# The caseinolytic protease complex component CLPC1 in Arabidopsis maintains proteome and RNA homeostasis in chloroplasts

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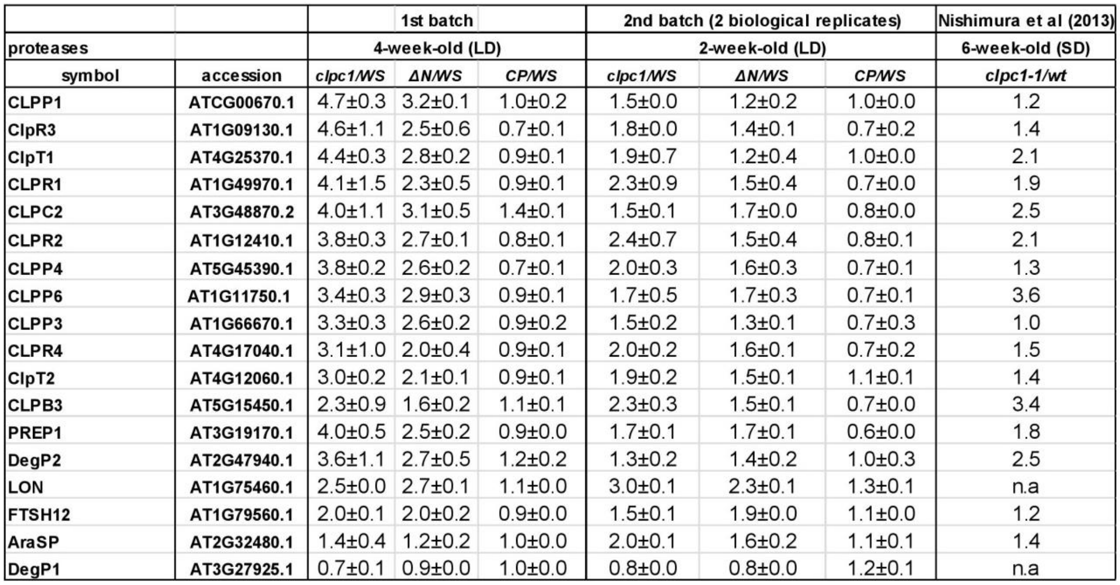
## Supplementary data

Table S1. Proteases accumulation in seedlings of different genotypes



| | | 1st batch | | | 2nd batch (2 biological replicates) | | | Nishimura et al (2013) |
|---|---|---|---|---|---|---|---|---|
| **proteases** | | **4-week-old (LD)** | | | **2-week-old (LD)** | | | **6-week-old (SD)** |
| **symbol** | **accession** | ***clpc1/WS*** | ***ΔN/WS*** | ***CP/WS*** | ***clpc1/WS*** | ***ΔN/WS*** | ***CP/WS*** | ***clpc1-1/wt*** |
| **CLPP1** | **ATCG00670.1** | 4.7±0.3 | 3.2±0.1 | 1.0±0.2 | 1.5±0.0 | 1.2±0.2 | 1.0±0.0 | 1.2 |
| **ClpR3** | **AT1G09130.1** | 4.6±1.1 | 2.5±0.6 | 0.7±0.1 | 1.8±0.0 | 1.4±0.1 | 0.7±0.2 | 1.4 |
| **ClpT1** | **AT4G25370.1** | 4.4±0.3 | 2.8±0.2 | 0.9±0.1 | 1.9±0.7 | 1.2±0.4 | 1.0±0.0 | 2.1 |
| **CLPR1** | **AT1G49970.1** | 4.1±1.5 | 2.3±0.5 | 0.9±0.1 | 2.3±0.9 | 1.5±0.4 | 0.7±0.0 | 1.9 |
| **CLPC2** | **AT3G48870.2** | 4.0±1.1 | 3.1±0.5 | 1.4±0.1 | 1.5±0.1 | 1.7±0.0 | 0.8±0.0 | 2.5 |
| **CLPR2** | **AT1G12410.1** | 3.8±0.3 | 2.7±0.1 | 0.8±0.1 | 2.4±0.7 | 1.5±0.4 | 0.8±0.1 | 2.1 |
| **CLPP4** | **AT5G45390.1** | 3.8±0.2 | 2.6±0.2 | 0.7±0.1 | 2.0±0.3 | 1.6±0.3 | 0.7±0.1 | 1.3 |
| **CLPP6** | **AT1G11750.1** | 3.4±0.3 | 2.9±0.3 | 0.9±0.1 | 1.7±0.5 | 1.7±0.3 | 0.7±0.1 | 3.6 |
| **CLPP3** | **AT1G66670.1** | 3.3±0.3 | 2.6±0.2 | 0.9±0.2 | 1.5±0.2 | 1.3±0.1 | 0.7±0.3 | 1.0 |
| **CLPR4** | **AT4G17040.1** | 3.1±1.0 | 2.0±0.4 | 0.9±0.1 | 2.0±0.2 | 1.6±0.1 | 0.7±0.2 | 1.5 |
| **ClpT2** | **AT4G12060.1** | 3.0±0.2 | 2.1±0.1 | 0.9±0.1 | 1.9±0.2 | 1.5±0.1 | 1.1±0.1 | 1.4 |
| **CLPB3** | **AT5G15450.1** | 2.3±0.9 | 1.6±0.2 | 1.1±0.1 | 2.3±0.3 | 1.5±0.1 | 0.7±0.0 | 3.4 |
| **PREP1** | **AT3G19170.1** | 4.0±0.5 | 2.5±0.2 | 0.9±0.0 | 1.7±0.1 | 1.7±0.1 | 0.6±0.0 | 1.8 |
| **DegP2** | **AT2G47940.1** | 3.6±1.1 | 2.7±0.5 | 1.2±0.2 | 1.3±0.2 | 1.4±0.2 | 1.0±0.3 | 2.5 |
| **LON** | **AT1G75460.1** | 2.5±0.0 | 2.7±0.1 | 1.1±0.0 | 3.0±0.1 | 2.3±0.1 | 1.3±0.1 | n.a |
| **FTSH12** | **AT1G79560.1** | 2.0±0.1 | 2.0±0.2 | 0.9±0.0 | 1.5±0.1 | 1.9±0.0 | 1.1±0.0 | 1.2 |
| **AraSP** | **AT2G32480.1** | 1.4±0.4 | 1.2±0.2 | 1.0±0.0 | 2.0±0.1 | 1.6±0.2 | 1.1±0.1 | 1.4 |
| **DegP1** | **AT3G27925.1** | 0.7±0.1 | 0.9±0.0 | 1.0±0.0 | 0.8±0.0 | 0.8±0.0 | 1.2±0.1 | n.a |

