

### **Description of Additional Supplementary Files**

**Supplementary Data 1:** Estimation of genome sizes of five *Flaveria* species using Flow cytometry

**Supplementary Data 2:** Investigation of chromosome numbers using Fluorescence in situ hybridization assays

**Supplementary Data 3:** Estimation of genome assembly completeness

**Supplementary Data 4:** Comparison of protein-coding genes from Taniguchi's assemblies and our assemblies

**Supplementary Data 5:** Determination of functional copies of C4 genes

**Supplementary Data 6:** C4 version of *PEPC-k* was absent in Fram plant sequenced in this study

**Supplementary Data 7:** Verification of three copies of PEPC1 in the C4 species Ftri

**Supplementary Data 8:** Investigation of tandem duplications of C4 genes in other C4 species

**Supplementary Data 9:** Analysis of transposable elements and their effects on duplicated FtriPEPC1

**Supplementary Data 10:** Comparison of transcript abundances based on RNA-seq data

**Supplementary Data 11:** Comparison of protein abundance based on proteomics

**Supplementary Data 12:** Comparison of protein-to-transcript ratio

**Supplementary Data 13:** Prediction of *cis*-regulatory elements using ATAC-seq 52

**Supplementary Data 14:** ERF *cis*-regulatory elements were abundant in photosynthesis related genes of different C4 species

**Supplementary Data 15:** Construction of gene regulatory networks