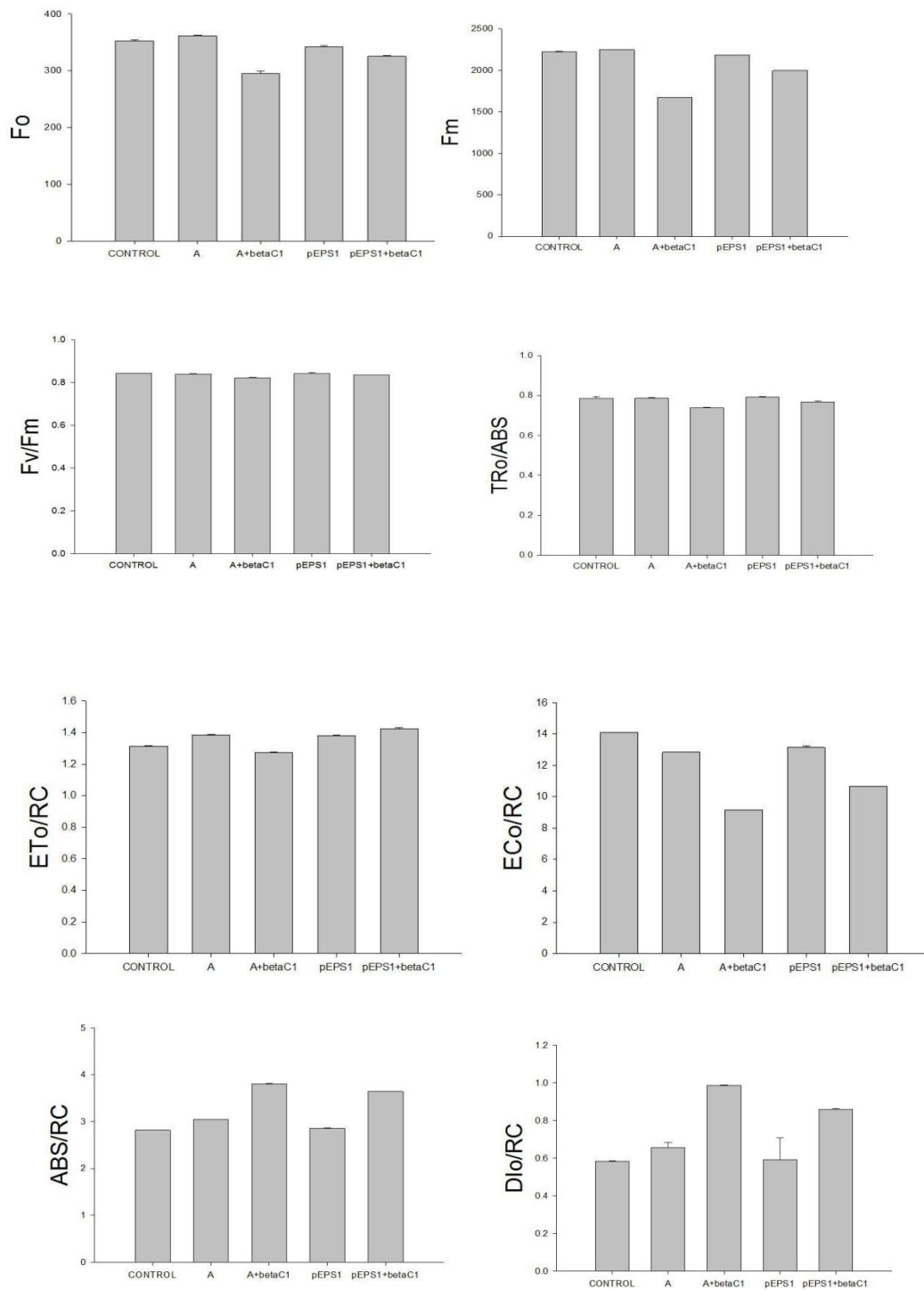
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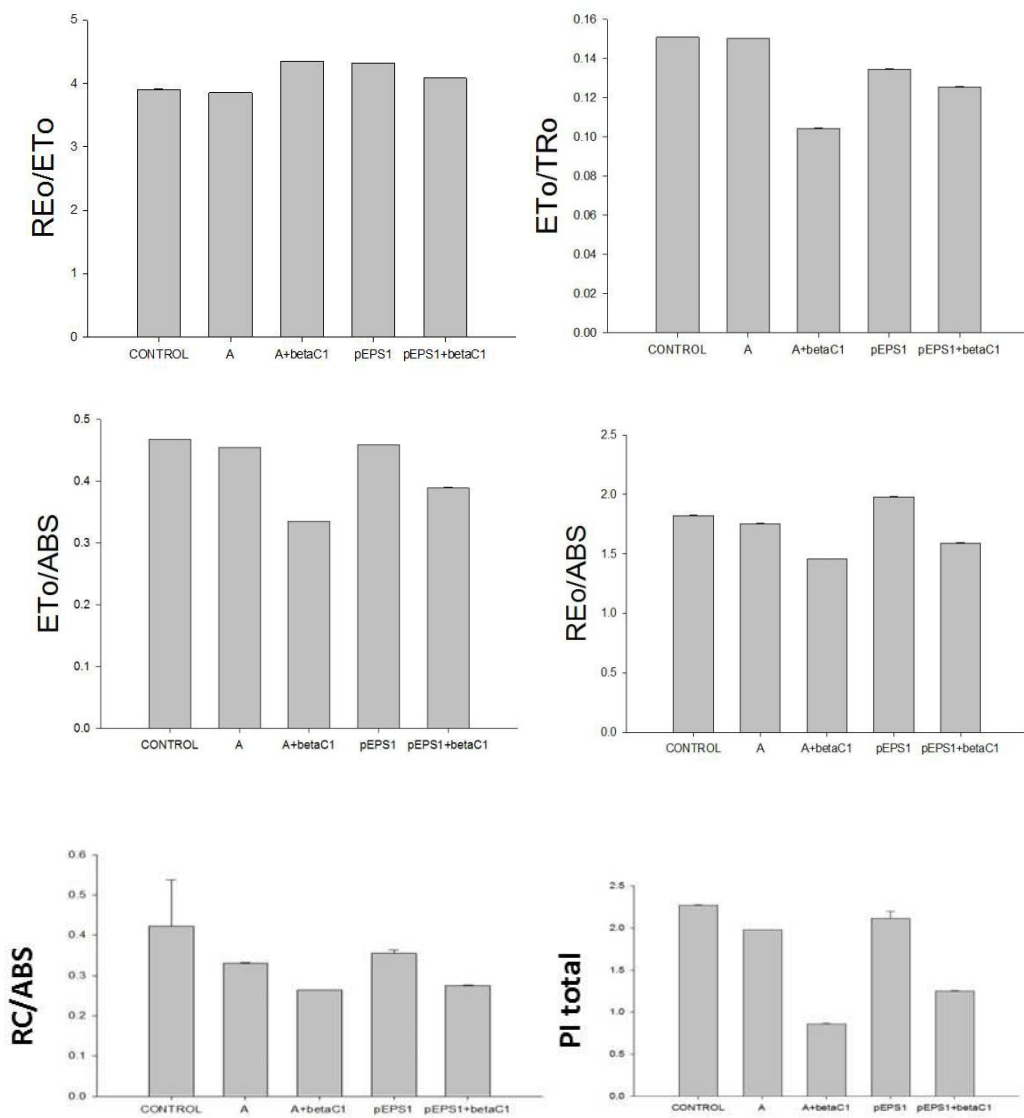
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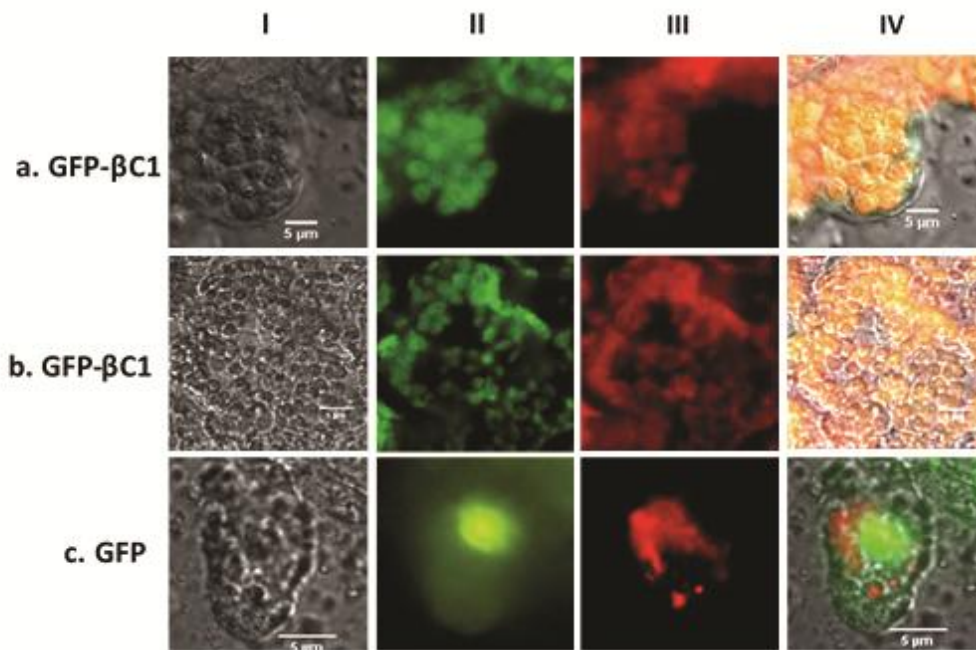
**Fig. S1.** Vein clearing symptom associated with yellow vein diseases caused by geminiviruses. (A) Ageratum yellow vein disease, (B) Bhendi yellow vein disease, (C) Mulvastrum yellow vein disease. Presence of betasatellites have been ascertained with each of these diseases.