Notes:

1. WS: wild type; *clpc1*: the *clpc1* mutant; ΔN, N-terminal (1-93 amino acid) deleted CLPC1 complementary line; CP, full-length CLPC1 complementary line, LD, long-day; SD, short-day.
2. The first batch dataset was from 4-week-old seedlings with three technical replicates. The second batch was from 2-week-old seedlings with two biological replicates and each biological replicate included 3 technical replicates.
3. Data are means and standard errors of protein abundance relative to the wild type (WS).
4. Data from Nishimura et al (2013) in [27].

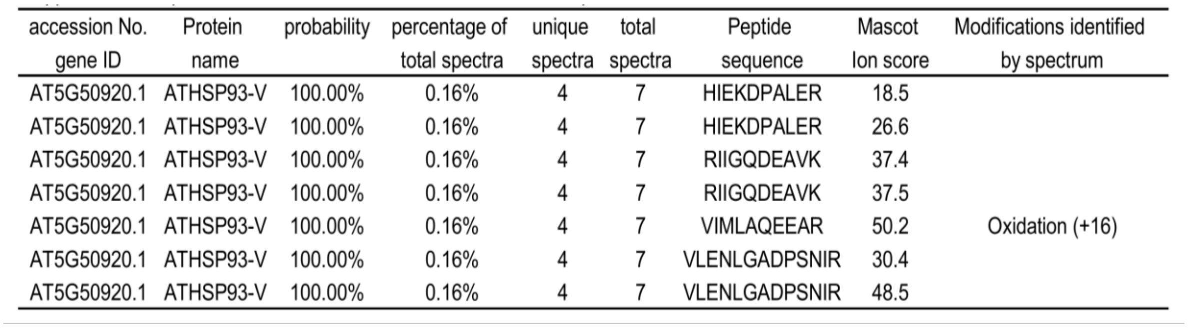
Table S2. Primers used in the study.

| | |
|---|---|
| CLPC1-F | CAATCGACTCCACCGTCTTT |
| CLPC1-R | TCCCATAAACCCTTGCATTCT |
| CLPC2-F | GAGGGTGTGTGATGAAGATGAG |
| CLPC1-R | TGTCAGAAGAGGTGCTTGTAAA |
| pClpP-F | CGACCCGATGTACAGACAATA |
| pClpP-R | TAGCGTGAGGGAATGCTATAC |
| ACT2-F | TGCTTATGTCGCTCTTGACTAC |
| ACT2-R | CTCTCAGCTCCGATGGTTATG |
| rbcL-F | GTGTTGGGTTCAAAGCTGGT |
| rbcL-R | CATCGGGTCCACACAGTTGTC |
| accD-F | TGTGGATTCAATGCGACAAT |
| accD-R | TTTTGCGCAGAGTCAATACG |
| atpA-F | CGGAAATCTTACCTCGACCA |
| atpA-R | ATGGGTGACGGTTTGATGAT |
| atpH-F | ATCCACTGGTTTCTGCTGCT |
| atpH-R | TTCCTTCTGCCTCAGGTTGT |
| atpI-F | ATTGGCAAATAGGGGGTTTC |
| atpI-R | GCCGTCGATTGGAATTGTTT |
| ccsA-F | CACAATAACTGCGCCAAGTG |
| ccsA-R | AACAGAGCGCCATAGCCTAA |
| cemA-F | TTTGCCCTGGTTGATCTCTC |
| cemA-R | TTGGATCGTTTCTTTGTGGA |
| ndhB-F | CCAGAAGAAGATGCCATTCA |
| ndhB-R | TCATCAATGGACTCCTGACG |
| ndhI-F | TTTGCCTGTTGTTGATTGGA |
| ndhI-R | ATTGGTAAACGACCCAAAGC |
| petA-F | CAGAGGGCGAATCCATTAAA |
| petA-R | GCCAAAACAACCGATCCTAA |
| petB-F | ATTGGGCGGTCAAAATTGTA |
| petB-R | AGACGGCCGTAAGAAGAGGT |
| psaA-F | GCCAAGAAATCCTGAATGGA |
| psaA-R | CATCTTGGAACCAAGCCAAT |
| psaC-F | GAGCATGCCCTACAGACGTA |
| psaC-R | CAGGCGGATTCACATCTCTT |
| psbL-F | CAATCAAATCCGAACGAACA |
| psbL-R | GAAATAATTCGAAAATAAAACAGCAA |
| rpL14-F | AGCGGGGCTAGAGAATTGAT |
| rpL14-R | ACTGCGGCATTGTCATCATA |
| rpL20-F | TCGGAGGCGTAGAACAAAAC |
| rpL20-R | CGATGAGCCGAAACTAAAGC |
| rpoA-F | GCGATGCGAAGAGCTTTACT |
| rpoA-R | CCAGGACCTTGGACACAAAT |
| rpoB-F | AAAAAGCACGGATACGGATG |
| rpoB-R | CTTCTTGAATGCCCCGATTA |
| rpoC1-F | TCGGATACGAAGATATCAAATGG |
| rpoC1-R | TTAGTTATGGGCCTAGCAAAAGA |

