**Table S1** Information of *CHUP1* associated genes in C3, C4, CAM, and SCC4 model plants

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plant type** | **Type** | **Species** | **Gene ID** | **Gene Name**  | **Genome location** | **Genomic position** | **Strand** | **CDS (bp)** | **Amino acids** |
| **Dicot** | **SCC4** | Bienertia | *Bsv0100-00036688* | *ABP* | 005074F | 79478-83961 | + | 1878 | 626 |
| *Bsv0100-00048649* | *TPR* | 009810F | 87009-93826 | + | 1902 | 634 |
| Suaeda | *GOSA\_00005395* | *ABP* | Contig 156 | 3076372-3081617 | + | 1839 | 613 |
| *GOSA\_00011445* | *TPR* | Contig 43 | 1555839-1549011 | - | 1995 | 665 |
| **C4** | Amaranthus | *AHYPO\_022001* | *ABP* | Scaffold 1206 | 51877-56043 | + | 1734 | 578 |
| *AHYPO\_001994* | *TPR* | Scaffold 10 | 436055-442560 | - | 1827 | 609 |
| *AHYPO\_004278* | Scaffold 27 | 259160-266086 | + | 1884 | 628 |
| **C3** | Quinoa | *XP\_021765375.1* | *ABP* | US | 1899882-1904524 | + | 1884 | 628 |
| *XP\_021749872.1* | US | 3442408-3446702 | + | 1887 | 629 |
| *XP\_021766074.1* | *TPR* | US | 1933640-1939082 | + | 1962 | 654 |
| *XP\_021749322.1* | US | 1033541-1038817 | + | 1953 | 651 |
| Arabidopsis | *NP\_564600.1* | *ABP* | Chr 1 | 19369089-19372121 | + | 1719 | 573 |
| *NP\_564524.1* | *HPR* | Chr 1 | 17835032-17837781 | + | 1674 | 558 |
|  |  | *NP\_193591.2* | *TPR* | Chr 4 | 10231218-10234693 | + | 1926 | 642 |
| **CAM** | Cactus | *Cgig1\_19563* | *ABP* | Scaffold 2273 | 28812-25420 | - | 1764 | 588 |
|  | *Cgig1\_14505* | Scaffold 3061 | 1458-5954 | + | 1884 | 628 |
|  | *Cgig1\_10838* | *TPR* | Scaffold 9690 | 59368-66464 | + | 1818 | 606 |
|  | *Cgig1\_08664* | Scaffold 11127 | 19773-13412 | - | 1626 | 542 |
| **Monocot** | **CAM** | Pineapple | *XP\_020088904.1* | *ABP* | Chr 5 | 1027105-1030983 | + | 1854 | 618 |
| *XP\_020101494.1* | *HPR* | Chr 13 | 11496196-11499219 | + | 1650 | 550 |
| *XP\_020098124.1* | *TPR* | Chr 11 | 10571857-10577577 | + | 1935 | 645 |
| **C3** | Rice | *LOC\_Os05g09380.2* | *ABP* | Chr 5 | 5266936-5270777 | + | 1851 | 617 |
| *LOC\_Os05g49820.1* | *TPR* | Chr 5 | 28588545-28593953 | - | 1977 | 659 |
| **C4** | Sorghum | *XP\_002439400.1* | *ABP* | Chr 9 | 7981764-7985560 | + | 1899 | 633 |
| *XP\_021306316.1* | *HPR* | US | 1722-5701 | - | 1386 | 462 |
| *XP\_021302756.1* | *TPR* | Chr 9 | 57783790-57779140 | + | 2022 | 674 |
| Maize | *Zm00001d037695.1* | *ABP* | Chr 6 | 134311545-134315819 | - | 1674 | 558 |
| *Zm00001d044594.1* | *HPR* | Chr 3 | 232914562-232917062 | - | 1263 | 421 |
| *Zm00001d039086.1* | *TPR* | Chr 6 | 169733422-169738386 | - | 2022 | 674 |
| **Outgroup** | **C3** | Amborella | *XP\_006826759.2* | *ABP* | US | 803189-806937 | + | 1905 | 635 |
| *XP\_006840355.1* | *HPR* | US | 1463253-1469687 | + | 1704 | 568 |
| *XP\_006852472.3* | *TPR* | US | 1732324-1747722 | + | 2007 | 669 |

US, Unplaced Scaffold.