**Fig. S2.** Graphical comparison of different parameters extracted from chlorophyll a fluorescence OJIP curve

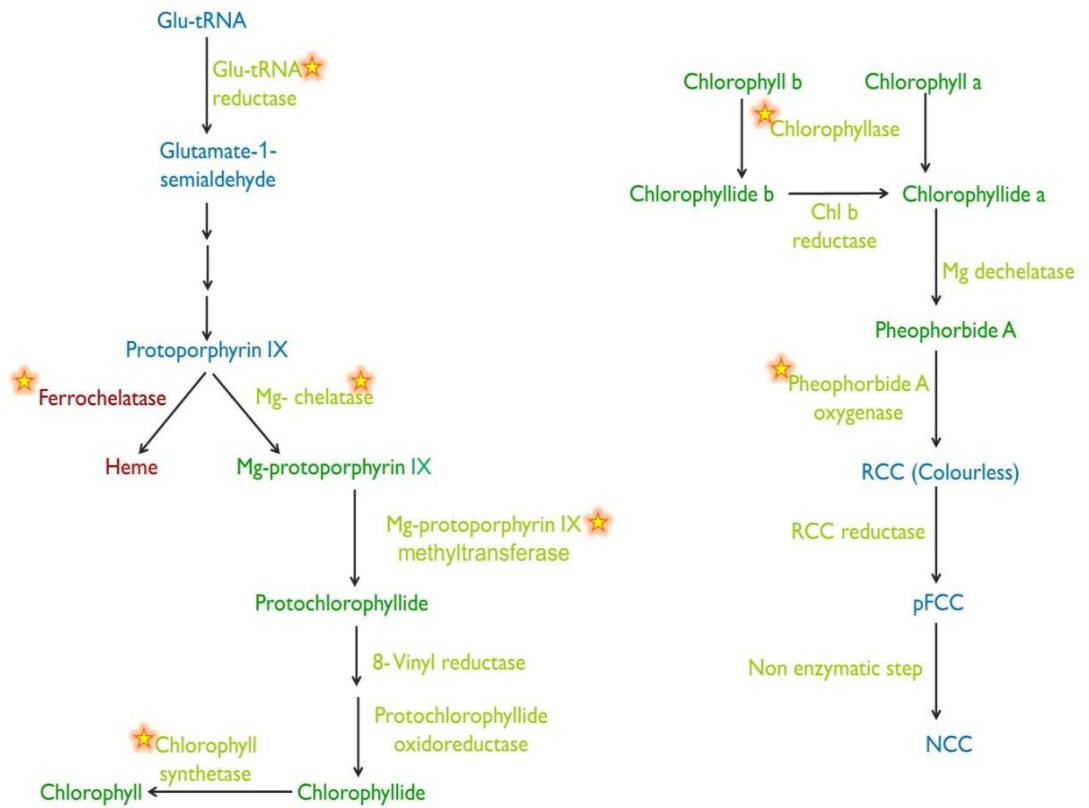


**Fig. S2.** Graphical comparison of different parameters extracted from chlorophyll a fluorescence OJIP curve

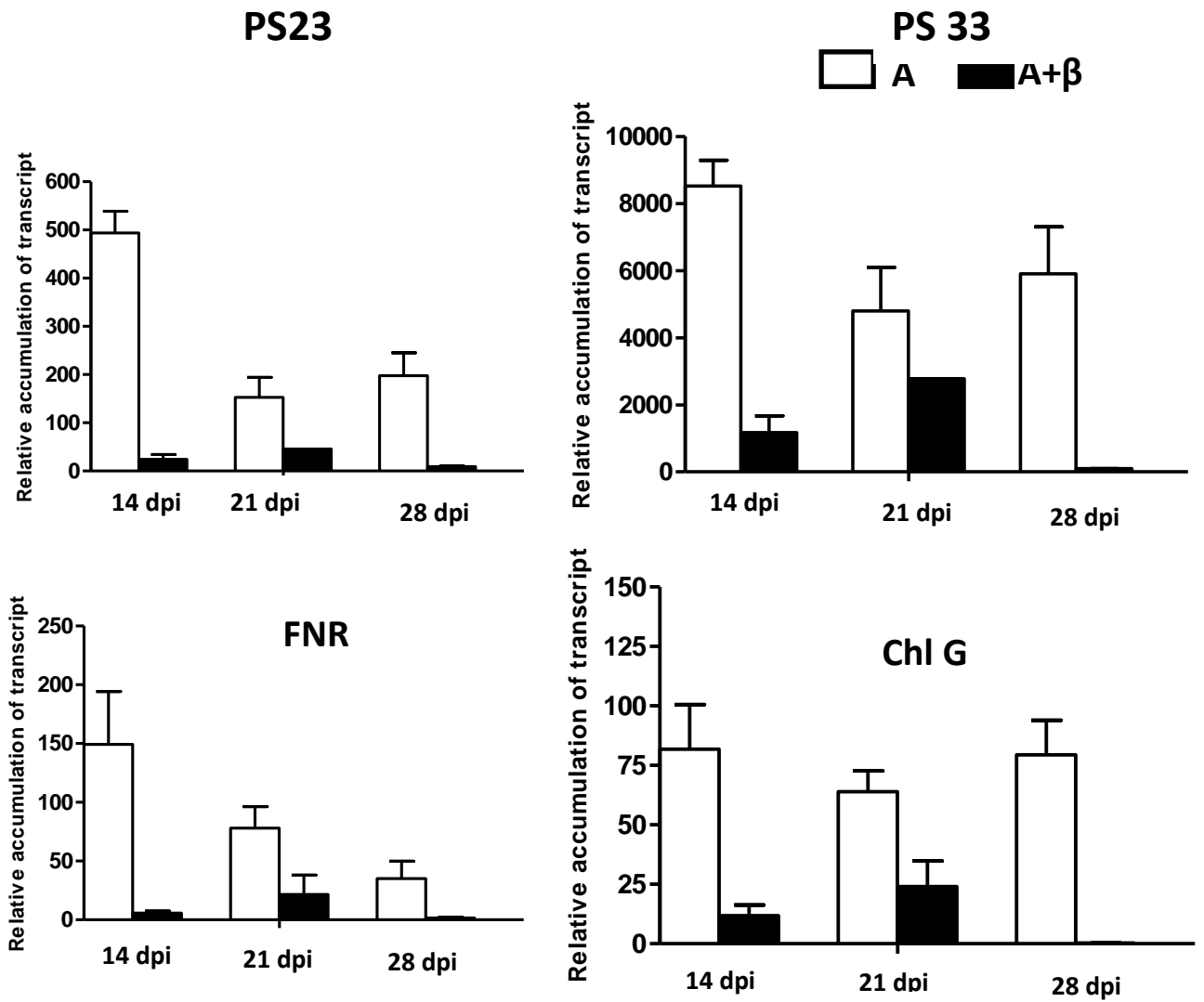


**Fig. S3.** Betasatellite encoded  $\beta$ C1 protein localizes in chloroplast of mesophyll cells of *N. benthamiana*.

Sections of *N. benthamiana* leaves, agroinfiltrated with pBIC-  $\beta$ C1 (row a and b) and pBIC vector (row c), were visualized under fluorescent microscope. Images of the cells (panel I) in bright field, (panel II) showing GFP fluorescence and (panel III) showing chlorophyll autofluorescence. Panel IV represents merged images of panel I, II and III.