| | |
|---|---|
| rpoC2-F | ATGGAGCCCGTAAAGGAGTT |
| rpoC2-R | CGTCTGCTAAGACACGACCA |
| rpS18-F | CAAGCGATCTTTTCGTAGGC |
| rpS18-R | AAAGCTCACTCTATTCACCCGTCT |
| ycf2-F | TAGCCCTCGGTCTATTGGTG |
| ycf2-R | GGATCCACTTTTTGGGGAAT |
| ycf3-F | TCCAATACTCAGCGGCTTG |
| ycf3-R | TTCGGGCATTAGAACGAAAC |
| ycf5-F | GCGAACAACCGGAGCTATTA |
| ycf5-R | CCGACATGCGTATTTTTGATT |
| 23s rRNA-F | GGGCGACTGTTTACCAAAAA |
| 23s rRNA-R | TTACCCGACAAGGAATTTCG |
| 16s rRNA-F | CGGTATCTGGGGAATAAGCA |
| 16s rRNA-R | GATTTGACGGCGGACTTAAA |
| atpB-F | CCGTTTCGTACAAGCAGGAT |
| atpB-R | CGGGGTCAGTCAAATCATCT |
| atpE-F | TCCACAAGAAGCTCAGCAAA |
| atpE-R | GTGTCCGAGCTCGTCTGAG |
| atpF-F | GCTCCTTCACGCAGTTCTTC |
| atpF-R | TACTTGGGTCACTGGCCATC |
| matK-F | ATCCTTTGTTGCCAGAATGC |
| matK-R | TTTTTCTACGCAAGCGGTCT |
| ndhA-F | TTGACGCCACAAATTCCAT |
| ndhA-R | TTAGGTGGTCTGCGAGCTG |
| ndhD-F | TGGAGAATGGGAATAGATGGAC |
| ndhD-R | TCCCGAGAAGAAAATGATCCTA |
| ndhK-F | GCAGTCCGCATATTGGAAAT |
| ndhK-R | CGTGGGACGATACTGGACTT |
| petD-F | TCCTTTTGCAACTCCTTTGG |
| petD-R | CCGCTGGTACTGAAACCATT |
| psaB-F | GGACCCCACTACTCGTCGTA |
| psaB-R | ATTGCTAATTGCCCGAAATG |
| psbA-F | GAGCAGCAATGAATGCGATA |
| psbA-R | CCTATGGGGTCGCTTCTGTA |
| psbB-F | CGTGCGACTTTGAAATCTGA |
| psbB-R | TAGCACCATGCCAAATGTGT |
| psbC-F | ACTTCCCCACCTAGCCACTT |
| psbC-R | AGCCCAAAACTGCAGAAGAA |
| psbD-F | CACAAATCTTTGGGGTTGCT |
| psbD-R | CCATCCAAGCACGAATACCT |
| rpL16-F | TGTACGACGTGGTGGAAAA |
| rpL16-R | GCATTTTTGATGCCGCTATT |
| rpL22-F | AAAGCTGAGGTGAACCAAGG |
| rpL22-R | TGTCCCATAGGCCTCCACTA |
| rpL32-F | CTCGAAAAAGCGTATTCGTAAAA |
| rpL32-R | TGAAAAAGCTTTCAACGATGTC |
| rpS2-F | GGGCTCGGTGTCATTATGTT |

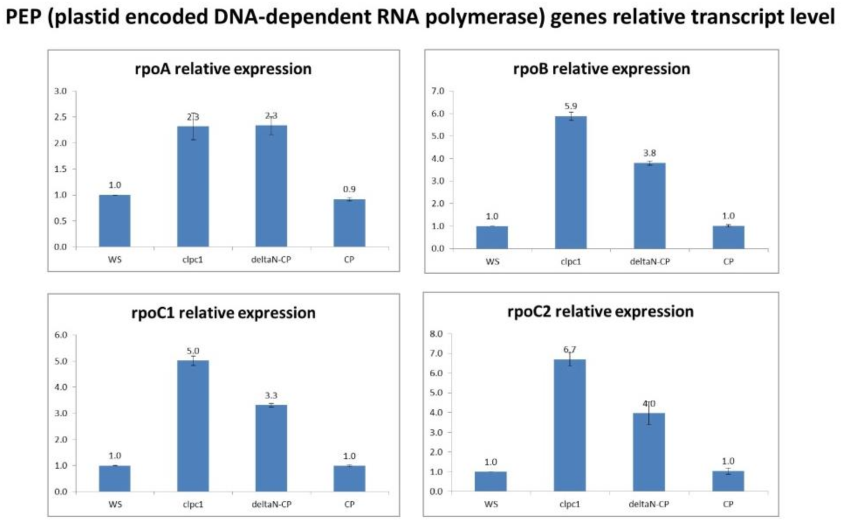
| | |
|---|---|
| rpS2-R | TCTTCAACACAGCTGCATCC |
| rpS4-F | CGATTGGGTATGGCTTTGAC |
| rpS4-R | ATGGTTTGGCAATTCCTCAG |
| rpS8-F | CGACCGGGTCTACGAATCTA |
| rpS8-R | ATTTCTCCGCCGATTCTTTT |
| rpS14-F | AATCCCCACCGCGTAATAGT |
| rpS14-R | AACATGCCTGAACCATTTCC |