Table S2. Subcellular localization of CHUP1 and its associated proteins of SCC4 plants species

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plant** | **Sequence ID** | **Description** | **Localization** | **Probability** |
|  |  |  |  | Cyto | Nucl | Extra | Cell mem | Mito | Plast | E.R. | Lyso/Vacu | Golgi | Perox |
| Bienertia | Bsv0100-00004053 | CHUP1 | Plast | 0.3 | 0.2 | 0.1 | 0.2 | 0.3 | **0.5** | 0.3 | 0.3 | 0.4 | 0.2 |
| Bsv0100-00048649 | CHUP1-like\_a | Cyto | **0.5** | 0.5 | 0.0 | 0.4 | 0.0 | 0.2 | 0.2 | 0.3 | 0.2 | 0.1 |
| Bsv0100-00036688 | CHUP1-like\_b | Cyto | **0.6** | 0.4 | 0.1 | 0.5 | 0.0 | 0.0 | 0.2 | 0.7 | 0.3 | 0.0 |
| Bsv0100-00030261 | ABP | Plast | 0.3 | 0.3 | 0.0 | 0.2 | 0.3 | **0.4** | 0.4 | 0.2 | 0.3 | 0.4 |
| Bsv0100-00002964 | TPR | Cyto | **0.5** | 0.4 | 0.0 | **0.6** | 0.0 | 0.3 | 0.2 | 0.4 | 0.3 | 0.0 |
| Suaeda | GOSA\_00026873 | CHUP1 | Plast | 0.3 | 0.2 | 0.1 | 0.2 | 0.3 | **0.5** | 0.3 | 0.3 | 0.4 | 0.3 |
| GOSA\_00011445 | CHUP1-like\_a | Cyto | **0.5** | 0.4 | 0.1 | 0.4 | 0.1 | 0.1 | 0.3 | 0.4 | 0.2 | 0.1 |
| GOSA\_00027286 | CHUP1-like\_b | Cyto | **0.6** | 0.4 | 0.0 | 0.4 | 0.0 | 0.0 | 0.2 | 0.7 | 0.4 | 0.0 |
| GOSA\_00025994 | ABP | Plast | **0.3** | 0.3 | 0.1 | 0.2 | 0.3 | **0.4** | **0.4** | 0.2 | 0.3 | **0.4** |
| GOSA\_00005395 | TPR | Cyto | **0.6** | 0.4 | 0.0 | **0.6** | 0.0 | 0.3 | 0.2 | 0.3 | 0.3 | 0.0 |

Note: Cyto, cytoplasm; Nucl, nucleus; Extra, extracellular; Cell mem, cell membrane; Mito, mitochondria; Plast, plastid; E.R., endoplasmic reticulum;

Lyso/Vacu, lysosome/vacoule; Golgi, golgi apparatus; Perox, peroxisome. ABP, actin-binding proteins; TPR, tetratricopeptide repeat.

Tool: DeepLoc - 2.0 Prediction of eukaryotic protein subcellular localization using deep learning (<https://services.healthtech.dtu.dk/services/DeepLoc-2.0/>).