**Fig. S4.** Schematic diagram of chlorophyll biosynthesis and degradation pathway. Genes selected for checking the transcript level are marked asterisk.



**Fig. S5.** Relative accumulation of host transcripts at earlier stages of accumulation

**Table S1.** Transient expression of  $\beta$ C1 reduces chlorophyll content in systemic symptomatic leaves

| Construct         | Chlorophyll a<br>mg / g<br>tissue (average) | Chlorophyll b<br>mg / g<br>tissue (average) | Total Chlorophyll<br>mg / g<br>tissue (average) |
|-------------------|---|---|---|
| pEPS1             | 1.15  | 1.256                                       | 2.4   |
| pEPS1+ $\beta$ C1 | 0.96  | 0.485                                       | 1.445   |



**Table S2.** Different biophysical parameters studied in relation to photosynthetic efficiency

| Parameters to analyse<br>Chl a fluorescence  | Significance  |
|--|---|
| PI ABS   | Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors                                     |
| PI total   | Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors  |
| ABS/RC   | Absorption flux (of antenna Chls) per RC  |
| Fv/Fm  | Maximum yield of primary photochemistry or maximum quantum efficiency of Photosystem II.  |
| TR <sub>0</sub> /CS <sub>m</sub> =<br>F <sub>m</sub> -F <sub>0</sub> /CS <sub>m</sub>  | Initial (maximum) flux of photochemically conserved (trapped) excitation energy per excited cross section   |
| TR <sub>0</sub> /ABS=  | Trapping flux (leading to Q <sub>A</sub> reduction) per absorption  |
| ET <sub>0</sub> /CS <sub>m</sub> = F <sub>m</sub> -<br>F <sub>j</sub> /CS <sub>m</sub> | Flux of electrons transferred between two photosystems per excited cross section.   |
| RE <sub>0</sub> / ET <sub>0</sub> ,  | Efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE) |
| RE <sub>0</sub> /ABS   | Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)   |
| ET <sub>0</sub> / TR <sub>0</sub>  | Efficiency/probability for electron transport (ET), i.e. efficiency/probability that an electron moves further than Q <sub>A</sub> <sup>-</sup>           |
| ET <sub>0</sub> /ABS   | Quantum yield for electron transport (ET)   |
| ET <sub>0</sub> /RC  | Electron transport flux (further than Q <sub>A</sub> <sup>-</sup> ) per RC  |
| DI <sub>0</sub> /CS <sub>m</sub> =F <sub>0</sub> /CS <sub>m</sub>                      | Measure of the energy dissipated as heat or fluorescence per excited cross section.   |
| DI <sub>0</sub> /RC  | dissipated energy flux per active reaction centre   |
| RC/ABS   | Q <sub>A</sub> -reducing RCs per PSII antenna Chl (reciprocal of ABS/RC)  |
| EC <sub>0</sub> /RC  | Electron transport flux (further than Q <sub>A</sub> <sup>-</sup> ) per RC  |

**Table S3.** Effect of betasatellite infection of on net photosynthetic (CO<sub>2</sub>exchange) rate, and stomatal conductance

| Treatment                             | Photosynthetic efficiency %<br>(w.r.t control) | Stomatal conductance %<br>(w.r.t control) |
|---------------------------------------|--|---|
| A inoculated (28 dpi)                 | 96   | 84  |
| A+ $\beta$ inoculated (28 dpi)        | 38   | 85  |
| pEPS1 vector infiltrated (7dpi)       | 94   | 62  |
| pEPS1+ $\beta$ C1 infiltrated (7 dpi) | 69   | 75  |