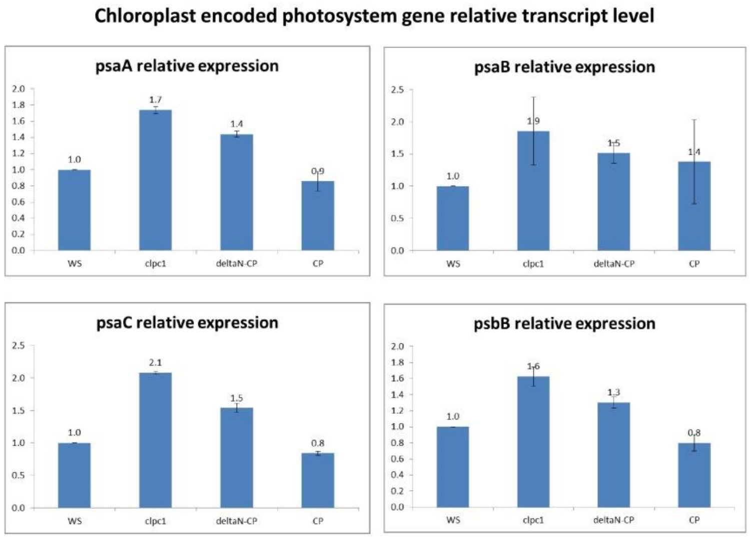
Table S3. Peptides identified of CLPC1 in SVR7-GFP CO-IP experiment



| accession No. gene ID | Protein name | probability | percentage of total spectra | unique spectra | total spectra | Peptide sequence | Mascot Ion score | Modifications identified by spectrum |
|---|---|---|---|---|---|---|---|---|
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | HIEKDPALER | 18.5 | |
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | HIEKDPALER | 26.6 | |
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | RIIGQDEAVK | 37.4 | |
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | RIIGQDEAVK | 37.5 | |
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | VIMLAQEEAR | 50.2 | Oxidation (+16) |
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | VLENLGADPSNIR | 30.4 | |
| AT5G50920.1 | ATHSP93-V | 100.00% | 0.16% | 4 | 7 | VLENLGADPSNIR | 48.5 | |

Figure S1. Relative expression levels of sense transcripts in the *clpc1* mutant and its complementation lines. Shown are means and SD from 3 replicates. qRT-PCR was conducted using gene-specific primers (Table S2) normalized against the expression of the *ACTIN2* gene. WS, the wild type; *clpc1*, the *clpc1* mutant; ΔN-CP, *clpc1* expressing N-terminus-truncated CLPC1; CP, *clpc1* expressing the full-length wild-type CLPC1.





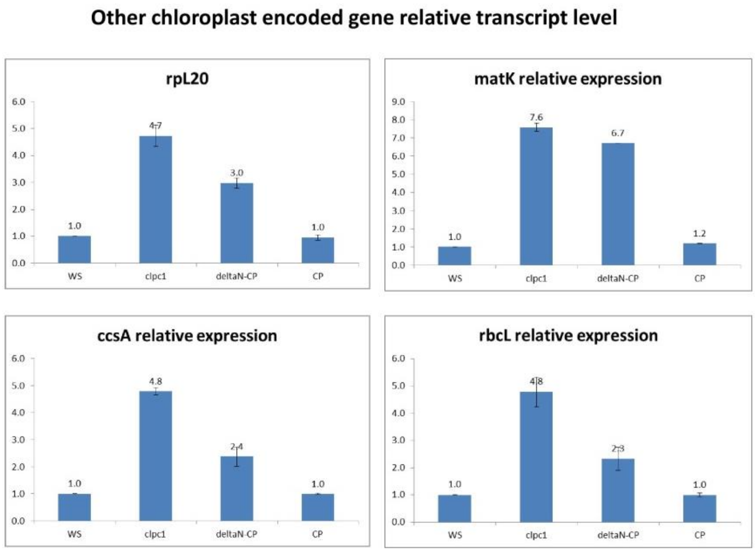


Figure S2. Over-expressing CLPC2 in *clpc1* mutant partially or fully restored the chloroplast RNA level.