**Table S3** Database links for the genomes used in this study

|  |  |
| --- | --- |
| **Plants** | **Database links** |
| *Amborella trichopoda* | https://genome.jgi.doe.gov/portal/pages/dynamicOrganismDownload.jsf?organism=Atrichopoda |
| *Zea mays* | http://ensembl.gramene.org/Zea\_mays/Info/Index |
| *Sorghum bicolor* | https://www.ncbi.nlm.nih.gov/genome/108?genome\_assembly\_id=321335 |
| *Oryza sativa* | https://genome.jgi.doe.gov/portal/pages/dynamicOrganismDownload.jsf?organism=Osativa |
| *Anana cosmosus* | https://www.ncbi.nlm.nih.gov/genome/13945?genome\_assembly\_id=271638 |
| *Arabidopsis thaliana* | https://www.ncbi.nlm.nih.gov/genome/4?genome\_assembly\_id=380024 |
| *Cactus gigantea* | https://datadryad.org/stash/dataset/doi:10.5061/dryad.72c2g |
| *Chenopodium quinoa* | https://www.ncbi.nlm.nih.gov/genome/12754?genome\_assembly\_id=278928 |
| *Amaranthus hypochondriacis* | https://genome.jgi.doe.gov/portal/pages/dynamicOrganismDownload.jsf?organism=Ahypochondriacus |
| *Suaeda aralocaspica* | http://gigadb.org/dataset/100646 |
| *Bienertia sinuspersici* | Unpublished |

**Table S4** Homology sequences used for proline rich repeats identification

|  |  |
| --- | --- |
| **Sequence ID** | **UNIPROT ID** |
| NP\_189197.2 | [Q9LI74](https://www.uniprot.org/uniprot/Q9LI74) |
| NP\_172192.1 | [Q9LMK4](https://www.uniprot.org/uniprot/Q9LMK4) |
| Zm00001d039104 | [A0A1D6MDK3](https://www.uniprot.org/uniprot/A0A1D6MDK3) |
| Zm00001d0351491 | [A0A3L6E5P0](https://www.uniprot.org/uniprot/A0A3L6E5P0) |
| Zm00001d028761 | [A0A1D6JZF5](https://www.uniprot.org/uniprot/A0A1D6JZF5) |
| Zm00001d047723 | [A0A1D6PCR1](https://www.uniprot.org/uniprot/A0A1D6PCR1) |
| XP\_020098505.1 | [A0A2H3ZAU1](https://www.uniprot.org/uniprot/A0A2H3ZAU1) |
| XP\_020099723.1 | [A0A199UI54](https://www.uniprot.org/uniprot/A0A199UI54) |
| XP\_020085709. | [A0A199VY21](https://www.uniprot.org/uniprot/A0A199VY21)  |
| Bsv0100-00004053 | [A0A0K9QIK8](https://www.uniprot.org/uniprot/A0A0K9QIK8) |
| Bsv0100-00030261 | [A0A0K9QHA8](https://www.uniprot.org/uniprot/A0A0K9QHA8) |
| Bsv0100-00002964 | [A0A0K9QVZ4](https://www.uniprot.org/uniprot/A0A0K9QVZ4) |

**Table S5** Primers used for real-time qPCR analysis *CHUP1* and its associated genes in *Bienertia sinuspersici*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene name** | **Gene ID** | **Forwards primer** | **Reverse primer** | **Amplicon size** |
| *CHUP1* | *Bsv0100-00004053* | TAGGCTTGTTGGTTGGTGCT | TGTGGTGGATAGTGGCTTCA | 123 |
| *CHUP1-like\_a* | *Bsv0100-00030261* | CCTGCTGAAGAAGTTGAGAAGA | TGGTGGAGTAGCCGTAGAA | 140 |
| *CHUP1-like\_b* | *Bsv0100-00002964* | GCATGCATATTTAGGCACATCTC | TCCACTAAGAAGGCCATTTCC | 131 |
| *ABP* | *Bsv0100-00036688* | AGAGGCACAGGTTTCTGATTAC | GCTCCGATTCGTACCTAATCTTC | 101 |
| *TPR* | *Bsv0100-00048649* | CGTCGAACAACTCCGTAACA | CAGCTTCAAGTGAGGGAACA | 101 |
| *GAPDH* | *Bsv0100-00017536* | CTCCAGGAATCCAGAGGAGATA | CTTTGCTCCACCCTTCAAATG | 118 |