**Table S4.** Host cellular proteins downregulated in response to betasatellite infection

| Spot no. | Protein homology                              | Acc. No.     | Theoretical/Experimental |            | % Change <sup>a</sup> | Coding site/ Localization     |
|----------|---|--------------|--------------------------|------------|-----------------------|-------------------------------|
|          |   |              | Mass                     | pI         |                       |                               |
| A01-05   | Rubisco LSU                                   | ABC86677     | 49.88/50.45              | 6.8/6.79   | 45±2.18               | chloroplast /chloroplast      |
| A06-08   | Rubisco SSU                                   | AAA34111     | 10.3/11.3                | 5.3/5.5    | 40±4.06               | nucleus /chloroplast          |
| A09      | ATP-dependent ClpP1 protease                  | ABF95892.1   | 37.16/37.1               | 9.45/9.9   | 33±2.14               | nucleus /chloroplast          |
| A10      | Phosphoglycerate kinase                       | NP_001147628 | 50.01/50                 | 6.07/6.1   | 35±0.3                | nucleus /cytoplasm            |
| A11      | NBS-coding resistance gene analog             | ACE79518.1   | 30.89/30.9               | 5.4/5.6    | 30±4.97               | nucleus /cytoplasm            |
| A12      | Leucine zipper protein-like                   | gi 56784542  | 44.6/43.2                | 6.84/7     | 27±2.10               | nucleus /cytoplasm            |
| A13      | Maturase K                                    | NP_566734    | 35.94/35.9               | 9.74/9.9   | 25±7.18               | nucleus /chloroplast          |
| A14      | Ribosome production factor 2-like protein     | NP_566734    | 34.4/37.5                | 9.1/8.7    | 20±1.27               | nucleus /cytoplasm            |
| A15      | Photosystem II protein T                      | ABQ15043     | 40.1/40.03               | 9.52/10.0  | 47±7.47               | nucleus /Chloroplast membrane |
| A16      | 33kDa protein of Oxygen evolving complex      | Q157M6       | 21.9/21.2                | 5.8/5.92   | 33±1.45               | nucleus /Chloroplast membrane |
| A17      | Allene oxide cyclase 3, chloroplast precursor | XP_002524412 | 28.71/21.7               | 9.32/9.7   | 25±1.24               | nucleus /Chloroplast membrane |
| A18      | Glutamate decarboxylase                       | XP_003616074 | 17.4/17.9                | 10.32/10.4 | 30±3.8                | nucleus /cytoplasm            |

<sup>a</sup>Proteins were considered as differentially expressed when a 1.2-fold increased or decreased accumulation was statistically confirmed by one-way ANOVA with a *p* value 0.05. The fold change was calculated comparing each sample versus the corresponding control. Differentially expressed proteins were identified by MALDI TOF/TOF.

**Table S5.** Host cellular proteins upregulated in response to betasatellite infection

| Spot no. | Protein homology                              | Acc. No.     | Theoretical/Experimental |            | % change <sup>a</sup> | Coding site/<br>Localization |
|----------|---|--------------|--------------------------|------------|-----------------------|------------------------------|
|          |   |              | Mass                     | pI         |                       |                              |
| B01      | Pathogenesis-related protein 1-1a             | gi 253796028 | 14.24/14.8               | 6.92/7.2   | 15±1.48               | nucleus<br>/cytoplasm        |
| B02      | N-acetyltransferase                           | XP_003597800 | 37.9/35.5                | 9.21/9.01  | 21±1.03               | nucleus<br>/cytoplasm        |
| B03      | Mitochondrial alternative oxidase 1b, partial | ACI28870     | 23.2/25.6                | 6.22/6.23  | 23±1.63               | nucleus<br>/mitochondria     |
| B04      | Fructokinase                                  | NP_564875    | 4.173/ 5.2               | 5.54/5.5   | 22±3.0                | nucleus<br>/mitochondria     |
| B05      | Small heat-shock protein                      | CAC81964     | 18160                    | 5.7/5.9    | 21±3.18               | nucleus<br>/cytoplasm        |
| B06      | Early responsive to dehydration               | gi 319993043 | 15.3/15.34               | 4.53/4.4   | 24±3.5                | nucleus<br>/cytoplasm        |
| B07      | Jasmonic acid methyl transferase              | AT1G19640    | 37.9/35.5                | 10.32/10.4 | 34±2.8                | nucleus<br>/cytoplasm        |
| B08      | Heat shock protein 17.0                       | CAA63901     | 16.95/16.5               | 5.78/5.6   | 20±1.8                | nucleus<br>/cytoplasm        |
| B09      | Pyruvate dehydrogenase                        | NP_174703    | 48.3/45.6                | 8.8/8.2    | 23±1.05               | nucleus<br>/cytoplasm        |

<sup>a</sup>Proteins were considered as differentially expressed when a 1.2-fold increased or decreased accumulation was statistically confirmed by one-way ANOVA with a *p* value 0.05. The fold change was calculated comparing each sample versus the corresponding control. Differentially expressed proteins were identified by MALDI TOF/TOF.