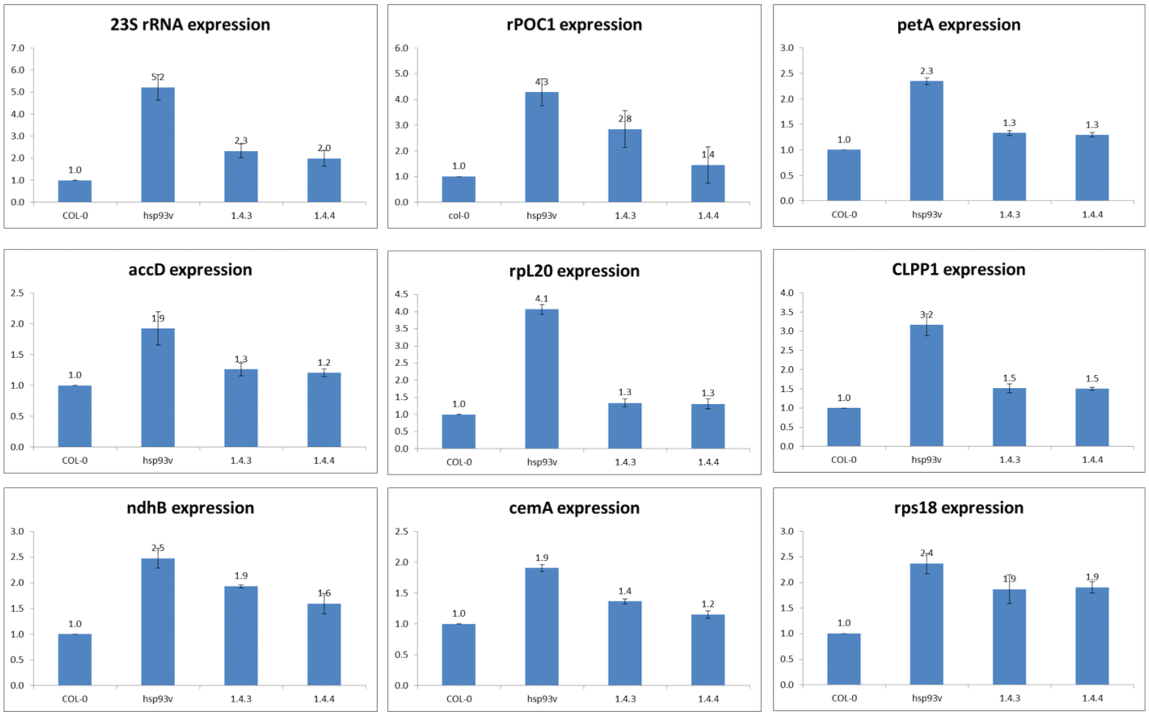
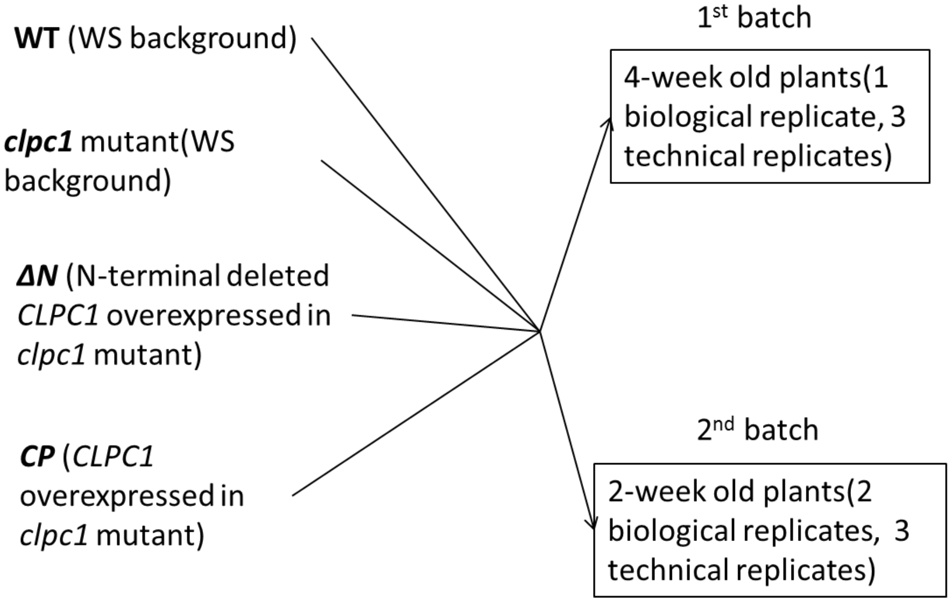


Figure S3. Schematic diagram to show the plant materials we used.



Note: Plant materials(all the plant materials used for proteomics are from Dr. Hsou-min Li (Chu & Li, 2012), and were grown at 21 degree with 16h light and 8h dark)

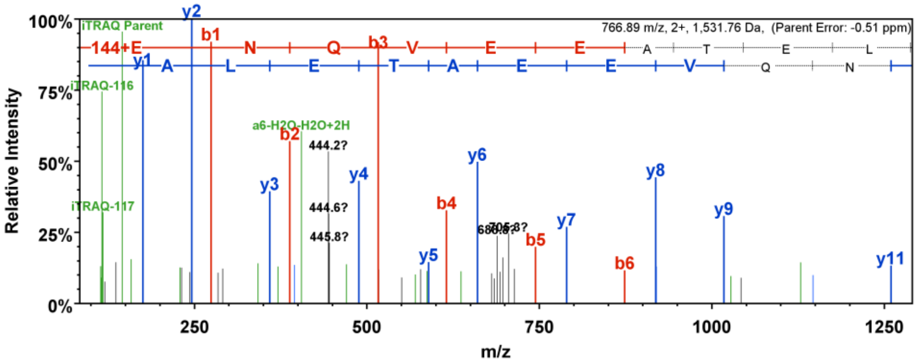
Supplementary dataset 1. **Spectral examples of 4 proteins from our iTRAQ-based proteomics analysis**

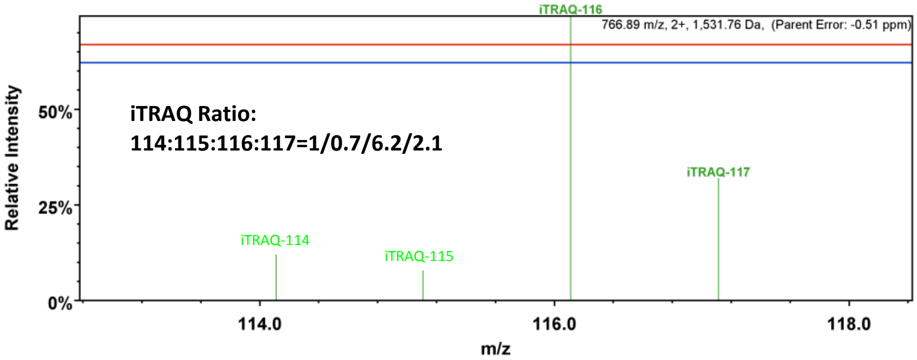
four proteins with each having two peptides and each peptide having two spectra (HCD and CID spectra) were shown below. The protein quantitation value was obtained by the weighted ratios of iTRAQ reporters from all identified peptides that belonged to the protein (enlarged iTRAQ reporter region was shown in the first spectral).

1. AT3G53700.1 [| Symbols: MEE40 | Pentatricopeptide repeat (PPR) superfamily protein |

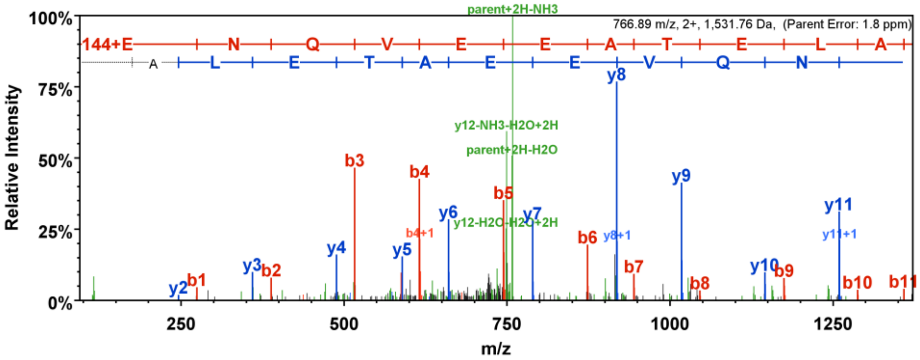
    a) Matched peptides: **ENQVEEATELAR**

HCD-spectral matched to TAIR10 database with iTRAQ reporter region (zoom in)



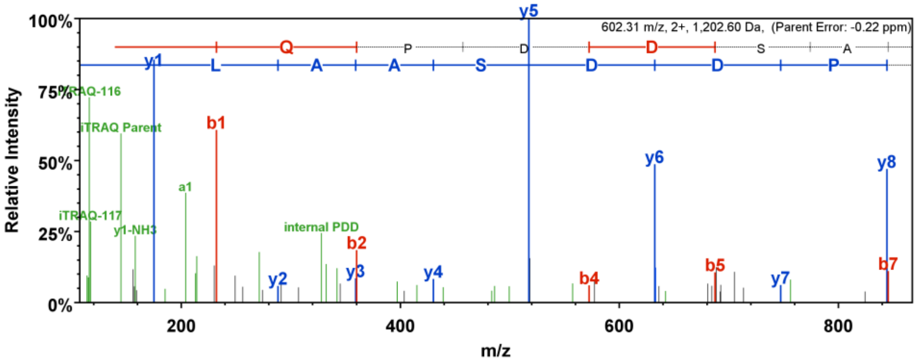


CID-spectral matched to TAIR10 database

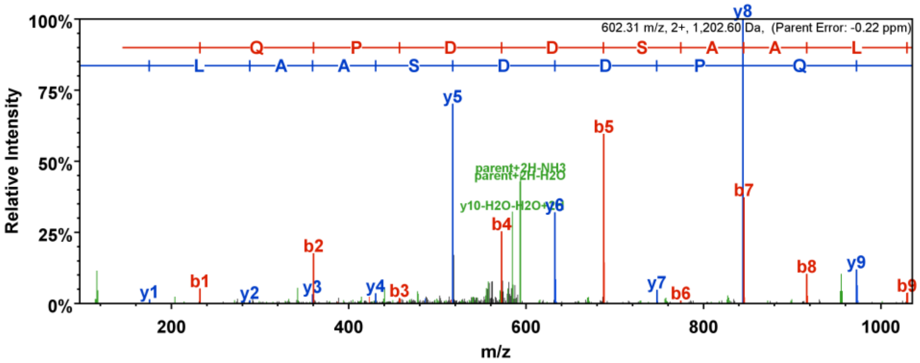


b) Matched peptides: **SQPDDSAALR**

HCD-spectral matched to TAIR10 database



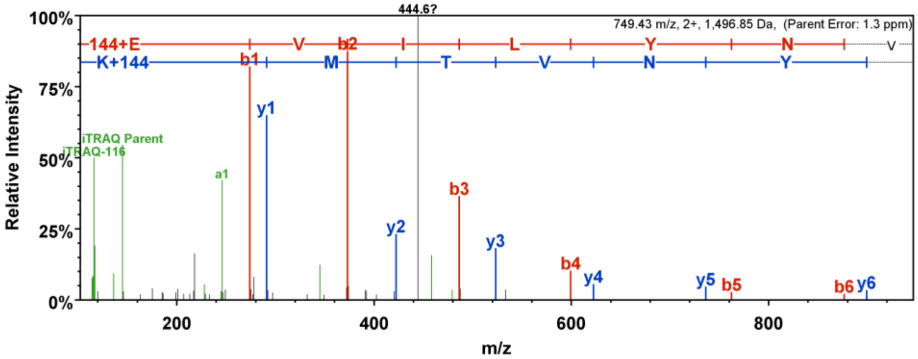
CID-spectral matched to TAIR10 database



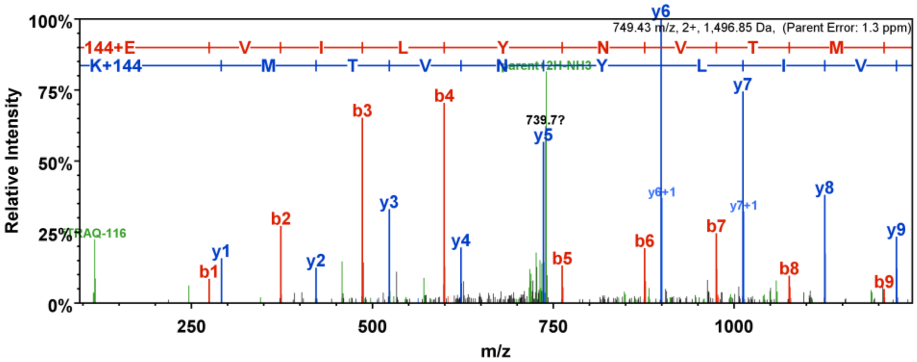
2. AT4G16390.1 [| Symbols: SVR7 | pentatricopeptide (PPR) repeat-containing protein