**Table S6.** Primers used for checking transcript level of selected host genes through qRT-PCR

| Target transcript   | Forward primer                                   | Reverse primer                                      | Amplicon size |
|---|--|---|---------------|
| Magnesium (Mg) protoporphyrin IX chelatase subunit I (CHLI) | NtRTCHLIFP<br>5'GGAAATCAACCTGCTC<br>AAGAACA 3'   | NtRTCHLIRP<br>5'ATCATCACACCTCC<br>AATCTTTGG 3'      | 150bp         |
| Mg protoporphyrin IX chelatase (CHLH)                       | NtRTCHLHFP<br>5'CCCTTTGGCTCCTTGTA<br>TGTATG 3'   | NtRTCHLHRP<br>5'GCCCAACAACAGG<br>AGCATT3'           | 110bp         |
| Mg protoporphyrin IX chelatase (CHLD)                       | NtRTCHLDFP<br>5'GGCCATTGACCGTGAG<br>ATAGG3'      | NtRTCHLDRP<br>5'CATTTGCCATTGAG<br>CCAACA3'          | 128bp         |
| HEMA1 encoding glutamyltRNA reductase                       | NtRTHEMAFP<br>5'GGGTCGGGTCAGACGA<br>TTG3'        | NtRTHEMARP<br>5'CAAGAGCAGAAAG<br>GCTGGAAGA3'        | 137bp         |
| Ferrochelatase  | NtRTFeChFP<br>5'GAGAAAAGCACTGCGG<br>GAAAG3'      | NtRTFeChRP<br>5'GAGGATAAAGAGG<br>GAGCACAACA 3'      | 140bp         |
| Mg-protoporphyrin IX methyltransferase (Chl M)              | NtRTChIMFP<br>5'AGTCCCCGGCACCAGT<br>AATG 3'      | NtRTChIMFP<br>5'CAGCCAATGACGCA<br>AGATGA 3'         | 150bp         |
| Chlorophyll synthase (CHLG)                                 | NtRTChIGFP<br>5'CCCTTTGCCTCTCATT<br>TTCC 3'      | NtRTChIGRP<br>5'TCCACTTGTCCGTTT<br>CTTGCT 3'        | 163 bp        |
| Chlorophyllase  | NtRTPhyllaseFP<br>5'CCCCATCAGAAGCAGG<br>AAAC 3'  | NtRTPhyllaseRP<br>5'TGCTCCTTCCACCA<br>AATATAACTG 3' | 146 bp        |
| Pheophorbide A oxygenase 1                                  | NtRTPheoFP<br>5'TTCAGCTCCTCAATCGA<br>GACTTAGT 3' | NtRTPheoRP<br>5'GATAAAGGAGCAA<br>GGCGATGA 3'        | 100bp         |
| PS23 OEC PSII   | NbRTPS23FP<br>5'CAATGCTTCTTGCACCA<br>TCAT 3'     | NbRTPS23RP<br>5'TTGAGGTGCAGACT<br>GTTTTTGG 3'       | 126BP         |
| PS33 OEC PSII   | NbRTPS33FP<br>5'CCCACATCATTACGGT<br>CAA 3'       | NbRTPS33RP<br>5'CGAATGGTCCCTCA<br>ATCTCA 3'         | 118BP         |
| Clp Protease  | NtRTClpFP<br>5'GCGGAAGAAGCTGCTGA<br>AACTG 3'     | NtRTClpRP<br>5'TTGGGCTTCTGTTG<br>CTGACA 3'          | 110 BP        |
| Ferredoxin-NADP reductase                                   | NtRTFdnrFP<br>5'GCTCCAAAAAGACAAC<br>ACCTTCA 3'   | NtRTFdnrRP<br>5'CCGCCAGACAATA<br>CCATCT 3'          | 110BP         |
| Dna K type molecular chaperone CSS1precursor chloroplast    | PsRTDnaKFP<br>5'TGATGGGCAGACAAGT<br>GTAGAAA 3'   | PsRTDnaKRP<br>5'GAGGAGCGGGAGG<br>GATACC 3'          | 110bp         |