a) Matched peptides: **EVILYNVTMK**

HCD-spectral matched to TAIR10 database

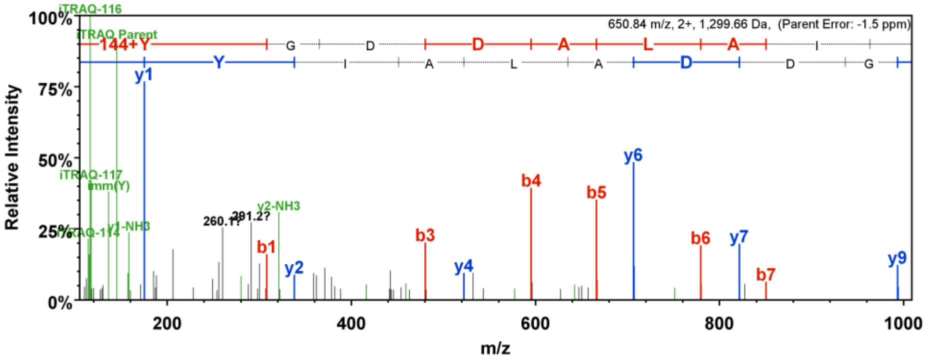


CID-spectral matched to TAIR10 database

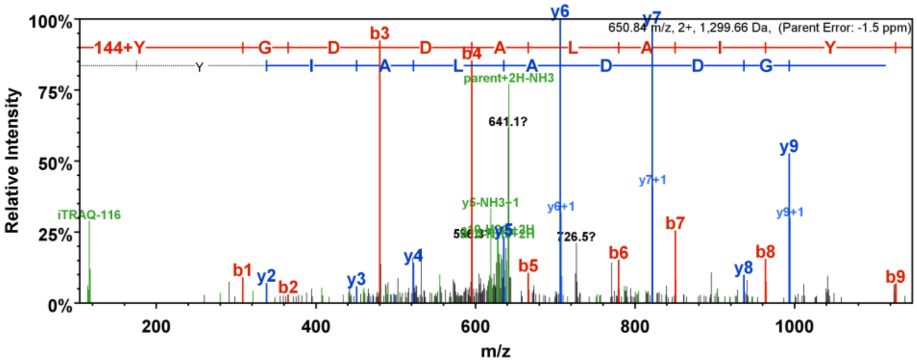


b) Matched peptides: **YGDDALAIYR**

HCD-spectral matched to TAIR10 database



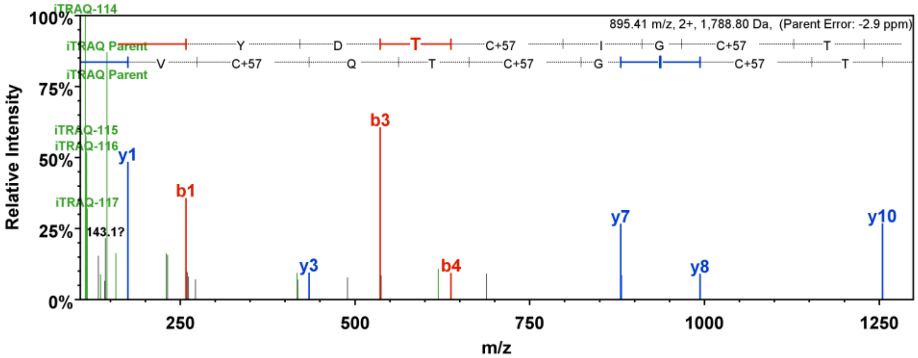
CID-spectral matched to TAIR10 database



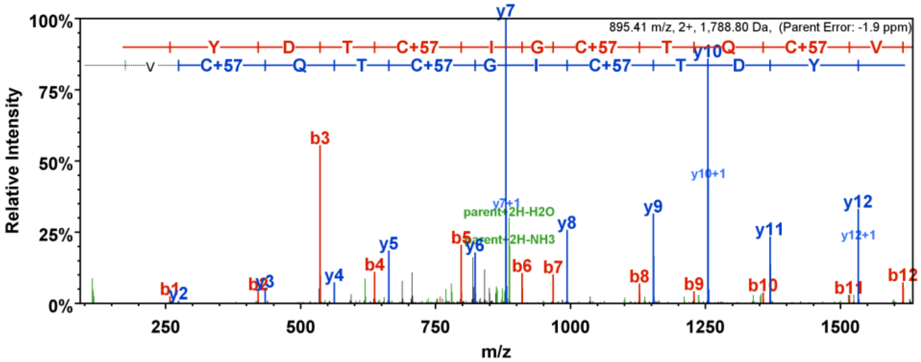
3. ATCG01060.1 [| Symbols: PSAC | iron-sulfur cluster binding;electron carriers;4 iron, 4 sulfur cluster binding

a) Matched peptide: **IYDTCIGCTQCVR**

HCD-spectral matched to TAIR10 database

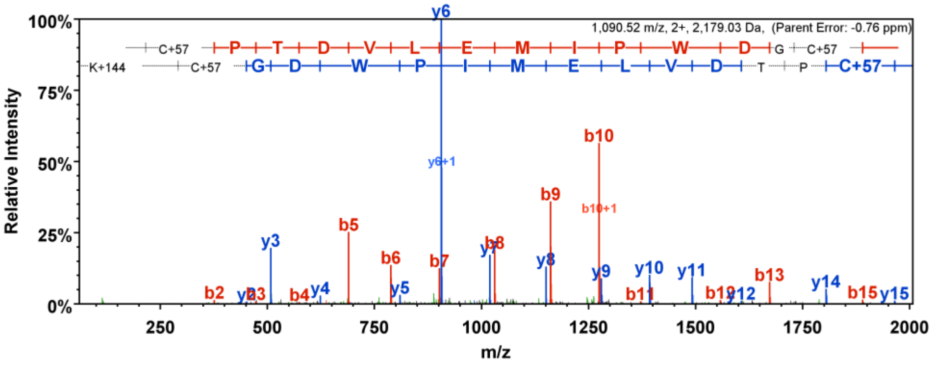


CID-spectral matched to TAIR10 database



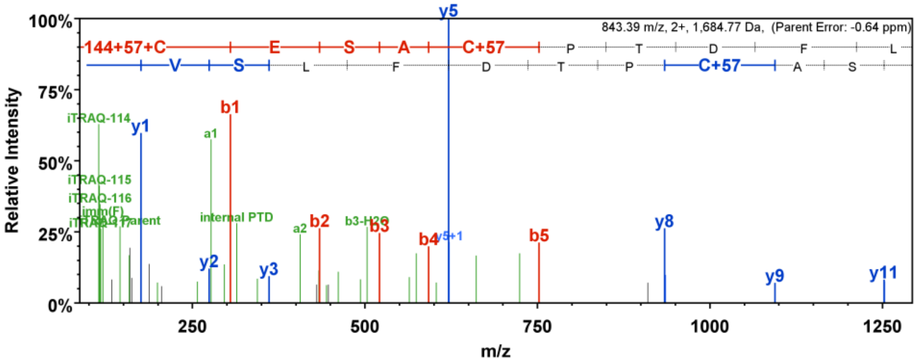
b) Matched peptide: **ACPTDVLEMIPWDGCK**

CID-spectral matched to TAIR10 database (HCD spectral is poor so no match to database)

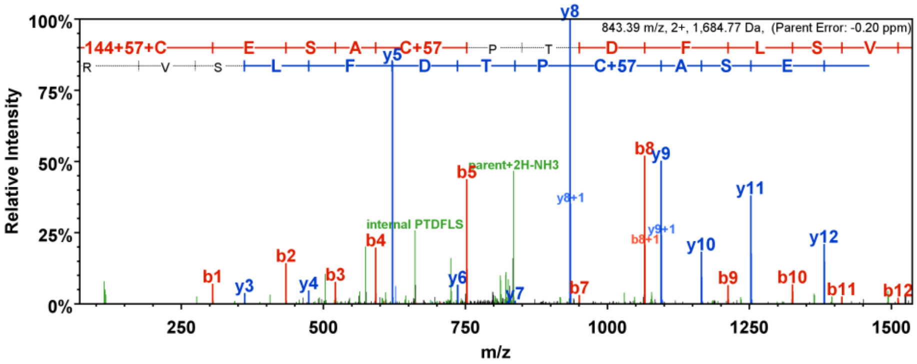


c) Matched peptide: **CESACPTDFLSVR**

HCD-spectral matched to TAIR10 database



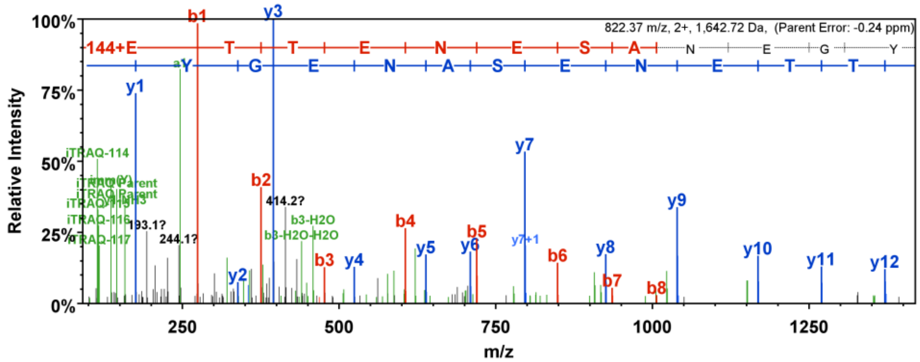
CID-spectral matched to TAIR10 database



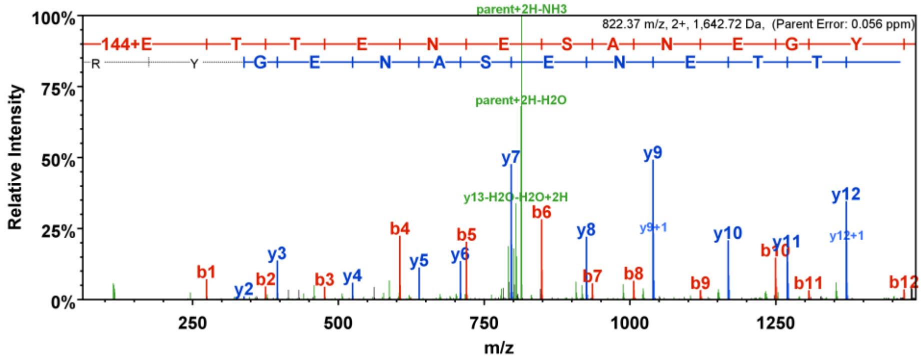
4. ATCG00020.1 [| Symbols: PSBA | photosystem II reaction center protein A

a) Matched peptide: **ETTENESANEGYR**

HCD-spectral matched to TAIR10 database

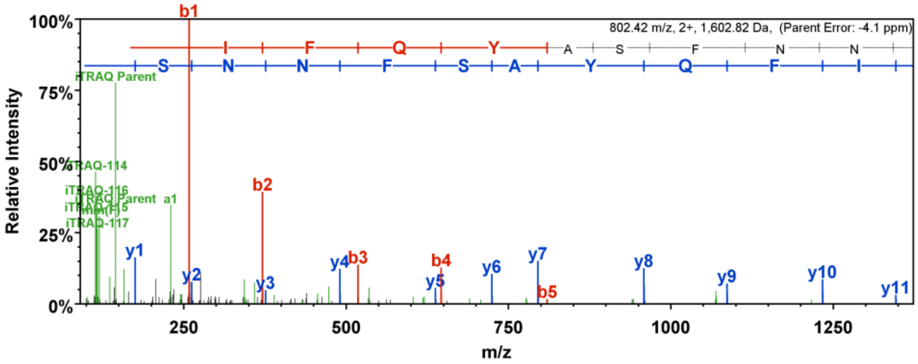


CID-spectral matched to TAIR10 database



b) Matched peptide: **LIFQYASFNNSR**

HCD-spectral matched to TAIR10 database



CID-spectral matched to TAIR10 database